CITY OF BOULDER
CITY COUNCIL AGENDA ITEM

MEETING DATE: November 8, 2018

AGENDA TITLE
Discussion and Direction for Updating and Improving the City's Mosquito Management Program

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EXECUTIVE SUMMARY
In the past five decades, mosquito populations have increased around the country by as much as 10-fold, while other insect populations around the world are plummeting. According to a recent study, the main drivers for this increase in mosquito populations are caused by humans—changing land use patterns, such as urbanization, and pesticide impacts to the environment. The same factors that favor mosquitoes are leading to biodiversity loss in many other species. While mosquitoes can readily breed in poor-quality sites created by human disturbance, other organisms—including many of the predators that naturally control mosquitoes—require higher quality habitat. Furthermore, mosquito control pesticides can alter already-imperiled habitats, which can

unintentionally contribute to conditions that favor mosquitoes, as well as impact non-target species.

The city's mosquito management program was implemented in 2003 to address the then-new threat of West Nile virus (WNv) to public health. Integrated Pest Management (IPM) principles were balanced with trying to eliminate as many of the mosquitoes that could potentially transmit WNv as possible. After the 2003 epidemic, models indicate that the risk from WNv decreased, while overall mosquito activity has risen in recent years despite efforts to control mosquitoes. In addition, new scientific information has become available on the detrimental effects of the larvicide currently used in Boulder, *Bti* (*Bacillus thuringiensis israelensis*). For these reasons, staff has evaluated the approach of the current mosquito program and studied different methods to better manage mosquitoes. The purpose of this memo is to provide this information to council and emphasize the need to revise the city's mosquito management approach to increase the overall effectiveness of the program, while better protecting ecosystems from the impacts of mosquito treatments and their unintended consequences.

An interdepartmental team of staff ecologists and expert consultants have analyzed historic data, reviewed the scientific literature and suggest the following changes for the city's program in these major categories:

1. **Managing mosquito breeding:**
    - Re-examine and update the original plan's mosquito breeding site categories to better reflect each site's characteristics and choose the most effective management option(s);
    - Use a comprehensive suite of mosquito management tools on a site-by-site basis;
    - Focus on preventing mosquito breeding sites by actions such as improving drainage, altering irrigation water release schedules, filling ruts, etc.
    - Develop new field protocols for two teams of specialized field technicians using either targeted *Bti* applications or ecological management tools;
    - Use the same treatment options to manage both the mosquitoes that can potentially transmit WNv and nuisance mosquito larvae; and
    - Use a holistic approach for mosquito larval treatments. Support ecological systems overall, using a range of appropriate treatments to help maintain healthy wetlands and work towards restoring degraded wetlands.

2. **Educating, training, and building awareness:**
    - Train operations crews from Public Works and Parks and Recreation to survey and identify urban mosquito breeding areas, such as storm drains, water-saturated areas in turf, clogged ditch grates, etc., and mitigate or treat with *Bti;*
    - Develop more comprehensive education and outreach materials for the public to reduce breeding sites on private property and use effective methods to avoid mosquito bites; and
    - Build awareness of the city's approach to reduce mosquitoes and protect public health.

3. **Continuous improvement using adaptive management based on field data:**
    - Gather site-specific data from field monitoring visits to better understand the factors that influence mosquito breeding;
    - Review field data at the end of each season and revise and improve management protocols as information is gathered and more is learned; and
    - Protect city, county and state sensitive species including, but not limited to northern leopard frog, northern redbelly dace, grassland-nesting birds, plains topminnow, bobolinks, American bitern and federally-managed species such as bald eagle.

Staff wetland and wildlife ecologists are currently categorizing larval breeding sites based on ecological value. Consultants are developing new field protocols for treatment options and to gather ecological indicators to optimize mosquito treatments based on site type.

If council supports the staff recommendation, staff will complete the work to update the mosquito management program, and will implement changes during the 2019 field season.

STAFF RECOMMENDATION

Staff recommends that the current mosquito program be revised using a comprehensive approach that more effectively manages mosquitoes and includes:

- An adaptive management plan with site-specific appropriate treatments, where the field data is reviewed after each field season to improve specific management strategies and the overall program is assessed each year to refine mosquito management the following season;
- Focus on prevention of artificial larval breeding sites by developing an irrigation management strategy, a program for urban monitoring and mitigation by utilities and parks operations crews, and public education and outreach to eliminate breeding sites on private property;
- Development of new field monitoring and treatment protocols that target *Bti* application to where it will be most effective and gathers ecological information and site attributes to utilize the best and most effective management option;
- Reduce the use of *Bti* as much as possible, while not compromising mosquito management outcomes by focusing on ecologically-sound treatment options.

COMMUNITY SUSTAINABILITY ASSESSMENTS AND IMPACTS

- Economic: The city’s environmental stewardship and ecologically-based management approach create beautiful, safe and award-winning athletic facilities, parks, streetscapes, urban forest, natural lands, local food production, and recreational and outdoor activities, that provide revenue for the city and businesses, and generates tourism. Ecosystem services are more difficult to monetize, but protection and enhancement of healthy ecosystems supports a sustainable economy and saves money and resources by providing natural pest control, mitigating the impacts of climate change and reducing the costs from extreme weather events.

- Environmental: Protection of plants, wildlife and biodiversity is crucial for maintaining ecosystem services. The city's management strategies improve the health of the community and the surrounding lands and waterways by reducing chemical inputs, utilizing ecologically-focused management, and restoring and protecting healthy ecosystems.

- Social: A healthy and safe environment encourages the public to go outdoors and participate in recreational and athletic activities, interact with nature and improves overall well-being. High mosquito activity can diminish these activities. The city's program seeks to protect public health and enjoyment of the outdoors, using an environmentally-sound and scientifically-based approach.

OTHER IMPACTS

- Fiscal - The 2018 contract for mosquito control services was $257,566, with annual increases expected in future years if the program is continued as it's currently structured. Consultant fees are approximately $40,000 for review, analysis and development of new field protocols.
- Staff time – A team of 10 staff ecologists/specialists have committed significant work plan time to analyze the program and develop new tools. Other staff have provided additional resources to address the concerns of residents throughout the city, and to make improvements to high-mosquito activity areas in surrounding fields and utility channels around the Greenbelt Meadows neighborhood.

BOARD AND COMMISSION FEEDBACK

Staff presented its recommendation to the Environmental Advisory Board (Oct. 3), Open Space Board of Trustees (Oct. 10) and Parks and Recreation Board (Oct. 22). All three boards unanimously support the staff approach for updating the mosquito program.

Feedback includes:

- Using a more ecological approach provides many other benefits to the city's wetlands that could be monetized;
- The program can better utilize funds to make the environment better;
- Add a way to report standing water through Inquire Boulder;
- The city should be more transparent so the public better understands the city's program;
- Communicate clearly that staff is proposing doing more to manage mosquitoes with changes to the current program, not doing less as it may first appear;
- The city should emphasize personal responsibility and provide more outreach and education to empower the public; and
- Continue outreach and education about WNv and the importance of people protecting themselves to avoid contracting the disease.

PUBLIC OUTREACH AND FEEDBACK

During the summer, staff held a public meeting, met with the community at Neighborhood Office Hours, and reached out to concerned citizens one-on-one about the underlying causes of high mosquito activity and the need to update the current program. The current staff recommendation had not been fully developed at that time. The response was mixed—some support the approach the city is using and some people from high mosquito-activity neighborhoods are frustrated and expect the city to lower mosquito activity.

Staff is updating the city's website, creating an educational video and working on outreach materials to provide information to the public about proposed changes to the program and to listen to peoples' concerns, suggestions and feedback. Public engagement about upcoming changes to the program will occur during Dec. 2018 to February 2019, and ongoing engagement will continue during the 2019 mosquito season as changes to the program are implemented.

BACKGROUND

With changing climate, habitat destruction/fragmentation, and contamination from pollutants, including widespread pesticide use, alterations in species composition and range are transforming the world's ecosystems with consequences that are yet to be fully understood. A February 1, 2018 Information Packet memo discusses the planet's biodiversity crisis and the city's Ecological Integrated Pest Management (IPM) Policy that uses a holistic approach that relies less on direct control methods of individual undesirable species and focuses predominantly on enhancing biodiversity and ecosystem balance to utilize the natural processes that keep populations of undesired species low.

When the city first developed a mosquito management program in 2002, the environmental and human health risks of using a range of pesticides to target mosquitoes at every life stage were found to be too high and were not in alignment with the city's IPM Policy and natural lands protection guidelines. As West Nile virus (WNv) was moving westward across the country at that time, it necessitated a thoughtful and effective plan to protect the public health. The city developed a plan to address the threat of WNv to the public, while minimizing the impacts to the environment.

The mosquito species that can vector or transmit WNv, *Culex* mosquitoes, breed in urban areas in objects or depressions that contain water, so outreach and education programs were initiated to encourage the public to drain standing water in residential yards and inform residents about the importance of personal responsibility to avoid mosquito bites.

Culex can also breed in natural areas, and many potential mosquito breeding sites are on city-managed open space and natural lands. City staff were concerned about applying mosquito control products that could disrupt wetland ecological balances. Therefore, the city's current program focused on limiting the amount of larvicide (*Bti*) applied to wetlands by treating only *Culex* larvae and leaving non-vector or nuisance mosquito larvae untreated to reduce *Bti* application and maintain an important food source for other animals. In 2007, a nuisance program was added to the WNv management program in high mosquito activity areas around city recreational facilities and neighborhoods. For more information about the city's program, identified gaps, and the approach for the current program update, please see the April 12, 2018 Information Packet memo.

Types of mosquitoes in Boulder

Fifty-seven species of mosquito have been identified in Colorado and around a dozen are caught in city mosquito traps. Mosquitoes are divided into two main categories based on their lifecycle and behavior—those that lay their eggs on standing water and those that lay their eggs on damp soil or mud, the floodwater mosquitoes. Vector (disease-transmitting) mosquitoes in Colorado are *Culex* species that lay their eggs on water. Non-vector mosquitoes are predominantly floodwater mosquitoes. This difference in biology is an important consideration for effectively managing mosquitoes. The following chart shows the major distinctions between the vector (*Culex*) and non-vector or nuisance mosquitoes.

Vector

(potential to transmit disease)

Culex species in Colorado are most important for transmitting West Nile viurs

Culex lay eggs directly on water

Adults are more likely to be close to larval breeding sites

Non-Vector

("nuisance" mosquitoes unlikely to transmit disease to people)

Most nuisance mosquitoes are floodwater species that lay eggs on mud and overwinter as eggs. Some species lay eggs on water and overwinter as larvae

Often have large synchronus emergence in spring

Adult mosquitoes are often found many miles from larval breeding sites - some species travel 25 miles or more

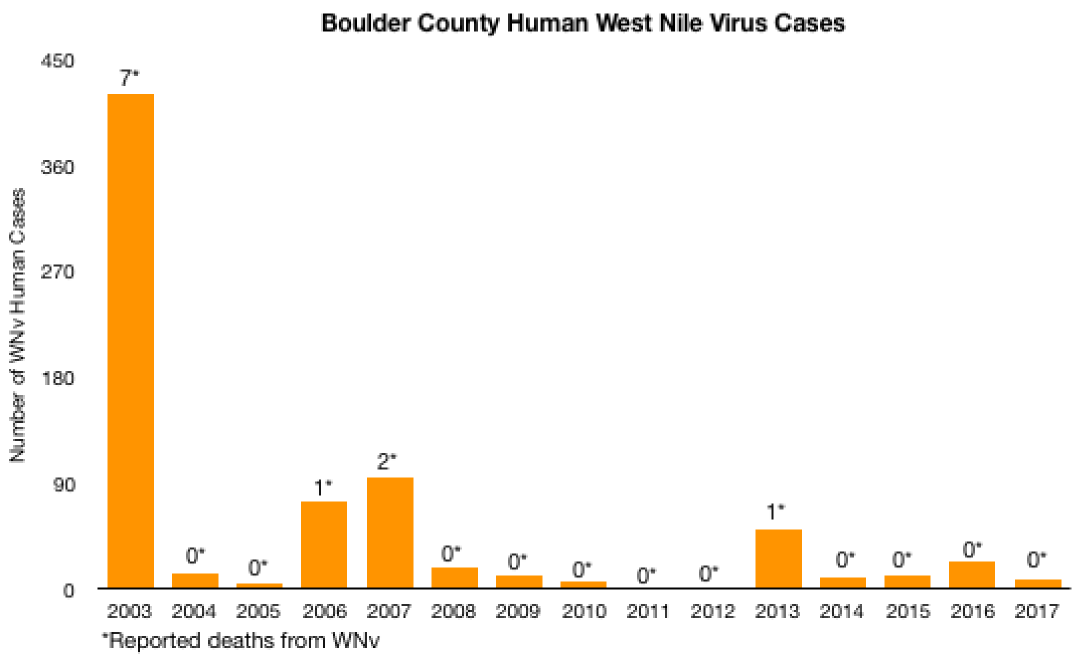
ANALYSIS

What has changed since the original mosquito management plan was developed?

Field Data – During the 15 years since the city's program was adopted, the city has collected weekly adult mosquito trap data that provides information about location, abundance and the species of mosquitoes that are present throughout the city. The density and mosquito larval type (*Culex* and non-*Culex*) are collected weekly from each breeding site. Staff and consultants continue to analyze this dataset to determine the patterns of adult mosquito activity, larval site breeding patterns and modeling the effect of different *Bti* application protocols on projected adult mosquito emergence.

Ecosystem Impacts - During the development of the city's WNv mosquito management plan, the scientific literature was reviewed to determine the impacts of different mosquito control products and all were found to have broad and unacceptable impacts, except for the bacterial larvicide product, *Bti*. A handful of studies that were available at the time showed non-target impacts and ecosystem alterations, since *Bti* kills all aquatic fly larvae, including hundreds of harmless species. Basic ecological principles would suggest that removal of a large component of the base of wetland food webs would impact multiple other species. A recent literature review revealed wide-ranging adverse impacts, both direct and indirect, to non-target species and demonstrated ecosystem-wide impacts from *Bti* use (see Attachment A). In light of this new information, staff and ecological consultants have been reassessing larval treatment protocols, and are developing a range of effective treatment options, so that *Bti* use can be reduced and following the [IPM process](), less impactful options can be considered first.

West Nile Virus Risk to People – Our understanding of the epidemiology and risk for WNv has changed since 2003, which should be used to inform the city's management approach. When WNv arrived in the Front Range in 2003, human cases reached epidemic levels and Boulder County had the highest number of cases in the nation. Cases declined sharply in 2004, and although WNv is now endemic with cases occurring every year, it has not reached epidemic levels since 2003.

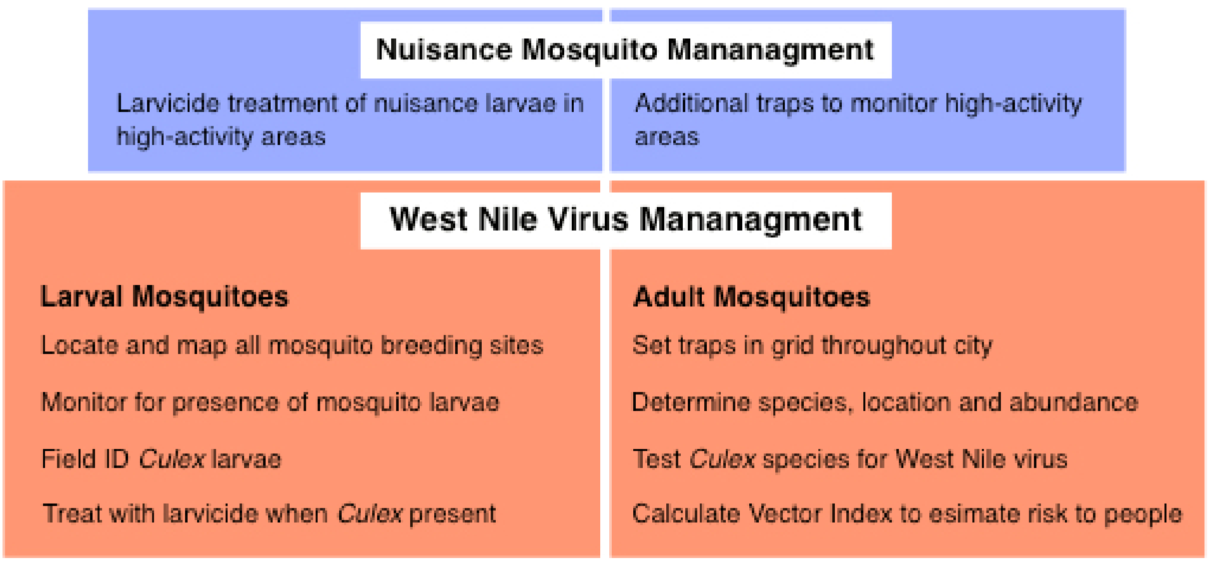


A [recent study]() modeled the driving forces of WNv human cases in 10 states, including Colorado, under current and future climate scenarios and identified the drivers, which vary depending on geographic region. WNv cases in Colorado are primarily driven by two factors—drought and human immunity. The majority—80 percent—of people who contract WNv have no symptoms and are unaware they were infected, but then develop immunity. The authors of this study suggest that population-wide protections from immunity are much higher than expected and their models predict that it is unlikely that Colorado will experience another WNv epidemic. However, it's important to keep in mind that susceptible individuals can become ill if bitten by an infected mosquito and public education about personal protection and larval site reduction is crucial for public health.

Appropriateness of Adult Mosquito Control Contingency Plan – The [WNv Mosquito Management Plan]() contains provisions for adult mosquito management that includes spraying/fogging with insecticides in the event of a WNv outbreak. Studies show this approach is ineffective with potentially adverse effects for human and environmental health. A WNv outbreak is also unlikely, and if it or another mosquito-borne disease were to reach concerning levels, staff has outlined a series of escalating risks and associated actions to reduce human exposure (Attachment B).

A team of interdepartmental staff ecologists and ecological consultants have been reviewing data, scientific literature and assessing ecologically-sound practices to reduce mosquito activity.

The city's current program has two major components – 1) larval site monitoring and larvicide treatment and 2) adult mosquito monitoring and WNv testing.



In addition to refining larval treatment protocols, there are other opportunities to improve mosquito management. Staff is proposing an adaptive management process that addresses each site individually, gathers data to assess adult and larval populations, relevant ecological parameters, and reviews the data each year to continuously improve the program.

The following table provides an overview of proposed changes to the program.

	Program Component	**Rationale**
Keep Unchanged	Adult mosquito trapping, monitoring and WNv testing	Provides valuable information about overall mosquito activity and WNv risk
Modify	Larval breeding site treatment	Site-specific treatments provide better mosquito management,

	Program Component	Rationale
	– Categorize sites by ecological quality – Pinpoint mosquito breeding habitat within each site – Choose from multiple treatment options to tailor the most appropriate/effective management for each site – Refine existing field treatment protocols for *Bti* application – Develop new protocols for ecological field technicians to monitor mosquito breeding, natural enemy presence, and other relevant site attributes	protect biodiversity and can provide more comprehensive, sustainable and effective management of mosquito populations based on current research and scientific/ecological principles
Modify	Adult mosquito control contingency plan	The original WNv management plan allowed for insecticide application for adult mosquitoes if certain thresholds were met. The potential harm of adulticide treatments outweighs any potential benefit. A chart of escalating WNv risk and associated city actions has been created that addresses risk and protect public health (Attachment B).
Modify	Improve public education and outreach	– Provide more outreach about the role of personal responsibility and actions to reduce mosquito breeding sites and prevent bites. – Improve complaint tracking to help ID potential mosquito problem spots and refine management to more effectively address. – Provide more information about city operations to increase transparency and better understanding about the city's program.

	Program Component	Rationale
Add	Integrated irrigation management and infrastructure maintenance strategy by interdepartmental team	Minimize mosquito breeding sites caused by irrigation by evaluating drainage from fields and trails, modify irrigation water release schedules where appropriate, coordinate between departments responsible for ditch maintenance or relationships with ditch companies to better respond to mosquito breeding issues – both prevention and responding to problems as they arise
Add	Train urban staff from Parks Operations and Public Works to recognize breeding problem spots in parks, storm water drains and other public areas	Crews will receive training to report or manage areas with stagnant water and respond in the field to drain or treat with *Bti*.
Add	Develop materials for code enforcement to provide to private property owners with standing water issues.	There is no ordinance to address standing water on private property. Code enforcement can provide educational materials in response to neighbor complaints about standing water issues.

Most significant proposed change – larval breeding site assessment and treatment options

Mosquito breeding sites cover a wide range of types from muddy depressions in soil, stagnant water in containers or storm drains to high quality wetlands. If breeding sites can be eliminated by inspecting and draining artificial sites, cleaning clogged trash guards in ditches or managing flood irrigation, this is the quickest and most effective approach. However, sites with high ecological function can possess built-in pest controlling organisms, such as fish, predatory insects, birds and spiders that can keep mosquito populations naturally low. *Bti* should be used where appropriate, but alternative treatments should first be considered.

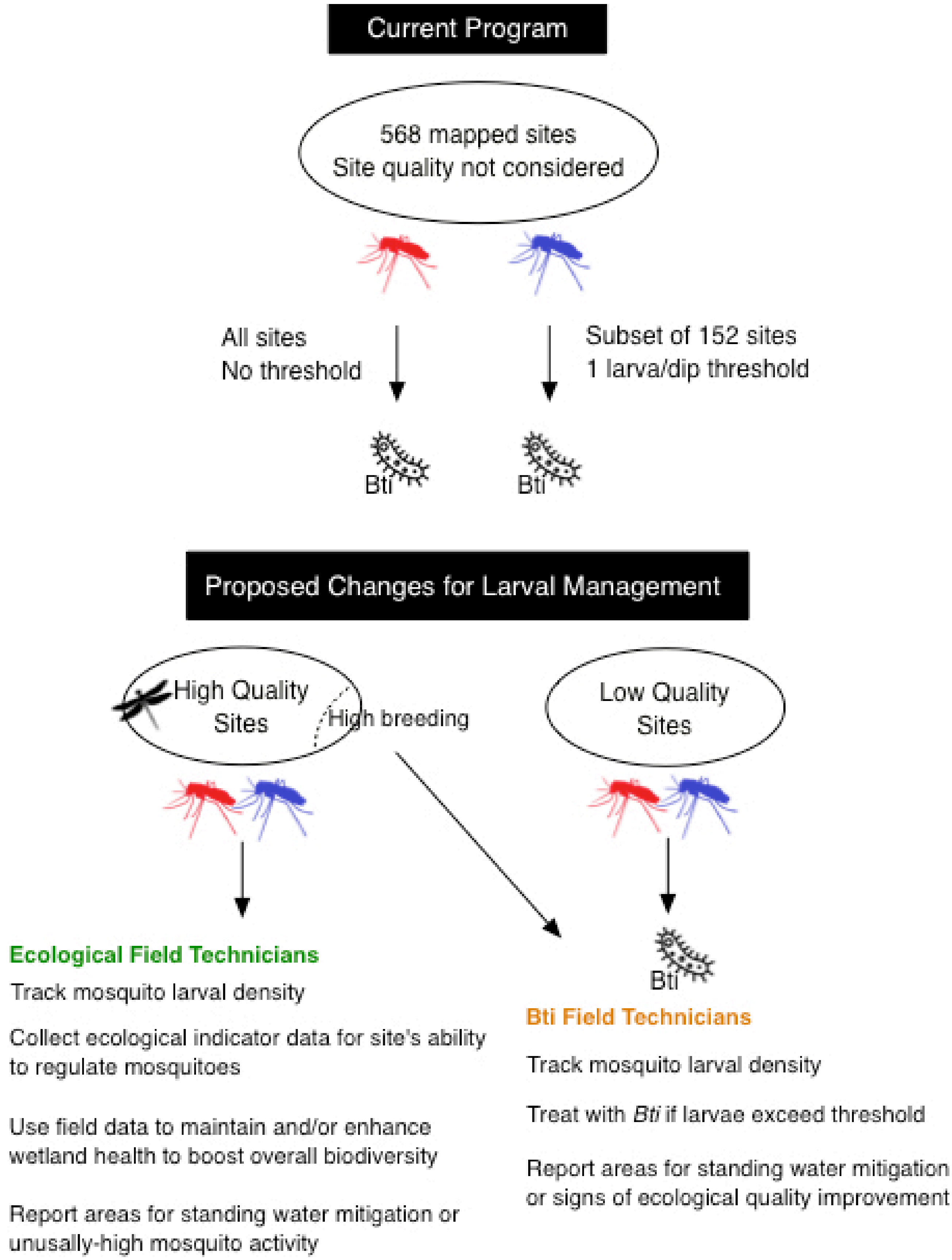
Staff is currently assessing the ecological significance of breeding sites **and** mosquito larval breeding history to develop a site-specific management plan. Breeding sites fall roughly into the following categories:

General Characterization of Mosquito Larval Breeding Types

The most challenging category is 4 – high quality/high breeding sites. Some of these sites may need to be treated with *Bti* in the short-term. However, high quality/high breeding sites are the most susceptible to disruption from repeated *Bti* applications and staff will be exploring alternative treatments to decrease mosquito breeding habitat and enhance predator populations.

A table of mosquito treatment options are provided in Attachment C. The current program treats all vector mosquitoes at all sites with *Bti*. Non-vector mosquitoes occur at the same sites as vector mosquito larvae approximately 50 percent of the time and are also treated by association. A subset of sites is treated when only non-vector larvae are present as part of the nuisance mosquito program. Sites in the nuisance program are also monitored earlier in the season, since they emerge before *Culex* mosquitoes.

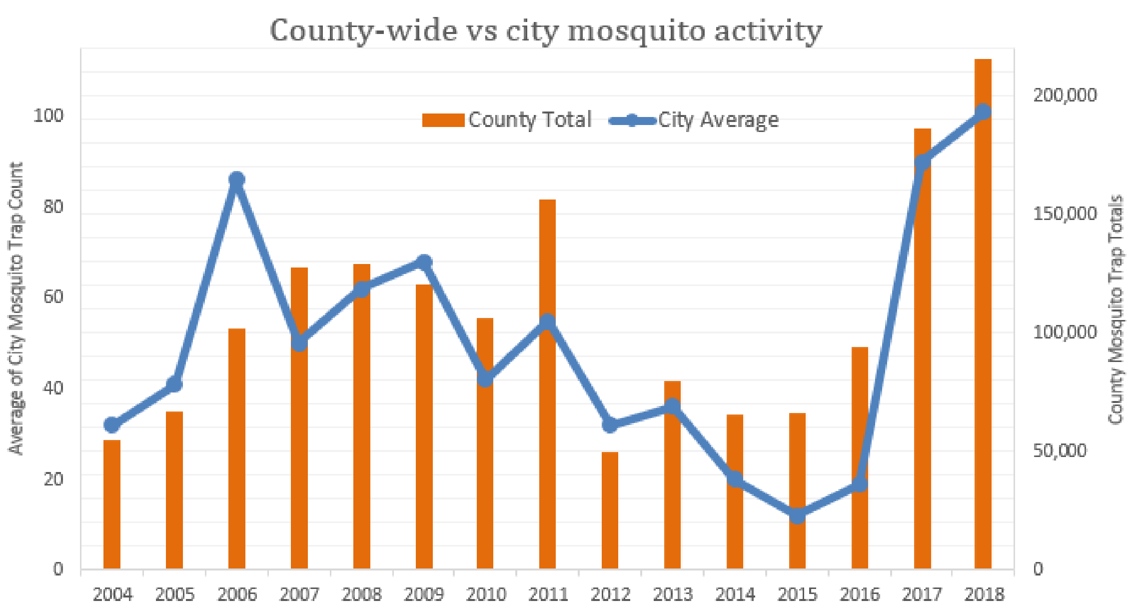
The current program relies on field technicians identifying the vector larvae in the field, which is time-consuming and adds cost to the overall program. Staff is exploring combining the vector and nuisance mosquito programs. Applying the same treatment thresholds to both groups of larvae may improve overall mosquito management, be more cost-effective and provide better protection for sites with high ecological value. This may also increase the treatment of some nuisance mosquitoes. The current program would be maintained by continuing to monitor and treat the sites currently designated as nuisance sites early in the spring, since floodwater mosquitoes emerge earlier than *Culex* mosquitoes. A consultant is currently determining the appropriate *Bti* treatment thresholds based on larval breeding patterns over the last 15 years. The characteristics of the breeding site itself will guide the treatment options and choices. The diagram below compares the current program to the proposed process.


Current Program
568 mapped sites
Site quality not considered
All sites
No threshold
Subset of 152 sites
1 larva/dip threshold
Bti
Bti
Proposed Changes for Larval Management
High Quality Sites
High breeding
Low Quality Sites
Ecological Field Technicians
Track mosquito larval density
Collect ecological indicator data for site's ability to regulate mosquitoes
Use field data to maintain and/or enhance wetland health to boost overall biodiversity
Report areas for standing water mitigation or unusally-high mosquito activity
Bti
Bti Field Technicians
Track mosquito larval density
Treat with *Bti* if larvae exceed threshold
Report areas for standing water mitigation or signs of ecological quality improvement

Mosquito Activity in Boulder and Surrounding Area

Mosquito activity in Boulder mirrors activity in the surrounding areas, particularly for floodwater mosquitoes that can travel great distances and can achieve high numbers on the northern and southeastern edges of the city. The mosquito larval breeding sites on city-owned properties have been inspected and treated for 15 years. The chart below shows the relationship between county-wide mosquito activity and average mosquito trap count for the City of Boulder. For the most part, when mosquito numbers are lower regionally, they are lower for the city and when mosquito numbers are high regionally, the city also experiences high numbers. One of the most important methods to reduce mosquitoes traveling from outside city management areas is to support and enhance biodiversity, particularly predators such as birds, dragonflies and spiders that can help to control adult mosquitoes. Robust biodiversity in wetlands can also reduce mosquito eggs and resulting larvae.

It is important to continue judicious use of *Bti* for low-quality sites, but highly-functioning wetlands can [provide mosquito control](), as well as many other benefits.



Greenbelt Meadows: The most challenging area in the city is the Greenbelt Meadows neighborhood, which experiences much higher mosquito activity than other parts of the city. Attachment D describes the extent of the issue in Greenbelt Meadows, actions that have been taken and proposals to address the issue during the 2019 season.

NEXT STEPS

- Staff and consultants will complete:
    - Breeding site categorization (Dec. 2018)

    - Breeding history analysis for individual sites (Dec. 2018)
    - Completion of field protocols for *Bti* application and ecological monitoring (Dec. 2018)
- Public engagement – Dec. 2018 – Feb. 2019
- Complete Request for Proposal for program components – Feb. 2019
- Hire contractor(s) – March 2019
- Implementation of revised program – April 2019
- Provide council with update after first year of implementation – November 2019

ATTACHMENTS

Attachment A: Review of Scientific Literature for Impacts of *Bacillus thuringiensis sub-species israelensis* (*Bti*) for Mosquito Larval Control
Attachment B: Actions for Escalating West Nile Virus Risk
Attachment C: Mosquito Treatment Options
Attachment D: Efforts to Reduce Mosquito Activity in the Greenbelt Meadows Neighborhood

Review of Scientific Literature for Impacts of *Bacillus thuringiensis sub-species israelensis* (*Bti*) for Mosquito Larval Control

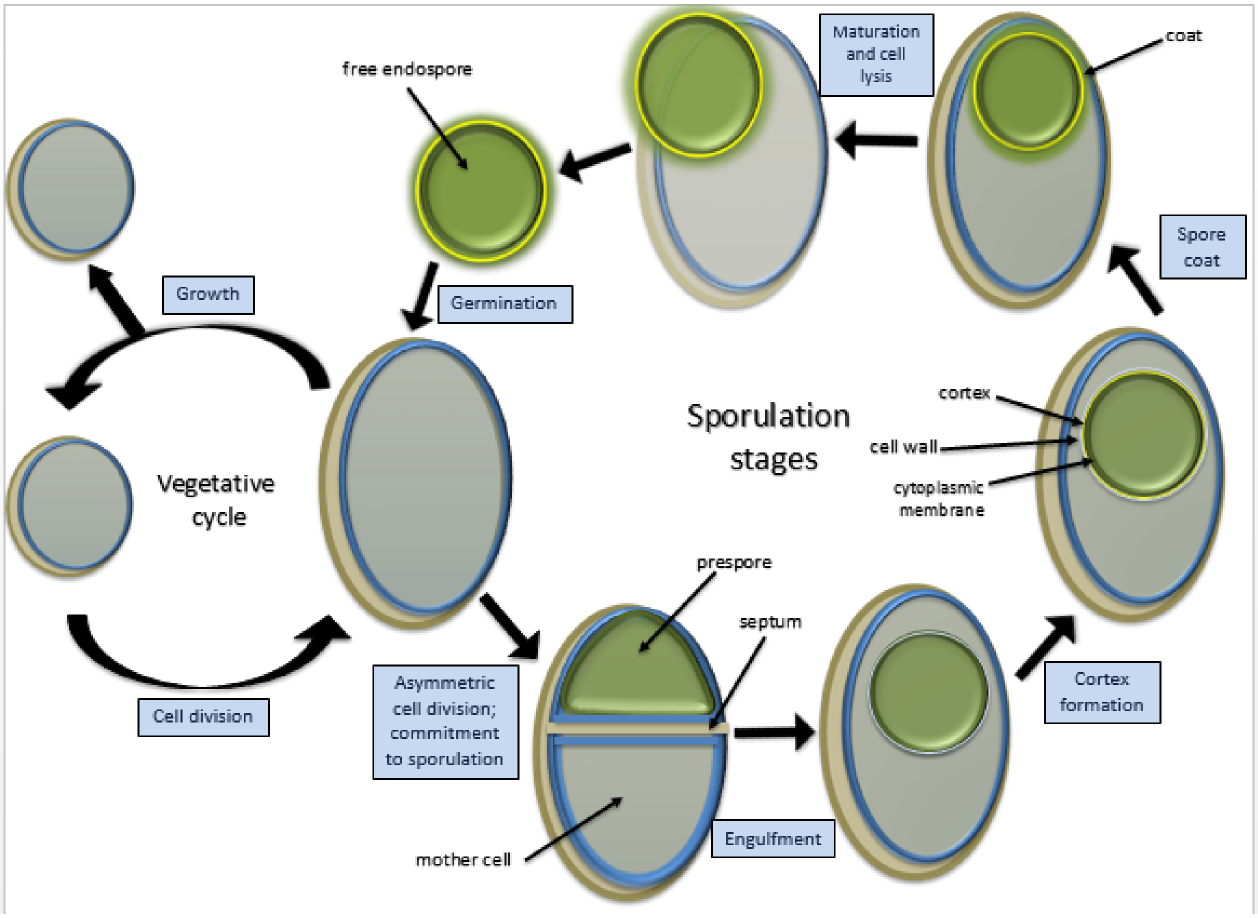
Summary
The larvicide, *Bacillus thuringiensis israelensis* (*Bti),* is the most targeted and least toxic product option for mosquito management. In most situations, *Bti* is effective at killing mosquito larvae. However, its use should be minimized due to direct toxicity to non-target organisms such as frogs and harmless and/or beneficial insects, as well as indirect effects, which can impact ecosystem function, from water quality to bird reproductive success.

Contaminants have been reported in formulated products, including pathogenic bacteria, toxins and endocrine disrupting activity. Although *Bti* resistance is not known to be widespread in mosquito larvae under field conditions, *Bti* has been shown to persist in the environment and it can "recycle" or replicate. *Bti* spores can be transported to untreated sites by adherence to animal bodies or through feces and cause potential non-target impacts at these untreated sites.

Bti has its place in mosquito management. Due to the potential for ecosystem-wide impacts, however, alternatives should be considered before *Bti* application—particularly in high-functioning wetlands and natural areas where *Bti* can disrupt ecosystem communities.

Background
Bacillus thuringiensis (*Bt*) is a gram-positive bacterium that forms toxin-containing protein crystal inclusions. When ingested by susceptible organisms, the crystals attack the gut. More than 67 *Bt* sub-species have been identified that are targeted to specific insect groups. The sub-species *Bacillus thuringiensis israelensis* (*Bti)* was discovered in 1976 and is toxic to aquatic fly larvae, including mosquitoes, black flies, craneflies, non-biting midges (chironomids), fungus gnats, filter flies and others in the sub-order Nematocera. When the *Bti* crystal inclusions are ingested by the larva, it binds to the gut, releases toxins, and forms pores that disrupt the tissues and osmotic balance, killing the insect. See Lacey, 2007 for details.

The *Bti* life cycle has a "sporulation cycle," that includes vegetative cell division and spore development. The vegetative phase is the living, replicating component of the lifecycle. Each vegetative cell divides into two daughter cells. When starved of nutrients, a daughter cell within the mother cell is walled off into an "endospore." When the mother cell dies, the spore is released. These spores are dormant and resistant to drying, heat and other environmentally adverse conditions. The protein crystals afford protection for the spores and also provide nutrients for germination when the spores are activated and convert to vegetative cells (Ibrahim et al., 2010).



Endospore formation and cycle (Attribution)

Production
Bti is produced by fermentation in large vats using a variety of materials/media that provide nutrients for the bacteria, which can influence its toxic properties and final formulation. For instance, some of the nutritional media can remain when the spores are recovered (Lacey, 2007). A few days before harvesting, nutrients are no longer provided, at which point the bacteria die, leaving dead cells, crystal proteins, and spores in the fermentation broth. The broth is processed into formulations of the final *Bti* product (Valent Biosciences). Additives are typically not disclosed by pesticide manufacturers and are considered proprietary information, but can include synergists, surfactants, sticking agents and UV protectants.

The potency is tested from each batch, which is measured in international toxic units or [ITU]/mg. However, there is no screening for metabolite or microbiological contaminants, and pathogenic bacteria have been found in *Bti* preparations (World Health Organization, 2009). Screening for endocrine disrupting properties is also not conducted on *Bti* formulations. However, significant estrogenic properties were found in three of five *Bti* formulations in laboratory assays, although it was not detected in field testing. These tests were conducted to try to determine the source of estrogenic activity in ground water near areas where *Bti* was applied. (Maletz et al., 2015).

Other toxic products, in addition to the protein crystals, can be produced by *Bti*:

> *During vegetative growth, various Bt strains produce an assortment of antibiotics, enzymes, metabolites and toxins, including Bc toxins, that may have detrimental effects on both target organisms and non-target organisms. Beta-exotoxin, a heat-stable nucleotide, is produced by some Bt subspecies during vegetative growth and may contaminate the products. Beta-exotoxin is toxic for almost all forms of life, including humans and the target insect orders* (World Health Organization, 2009).

Efficacy
Larvicides are considered the most effective and important component of mosquito control programs, since treatments can be applied to known breeding sites where mosquitoes are concentrated, and larviciding prevents the emergence of adult mosquitoes. *Bti* is also one of the most targeted and least acutely-toxic product options. Different formulations are designed to make contact with mosquito larvae in different types of habitats and include powders, liquid suspensions, granules, tablets, and briquettes. Multiple variables effect the lethality of *Bti*, including the insect's instar (age), density, organic content of the water, temperature, susceptibility of the target species, etc. (Laurence et al. 2010). Larvae that ingest *Bti* die rapidly—usually within a few hours.

Persistence, Proliferation and Resistance
Problems can arise from the use of all insecticide products, whether synthetic, natural or microbial—broad-spectrum or targeted. As pest managers become more reliant on regular use of pesticidal products, the pesticide can accumulate in the environment and insects can develop resistance.

Resistance: Field resistance to *Bt* sub-species that target beetles or lepidopterans has been reported and the underlying genetic mechanisms have been studied. The changes in genetic expression that allow insects to develop resistance have also been examined in mosquito larvae (Tetreau et al., 2012). Because *Bti* has four major toxins and additional minor toxins, the development of resistance is complex and requires the involvement of multiple genes (Bonin et al., 2015, Ben-Dov, 2014). This is thought to be the reason why resistance is developing slowly in natural mosquito populations. There are, however, cases in the literature of confirmed resistance in the field. A high level of resistance to *Bti* was detected in a population of *Culex pipiens* in New York (Ayesa et al., 2005).

Persistence: Insecticides that break down slowly and persist in the environment chronically expose both target and non-target organisms. Persistent pesticides prolong exposure of the pest, and if the pest remains susceptible, the pesticide will continue to control it. But long-term exposure can drive resistance and contribute to undesirable non-target impacts that can alter ecosystem dynamics. Studies show a range of activity for *Bti* under field conditions. Although it's generally thought that *Bti* is gradually deactivated and does not persist, several studies show that it can and does persist and remain toxic. *Bti* leaves the water column relatively quickly, after which the spores settle out of the water and bind to the soil substrate or particulates. When the soil or particulate substrates were stirred and filtered three weeks later, the suspension retained toxicity (Ohana et al., 1987) A study with simulated field conditions showed *Bti* residual activity for 20 weeks (Marcombe et al., 2011). Decaying leaf litter collected from ponds treated with *Bti* was found to be highly toxic to mosquito larvae months after application (Tetreau et al., 2012).

Bti spores can persist for months in the environment. The number of treatments, the type vegetation, and the presence of organic matter are all associated with persistence of the spores. Change in water level or salinity does not appear to affect spore persistence. "Recycling" or proliferation is when the spores germinate and return to vegetative growth, replicate, sporulate and produce toxins. *Bti* can kill mosquito larvae and then proliferate from their carcasses (Aly et al., 1984). Pupae can also recycle *Bti*. Older forth instar larvae that ingested *Bti* and completed pupation, later died as pupae from *Bti* infection and the carcasses of the pupae were found to recycle *Bti* (Khawaled et al, 1989).

One study showed no evidence that recycling occurs in sediment or other substrates and found that mosquito larvae must be present for recycling to occur (Duchet et al., 2013). However another study found much higher levels of spores in leaf litter than expected from *Bti* application alone and the researchers suggest that proliferation is occurring, as well as spore persistence (Tetreau et al., 2012). *Bti* has even been detected from untreated sites at high levels in decaying leaf litter. A high number of viable spores correlated with toxicity of the leaf litter samples to mosquito larvae. The researchers suggest that the bacteria could be germinating and proliferating in the natural environment (Tilquin et al., 2008). Spores can be transported to untreated sites by animals in two ways. The spores can adhere to the bodies of animals or be excreted after ingestion in the feces. The excreted spores maintain toxic properties and mosquito larvae are killed when exposed to them (Brazner and Anderson, 1986, Snarski, 1990).

The variability in studies shows that analytical techniques, field conditions, formulations and many other factors determine the persistence of *Bti*, and unlike most pesticides, since *Bti* is a microorganism, it does have the ability to replicate. This raises concerns about resistance developing in mosquito larvae, as well as impacts to food webs and habitat quality.

Ecological Impacts

Bti is a biopesticide and it is commonly thought to be safe and non-toxic to vertebrates and non-target invertebrates. This assumption is based on a number of studies in the past that found no secondary or indirect impacts from *Bti* treatments. During that same time period, some studies did record concerning impacts from *Bti*. One study measured significant losses in biomass at sites treated with *Bti*. In a three-year study, insect densities were reduced by 57 to 83% and biomass was reduced by 50 to 83% (Niemi et al., 1998). The researchers emphasized the potential impacts from the magnitude of these losses:

> *The prevailing knowledge of wetland ecosystems is too limited to fully assess the ramifications of these declines in aquatic insect communities for other food web components or for the overall functions of these wetlands. The application of these insecticides can certainly be viewed as changing the function and structure of these wetlands because of large reductions in insects, a major component of wetland food webs. It is difficult to believe that reductions of insect density and biomass in the range of*

50 to 80% would not eventually have major effect on these wetlands. Their ultimate effects remain unclear.

Direct Ecological Impacts
Several recent studies indicate a wide range of impacts from *Bti* treatments at all trophic levels of wetland ecosystems.

Impacts to non-target flies: *Bti* kills mosquitoes and other aquatic fly larvae from the sub-order Nematocera. Therefore, it would be expected that populations of non-target flies will be impacted from *Bti* use. The non-biting midges, or Chironomidae, are a diverse group of flies and can make up more than half of the species in wetland systems and dominate flying insects. A recent study that surveyed male chironomids in Colorado's Fountain Creek Watershed identified 151 different species (Hermann et al., 2016). Although different studies have shown a range of impacts to chironomids from *Bti* application, some have shown no impacts. One found no difference from *Bti* treatment for two common chironomid species in natural wetlands (Duchet et al., 2015). Another study cautioned that toxicity to *Bti* in chironomids varies greatly throughout their development and that many studies likely underestimate risk. Toxicity to larvae of *Chironomus riparius* was 209 times greater for first instar larvae and 90 times greater for second instar larvae than the lowest field application rate used in mosquito control (Kästel et al.,2016). Another study of temporary flooded wetlands found rich biodiversity of chironomid species with high turn-over between years in these unstable habitats. *Bti* treatment did not lower species richness. However, treated sites had a significant difference in species turnover and colonization dynamics were affected (Lundström et al., 2009). One study showed a significant decrease in the density of chironomids from *Bti* treatment (Pauley et al., 2015) and another long-term study in natural wetlands showed a 78% reduction of chironomid and related aquatic flies in treated areas (Jakob and Poulin, 2016).

Impacts to non-fly invertebrates:
Although no acute toxicity to *Bti* was observed, the amphipod, *Gammarus lacustris,* ingested *Bti* and spores were found in its feces. The amount of time that *Bti* remained in the gut was much longer than expected. *Bti* spores were also found in the guts of newborn progeny that were born at least a week past the last *Bti* exposure of the parent (Brazner and Anderson, 1986).

Bti is not just toxic to aquatic flies. A review paper listed an expanded host range of species that are susceptible to *Bti* that includes terrestrial flies, moths, beetles, nematodes and flatworms (Ben-Dov, 2014).

Five species of zooplankton or microcrustaceans that coexist with mosquito larvae in coastal wetlands were exposed to a range of *Bti* concentrations and were examined for acute and chronic effects. Crustaceans were chosen for this study with a range of different feeding behaviors, including predators, herbivores, filter feeders and benthic scrapers. As concentrations of *Bti* increased and over time, there was a pattern of increasing mortality (Olmo et al, 2016). Another study found Cladocera (waterfleas) were significantly affected by *Bti* treatment (Pauley et al., 2015).

Impacts to amphibians:
Bti is said to be nontoxic to vertebrates. However, recent studies show direct toxicity to tadpoles (*Hyla versicolor*). Short-term exposure of tadpoles to *Bti* affected their locomotion. Compared to controls, exposed tadpoles spent more time motionless, spent less time swimming and traveled shorter distances (Junges et al., 2017). When predators (dragonfly larvae) are present, *Bti* treatment significantly decreases tree frog tadpole survival (Pauley et al., 2015). Tadpoles (*Leptodactylus latrans*) showed dose-dependent sensitivity to *Bti* and 100% died after 48 hours of exposure to the highest dose, which is at the top range of recommended field rates. Exposure to lower doses of *Bti* induced intestinal damage (See figure below). Changes to enzymes created oxidative stress, leading to genotoxicity, which could be the cause the intestinal disruption (Lajmanovich et al., 2015).

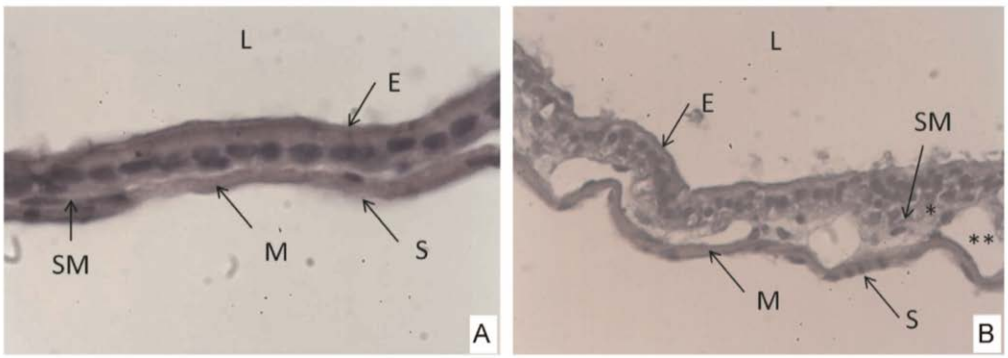


Fig. 5. Light micrographs of intestinal walls of *L. latrans* tadpoles at premetamorphic stages. (A) Control; (B) 48- h exposure to *Bti*-AS (2.5 mg/L). L: lumen; E: layer of cuboidal epithelial cells; M: muscle layer; SM: submucosa; S: serosa. (*) Infiltrate in the connective tissue underlying the epithelium. (**) Dilation of blood vessels. Hemotoxylin-Eosin, 100 ×.

From Lajmanovich et al., 2015.

Indirect Effects
The organisms that are directly affected from *Bti* are members of complex wetland communities and impacts to one component can have cascading effects that indirectly impact other organisms. Studies that assess indirect impacts are challenging to conduct. The majority of studies related to *Bti* effects measure efficacy for killing mosquito larvae. There are several studies looking at direct impacts to non-target organisms, but very few on indirect effects or persistence (Poulin, 2012).

Impacts to micro-organisms:
Very low concentration *Bti* treatment (too low to kill mosquitoes) in freshwater microcosms caused no measurable impacts to microorganisms, nutrients or suspended particles. Two weeks after application of high dose *Bti*, mosquito larval and microorganism density were decreased—the most abundant bacteria species were suppressed. After 44 days post-treatment, cyanobacteria was significantly reduced, showing changes in microbial community composition, reduced nutrients and algae (Duguma et al., 2015).

In a large study of natural wetlands, *Bti* application increased the density of protozoans by 4.5

times and the taxonomic richness increased by 60%. Mosquito larvae feed on protozoans and both mosquitoes and protozoans feed on bacteria (Östman et al., 2008).

A study showed that *Bti* is not directly toxic to phytoplankton. Mosquito larvae feed on phytoplankton, which decrease in a curvilinear fashion as mosquito density increases. Primary producers are indirectly impacted when mosquito larvae and related species are killed and removed from the ecosystem from *Bti* application (Duguma et al., 2017).

Impacts to macroinvertebrate mosquito predators:
A large-scale, five-year study of adult Odonata (dragonflies and damselflies) monitored richness and abundance in natural wetlands. A five-fold reduction in abundance and three-fold reduction in richness was found in *Bti*-treated sites. This was thought to be due to depletion of food availability from an 87% reduction of aquatic flies from treated sites (Jakob and Poulin, 2016).

Impacts to vertebrates:
House martins were assessed for three years between control and *Bti*-treated sites for diet, clutch size, and fledging survival. Insect prey at untreated sites was mainly spiders and dragonflies. Prey items were significantly smaller at treated sites and more flying ants were eaten. Reproductive success was lower at treated sites with decreased clutch size and fledging survival (Poulin et al., 2010).

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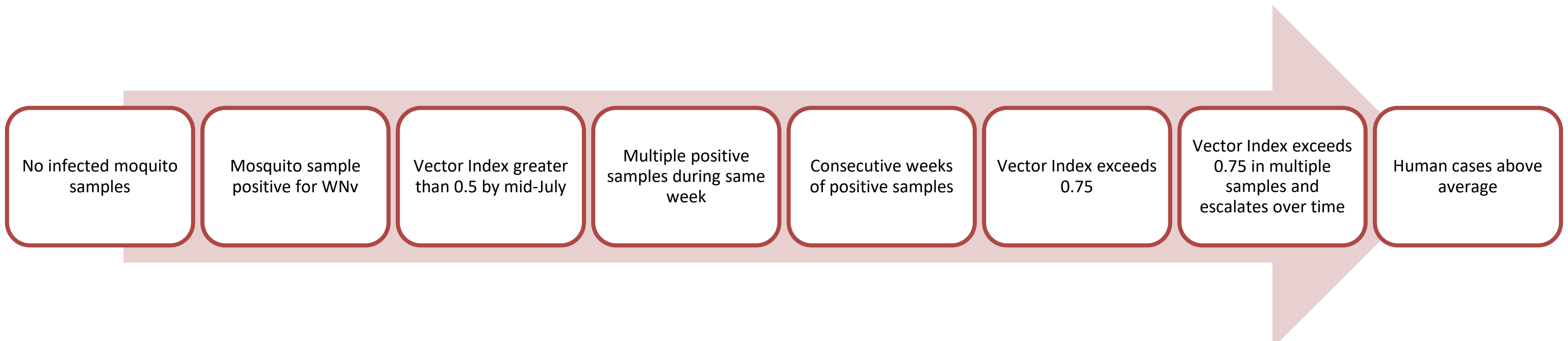
Conditions that Escalate Risk of West Nile Virus to People



Actions to Reduce Risk of West Nile Virus to People

Attachment C – Mosquito Treatment Options

The following table provides a range of mosquito treatment options. Some may be implemented over time as program changes are evaluated.

Mosquito Treatment	Pros	Cons
Bacterial larvicide - *Bti* (*Bacillus thuringiensis israelensis*)	– Effective at killing mosquito larvae – Less impactful than synthetic chemical pesticides, surface oils and methoprene (insect growth regulator) – Proven industry standard	– Body of literature shows direct impacts to non-target organisms, including amphibians – Indirect impacts to non-targets – Ecosystem-wide impacts – Persistent in sediment – Spores can be transferred to untreated sites – Can reproduce – Formulations can contain contaminants – Evidence that resistance can develop in some mosquito species
Predator complexes that occur naturally	All mosquito life stages are prey items for many groups of animals in both aquatic and terrestrial systems. – Can be highly effective – Cost-effective – Part of thriving ecosystem that provides many other benefits, including wetland ecosystem services – Complies with IPM policy and integrated ecosystems strategy – Manages for increases in distribution and abundance of sensitive species	– Variable and complex depending on type of site – Highly site specific – Colonization rates vary for mosquitoes and predator groups – Requires resources for monitoring and data analysis
Encouraging predators by creating habitat or enhancing existing wetland health	– Attracting wide variety of invertebrate and vertebrate species provides free, efficient pest control – Provides other important ecological benefits and services – Improves biodiversity	– Must align with site-specific objectives – Not appropriate for all sites – Takes time to implement - would have to be transitioned over time

Mosquito Treatment	**Pros**	**Cons**
	– Studies provide guidance for improving habitat (e.g. plantings to attract spiders, birds) – Terrestrial predators reduce adult mosquitoes migrating from outside city properties	
Introducing predators as biocontrol agents	– Option for low or mid-quality wetlands – Can rear some biocontrols (e.g. copepods) – May be able to purchase – Potential to transfer from other sites	– Important to source/use native predators – No experience using this method – May need to gather data about a candidate site before initiating – Requires tracking and monitoring – May require special permits
Vegetation management and prescribed burning	– Vegetation management can decrease harborage for adult mosquitoes, breeding habitat for larval mosquitoes and attract predators. – Improves invasive species management and improves overall biodiversity and habitat quality – Advances other city site objectives.	– Takes time to learn vegetation management specifically for mosquito management – May not be appropriate for other site management objectives
Create new healthy wetlands	– Can control large numbers of both larval and adult mosquitoes – Provides other important ecological benefits and services – Sequesters carbon – Provide resilience to reduce impacts from extreme weather events	– Expensive – May need to secure water rights - can be costly, lengthy process – Must align with other objectives to justify cost

Mosquito Treatment	Pros	Cons
Filling in artificial depressions (e.g. wheel ruts, cattle hoof prints)	– Eliminates poor quality sites that readily breed mosquitoes – Reduces *Bti* application	– Labor-intensive/costly – Logistically difficult to implement – At some sites, could adversely impact surrounding area if near sensitive habitat – Depending on cause, may be more cost-effective to prevent (e.g. herd management) – May require permits to fill if in jurisdictional wetlands
Herd Management	– Minimize the overlap of grazing and flood irrigation to prevent hoof disturbance of wet soils. – Cost-effective and preventive – Reduces *Bti* application	– Requires coordination with staff, lessees (water release management, cattle grazing, etc.) – In some situations, may not be feasible – Heavy rain can create same issue as irrigation
Optimize irrigation practices	– City staff has worked with OSMP agricultural lessees to alter water release and scheduling to decrease standing water. – Land and irrigation management practices can be viewed through the lens of mosquito breeding to decrease potential breeding sites – Flood irrigation could be tool to decrease floodwater mosquitoes by allowing hatching and then draining to kill larvae before they can emerge as adults.	– In some cases, runoff can create good wetlands, which needs to be balanced with standing water that becomes a breeding site – Can be logistically difficult to coordinate all players – Water rights are administered by the State of Colorado and there are limitations on what can be achieved regarding modifying irrigation schedules and quantities that may impact mosquito breeding.
Drain artificial breeding sites	– Train staff to check equipment that can fill with water and store to prevent and dump or drain when holding water – Train staff to avoid overwatering and notice when ground, particularly turf, is saturated – Check gutters and maintain to keep clear and flowing – Train staff to check storm drains	Requires resources and training.

Mosquito Treatment	Pros	Cons
	and other areas that could become clogged and hold water	

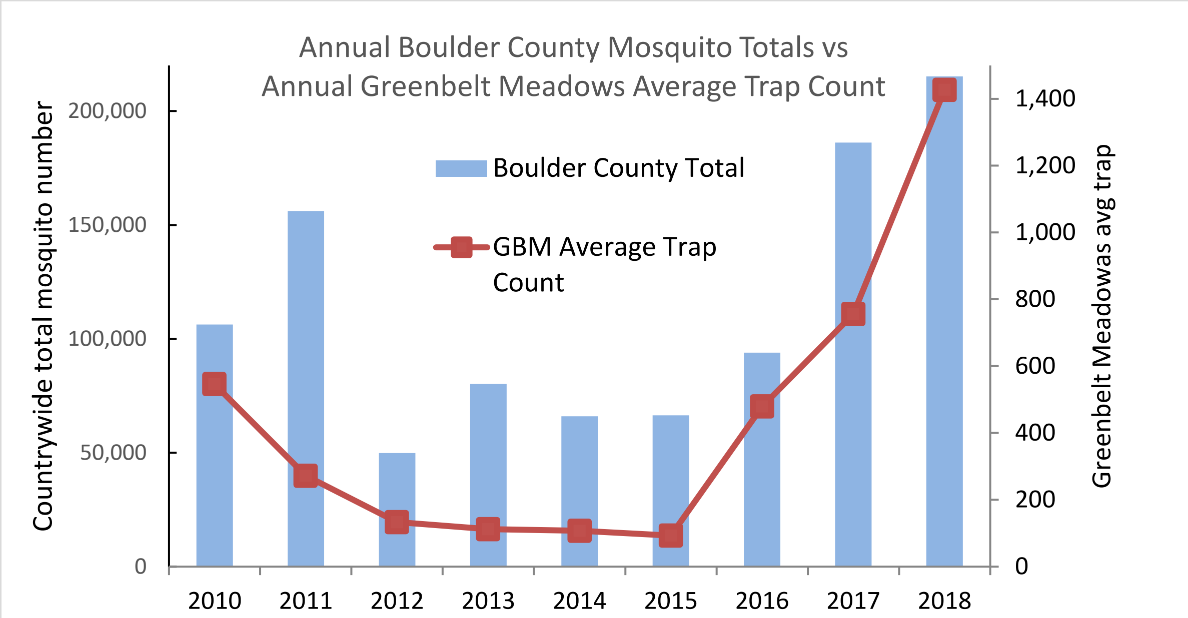
Attachment D: Efforts to Reduce Mosquito Activity in the Greenbelt Meadows Neighborhood

Background:
Mosquito activity varies greatly across different parts of the city—both the number of mosquitoes, as well as the particular species of mosquito. The central and west sides of town experience relatively low mosquito activity with a higher proportion of *Culex* species, which can potentially transmit West Nile virus. *Culex* breed in urban sites like containers and clogged gutters and tend not to travel very far from where they emerge.

The areas in the northern and southeastern edges of town often experience high mosquito activity and most of these mosquitoes are floodwater species. While they don't transmit disease, these mosquitoes are voracious biters, can travel long distances and can synchronously emerge, resulting in large populations that seem to appear overnight. Among these areas of high mosquito activity, Greenbelt Meadows (located in southeast Boulder, north of the intersection of 55th Street and South Boulder Road) is an outlier, particularly over the last two years when mosquito numbers consistently reached exceptionally high numbers week after week. Areas near Greenbelt Meadows are prime breeding habitat for floodwater mosquitoes. The city's mosquito contractor and city staff have focused on a one-mile radius surrounding Greenbelt Meadows, scouring the area for any missed breeding sites, treating all areas where mosquito larvae are found each week and conducting structural improvements to ditches, drainages and utilities channels.

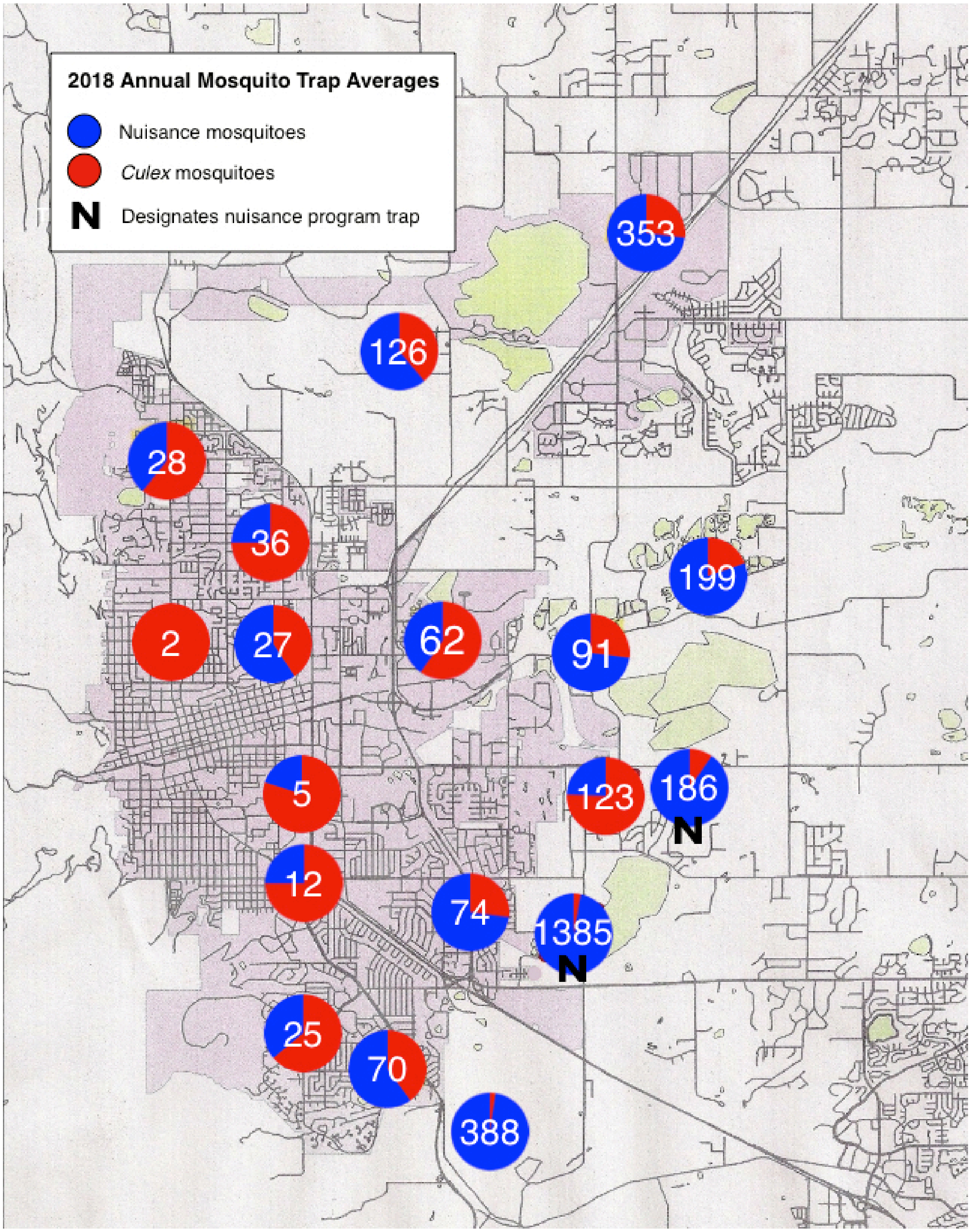
Despite these efforts, mosquito numbers doubled in 2018 from already high numbers in 2017. The residents of this neighborhood have frequently expressed frustration and concern that they are unable to enjoy the outdoors. City staff have spent hours of staff time and resources attempting to address the high mosquito activity in this area.

Mosquito Activity in Greenbelt Meadows Compared to the City and County
Over the past two years, mosquito activity reached record levels across the region. A chart on the last page of the memo shows total mosquito numbers from all of Boulder County (all municipalities and unincorporated areas) compared to the average trap count for the City of Boulder. Mosquito activity in the city tends to mirror regional activity. The chart below shows that the same pattern has been recorded in Greenbelt Meadows. Mosquito trap averages from other parts of the city rose by three times the historic average during the last two years, including Greenbelt Meadows in 2017. During the 2018 season, the rest of the city experienced a pattern similar to 2017. But during 2018, the Greenbelt Meadows trap average rose more than five times their 2010-2016 average.



During the first week of June, 3,000 mosquitoes were captured in the Greenbelt Meadows trap and these numbers remained consistently high throughout most of the summer, peaking at 5000 in mid-July. Although a new trap location could have some influence on the higher number of mosquitoes captured, these numbers are orders of magnitude higher than anywhere else in the city. The average trap count over the entire season was 1,385 compared to much lower numbers in other high-mosquito-activity areas of the city.

The map below shows the average number of mosquitoes caught in traps during 2018 season. Vector or *Culex* mosquitoes are shown in red and nuisance mosquitoes in blue. Greenbelt Meadow's average was 3.6 times higher than the next highest trap.



<u>Actions Taken to Address Mosquito Activity</u>

As stated above, the city's mosquito contractor has rigorously assessed the fields around Greenbelt Meadows several times during each season, checking for any standing water or breeding sites that might have been missed. The high mosquito trap numbers began before irrigation water was turned on in 2018, so irrigation was not the direct source of the large numbers of mosquitoes during the first half of the season. At any point during the season, if standing water were present, it was monitored for mosquito larvae and treated with the larvicide *Bti* if they were found.

In addition, staff performed the following work in 2018 to mitigate standing water issues:

1. Open Space and Mountain Parks (OSMP) coordinated with Utilities and worked with a jail crew to re-establish the drainage channel from Greenbelt Meadows to South Boulder Creek. The crew removed large woody debris deposited from recent storms, weed whipped and dug out the original channel and drainage pipe to the creek. Utilities used a level to make sure the drainage had the proper grade to drain effectively.

2. Utilities replaced several shifted and cracked panels in the concrete stormwater swale that runs on the north side of the neighborhood with new concrete to better accommodate the shallow slope through that area. Additionally, the stormwater piping was cleaned with a jet machine to remove any debris and the concrete swale was cleaned out several times during the summer with firehoses and brooms.

3. Infrastructure under Howard Ditch was inspected and appears to have been abandoned and will be reassessed during the winter. The ground water was too high during the summer to fully inspect the infrastructure. Crews pumped one of the manholes for a full day without making any headway.

4. There was some confusion about an area called the “blind ditch” by residents, who assumed it was stagnant and therefore breeding mosquitoes. This lateral ditch carries water from Howard ditch to a siphon pipe that takes water under South Boulder Creek and irrigates land on the east side of the creek. The water flowing in this lateral moves slowly because of the siphon pipe. It has been inspected with a camera to ensure it is flowing and functioning properly and it is not a source of mosquitoes. An educational sign was made and installed by the Bobolink Trail to provide visitors with information about its function.

5. OSMP staff initiated a drainage study, which will assess the best way to route tailwater generated by irrigation south of South Boulder Road. Improvements recommended by this project will be completed in coordination with the trails workgroup as some of this tailwater can accumulate on the trails in the area.

6. After rainfall events, OSMP staff inspected the earthen storm water drainage slough on the west side of the creek, which carries storm water from Greenbelt Meadows across the Howard Ditch and onward towards the creek. Water seems to be draining much more effectively since it was re-graded by jail crews.

7. OSMP regularly performs inspections of the irrigation and ditch system, cleans culverts and swales, maintains and checks the flow of laterals and works with lessees to alter water release schedules to decrease the amount of standing water in the fields.

8. Parks and Recreation hired a wetlands ecologist/entomologist to survey ponds near East Boulder that were closed to larvicide treatment to protect frog breeding and found no mosquito larvae and a complex of macroinvertebrate mosquito predators.

High floodwater mosquito activity occurs along the South Boulder Creek riparian area and ditch corridors. Adult mosquitoes travel along waterways to seek nectar sources, blood meal hosts, egg-laying sites and mates. Mosquitoes find shady areas in trees and other plants to hide during the heat of the day. The mosquito contractor noted that thousands of mosquitoes were aggregating in large crack willow trees on the east side of the Greenbelt Meadows common area between the HOA's property and the creek. The mosquito trap was set at this location through 2017. The trees spanned the property line of the HOA and city open space. Staff met with HOA officers to discuss options and offered to pay for removal if the HOA chose to have the trees removed. The mosquito contractor emphasized that removing the trees may or may not help reduce mosquito activity in the neighborhood and it could take several seasons to see the ultimate impact of the tree removal. The HOA gave permission to the city to remove the trees at a cost of approximately $8,000 and replanting cost another $6,000. The trap, which was previously placed in the crack willows that were removed, was re-located in 2018 a short distance south on the eastern edge of the neighborhood. Although this could be a factor in the higher trap counts in 2018, reports from neighbors indicate that mosquito activity was reflected well by mosquito trap results.

<u>Next Steps for 2019 Season:</u>

Staff will continue to make drainage improvements to prevent mosquito larval sites in the areas around Greenbelt Meadows. However, since these sites are regularly inspected and treated, it is likely that mosquitoes are traveling to Greenbelt Meadows from outside the immediate area (one-mile radius around the neighborhood). The challenge is first pinpointing the source of these mosquitoes that could be coming from several miles away and then taking steps to reduce breeding from those areas.

To try to determine the source of the mosquitoes, staff proposes setting two to three additional traps in areas outside the one-mile radius of Greenbelt Meadows to better understand the dispersal patterns of mosquitoes. Staff will develop a plan over the winter to implement this in the 2019 season. These will be "floating" traps that can be placed in different locations to track mosquito activity. If any city properties in outlying areas are discovered to be potential sources of mosquitoes, these will be managed to reduce mosquito breeding. If private property or county-owned property is suspected to be the source, the city will work with the owners to develop solutions for reducing mosquito activity.

For those mosquitoes that travel to the general area around Greenbelt Meadows, an important line of defense is to increase overall biodiversity to strengthen predator populations that can prey on mosquitoes that enter the area. Staff will assess the potential to attract more wildlife to the area and continue to research methods for achieving higher mosquito predator populations.

Staff is also exploring and designing an efficacy study of prescribed burning on city lands to determine if these treatments have beneficial effects on reducing dormant floodwater mosquito eggs on the ground as well as reducing adult mosquito harborage.