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Mosquitoes of Wisconsin: The Biology, Life History, Identification and Control

Timothy Wucherer

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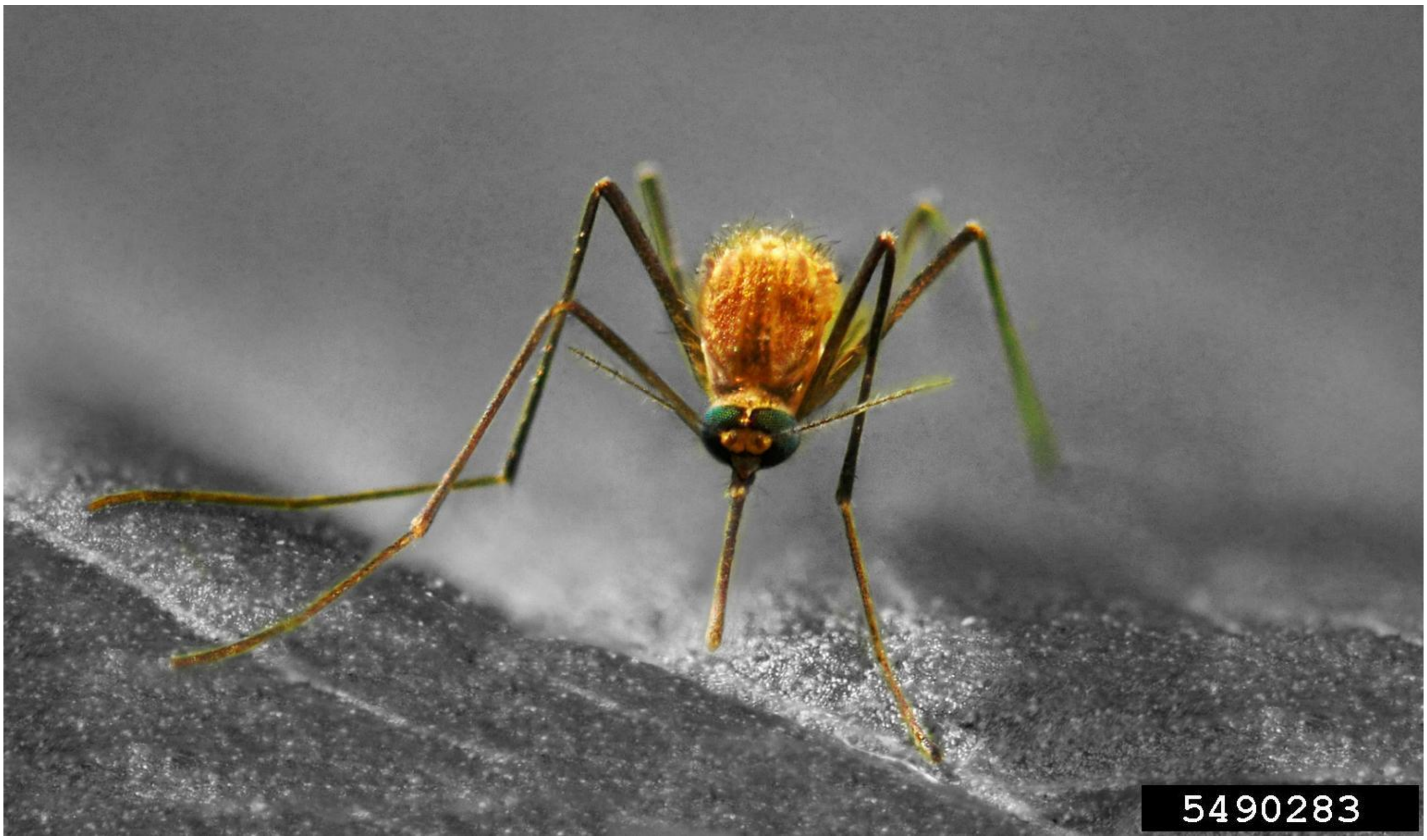

Photo: Credit: Ary Farajollahi, Bugwood.org

Timothy Wucherer

University of Nebraska-Lincoln

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Introduction

In the past 60 years, weather in Wisconsin has generally been getting warmer, and in many areas of the state, wetter (Moran et al., 2015). Long-term analysis of Wisconsin's climate has indicated an increase in average annual precipitation, longer growing seasons, and an increase in average temperatures (Moran et al., 2015). Increases in temperatures and rain have been directly correlated with increases in vector-borne diseases such as pathogens carried by mosquitoes (Moran et al., 2015). With this, Wisconsin communities should prepare for possible increases in mosquito activity and the health and veterinary concerns that come with direct exposure to certain species (Moran et al., 2015). The purpose of this project is to review the biology and life history of mosquitoes, identify key species in Wisconsin, and review management practices used to control mosquito populations to acceptable levels.

Biology of Mosquitoes

There are approximately 3530 species of mosquitoes worldwide, with around 56 species in Wisconsin (Service 2012; UW-Madison, 2021). Mosquitoes belong to the order Diptera (True Flies) and the family Culicidae. Within the family Culicidae, they are divided into three subfamilies: Toxorhynchitinae, Anophelinae (Anopheline), and Culicinae (Culicines), with Anophelinae and Culicinae species being important in Wisconsin (Service, 2012; UW-Madison, 2021). Important culicines in Wisconsin consist of mosquitoes in the genus *Aedes*, *Culex,* and *Coquillettidia* (UW-Madison, 2021). Anopheles is the only genus of anopheline mosquitoes (Service, 2012).

Morphology

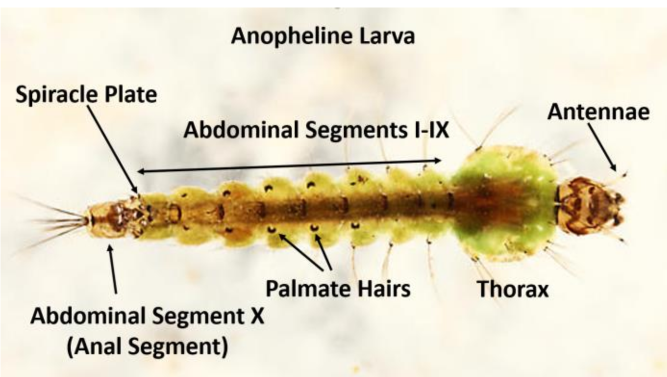
Eggs of most mosquito species are elongate, ovoid, or spindle-shaped, with a few being spherical or rhomboid (Mullen & Durden, 2018). The outermost layer of the egg consists of the eggshell or the chorion. When first laid, the eggs are white, with most turning dark within hours (Mullen & Durden, 2018). In the genera *Anopheles*, the eggs are boat-shaped, and the chorion has transparent air-filled compartments at the side of the egg called floats (Mullen & Durden, 2018). In *Culex* genera, the eggs have a cup-shaped corolla at one end, which allows the eggs to sit vertically on the water in a raft, with the dorsal side of the egg raft having apical droplets of a chemical, allowing them to stay upright (Mullen & Durden, 2018).

The mosquito larvae pass through four instars that closely resemble each other in appearance apart from size (Mullen & Durden, 2018). The head of the larval stage has a distinctive capsule, with a pair of "eyes" composed of lateral ocelli (simple eyes) clusters (Mullen & Durden, 2018). The head capsule also contains a pair of antennae that vary in shape and length (Mullen & Durden, 2018).

Chewing mouthparts include a variety of brushes, combs, and sweepers that are used for feeding (Mullen & Durden, 2018). The palatal brushes arranged laterally on the lower lip (labrum) can create water currents that will draw in floating or suspended particles towards the mouth (Mullen & Durden, 2018). The sweepers and brushes on the mandibles are believed to collect and pack particles to create a bolus of food in the pharynx (Mullen & Durden, 2018). In predatory larvae such as in the genera *Toxorhynchites*, the mandibles or maxillae are heavy and sharply toothed for capturing prey. The thorax of the larval stage is broad with three

indistinguishable, legless segments (Mullen & Durden, 2018).

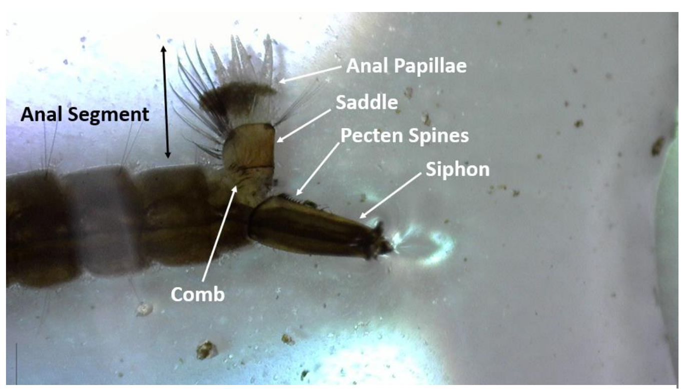
The larvae's abdomen is narrower than the thorax, cylindrical in shape, and comprised of eight distinct segments, with the second to the last segment being a combination of segments 8 and 9 (Mullen & Durden, 2018). In *Culex* and *Aedes* (Culicines) species, segment 8 contains an elongated air tube extending dorsally from the abdomen (Becker et al., 2020, Service, 2012). The siphon of *Coquillettidia* is short and has a heavily sclerotized point with a saw-like edge on the dorsal side used for piercing and attaching to plant tissues (Mullen & Durden, 2018). In *Anopheles* genera (Anopheline), the siphon is lacking, and the spiracles are situated on a short spiracle plate (Mullen & Durden, 2018). Anopheline Larave also have palmate hairs on the abdomen with this being lacking on culicine larvae (Service, 2012)



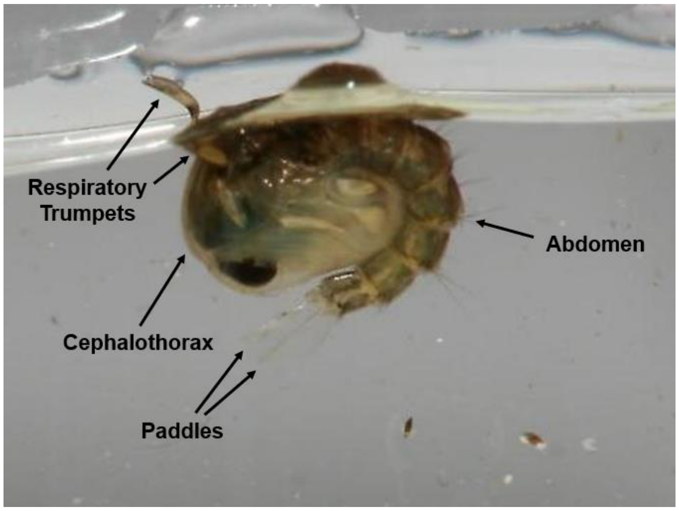
Anopheles punctipennis Larva
Photo Credit: Tom Murray

Culex pipiens Larva
Photo Credit: Omar Fahmy

The anal segment or segment 10 extends ventrally at an angle from the rest of the abdomen (Mullen & Durden, 2018). This segment usually possesses four anal papillae used primarily for osmoregulation (Mullen & Durden, 2018). The larval stage bears several structures at the terminal end of the abdomen that can be useful for identification (Mullen & Durden, 2018). These structures can include comb scales on segment 8, pecten spines on the siphon, and an exoskeleton plate (Saddle) that encircles the anal segment (Mullen & Durden,, 2018).



Culicine Larva Anal Segment
Photo Credit: Timothy Wucherer

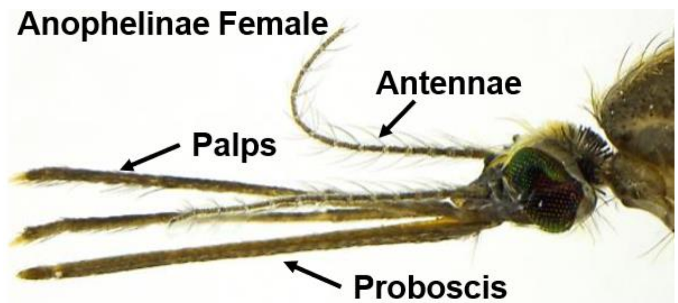


Mosquito Pupa
Photo Credit: Omar Fahmy

The pupa of mosquitoes is comma-shaped, with the head and thorax fused to form the cephalothorax with the abdomen curled below it (Mullen & Durden, 2018). Projecting from the dorsal side of the cephalothorax is a pair of respiratory tubes or air trumpets (Mullen & Durden, 2018). The respiratory trumpets obtain oxygen at the water surface for the pupa or in some species used to acquire oxygen from submerged vegetation (Mullen & Durden, 2018). The developing appendages of the coiled adult head and thorax can be seen through the cephalothorax (Mullen & Durden, 2018). The cephalothorax envelopes an air pocket ventrally that allows buoyancy and for the pupa to remain at the water surface when resting (Mullen & Durden, 2018). Attached to the eighth segment of the abdomen are two broad paddles which allow the pupa to propel through the water when disturbed (Mullen & Durden, 2018).

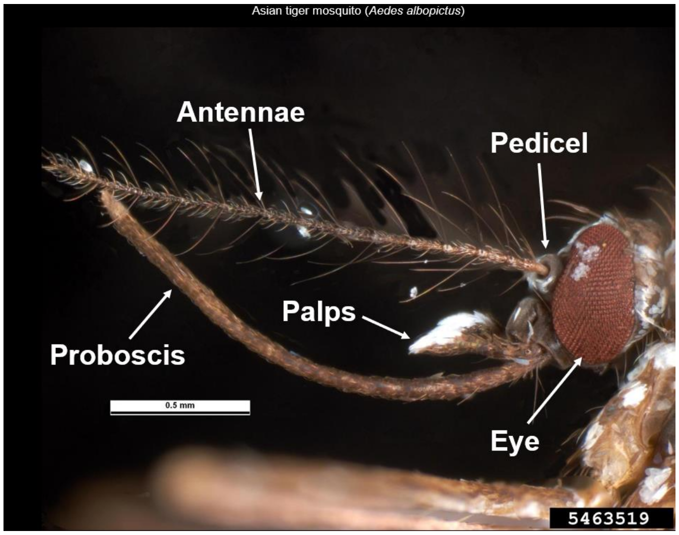
Adult mosquitoes can be distinguished from other flies that look similar by the possession of a prominent, forward-projecting proboscis and numerous appressed scales present on the legs, abdomen, and wing veins (Service, 2012). Generally, mosquitoes are relatively small and slender insects that usually measure 3-6 mm in length, with the body divided into a head, thorax, and abdomen (Service, 2012).

The head contains a pair of kidney-shaped compound eyes (Service, 2012). Between the eyes are a pair of filamentous and segmented antennae (Service, 2012). The pilose antennae in female mosquitoes have a whorl of short hairs (Service, 2012). Unlike the female mosquito, the male antennae have a feathery or plumose appearance. This feature on mosquitoes can help determine the sex of an individual (Service, 2012). It is important to note with males, except for a few genera, are of no medical importance (Service, 2012). At the antennae base is a large globular structure called the pedicel, which contains the Johnston's organ (Foster & Walker, 2018). The Johnston organ is a mass of radially assembled mechanoreceptors that will respond to the vibrations of the flagellum generated by sound (Mullen & Durden, 2018).

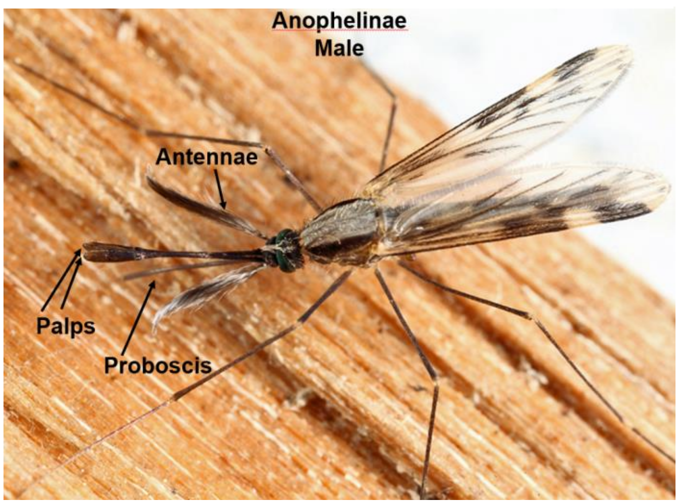


Anopheles Female
Photo Credit: Salvador Vitanza, Texas A&M Univeristy Extension

The palps are just below the antennae (Service, 2012). Both the male and female anopheline mosquitoes have palps that are about as long as the proboscis, with females being pointed apically and males being broadened (Mullen & Durden, 2018). In culicine species, the females have short



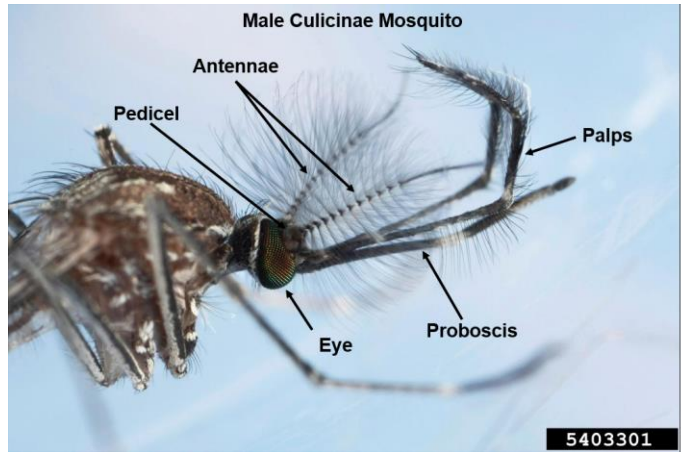
Aedes albopictus Female
Photo Credit: Pest and Diseases Image Library , Bugwood.org



Anopheles punctipennis Male
Photo Credit: Tom Murray

palps, with the males having long curved or brush-like palps (Mullen & Durden, 2018).

Projecting anteriorly between the palps is the proboscis (Mullen & Durden, 2018). The proboscis is at least two-thirds the abdomen's length and consists of the following mouthparts: labrum,



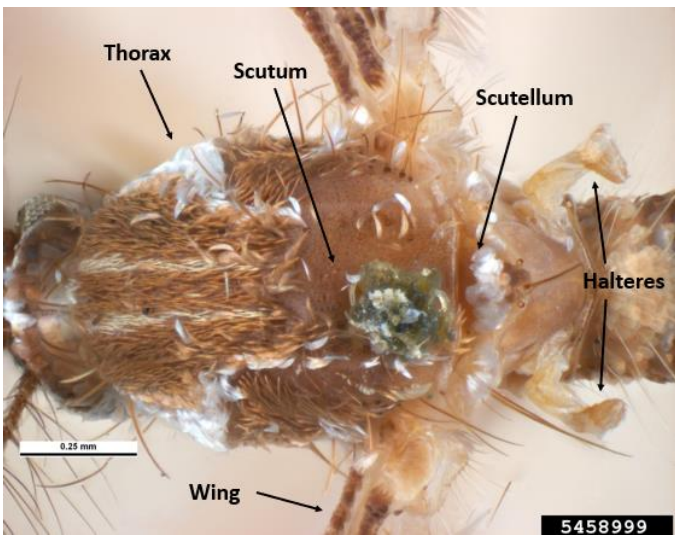
Culex tarsalis Male
Photo Credit: Joseph Berger, Bugwood.org

paired mandibles, hypopharynx, paired maxillae, and the labium (Mullen & Durden, 2018). The first four structures mentioned above have evolved into fine stylets, which form a tightly fitting fascicle

(Mullen & Durden, 2018). The stylets are cradled within a groove of the large and prominent labium, which comprises most of the mass (Mullen & Durden, 2018). The tip of the labium (lower lip) contains a pair of small labella that are taste sensitive (Mullen & Durden, 2018). These structures allow for water and sugar solutions to make contact with the inner surface of the labella (Mullen & Durden 2018). The hypopharynx and mandibles are pointed at the tip while the maxillae's tip ends in serrated blades (Mullen & Durden, 2018). The mandibles and maxillae both puncture the skin and advance the fascicle within the host's tissue (Mullen & Durden, 2018). The hypopharynx acts as the salivary channel that delivers saliva into the tissue during probing (Mullen & Durden, 2018). The labrum curls laterally during feeding to form a food canal to extract the host's blood or sugar solution up the proboscis (Mullen & Durden, 2018). In males and females of non-blood feeding species, the mandibles and maxillae are reduced (Mullen & Durden, 2018). In species of *Toxorhychites,* both

males and females have a non-piercing proboscis that curves downward (Mullen & Durden, 2018).

The thorax contains the locomotor units such as the six legs, a pair of forewings with the hindwings modified into knob-like structures called halteres (Mullen & Durden, 2018). The halteres are used for flight control and are located behind the insertions of the wings (Triplehorn & Johnson, 2005). The forewings are long and narrow, with scales along the veins and wing margins (Weissling, 2020). At the distal part of the wing, there is a unforked vein between two forked veins (Weissling, 2020).



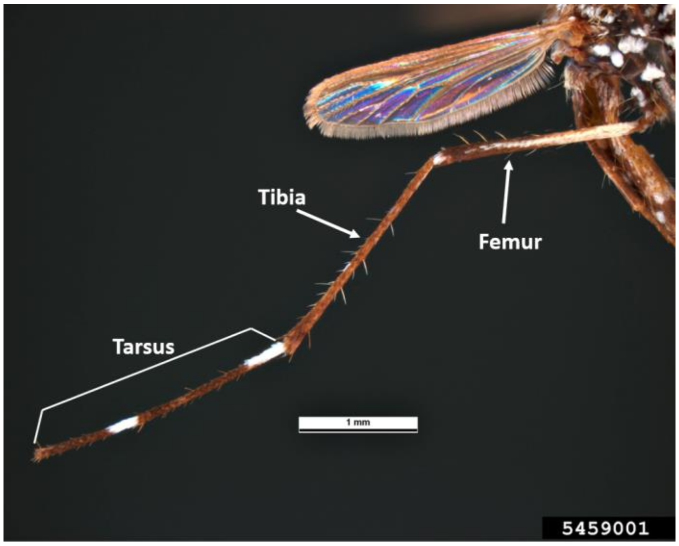
Yellowfever Mosquito (*Aedes aegypti*)
Photo Credit: Pest and Diseases Image Library , Bugwood.org

The legs are slender and are attached close together on the underside of the thorax by the long and downward projecting coxae (Mullen & Durden, 2018). At the distal portion of the legs, the tarsi are tipped with a pair of claws (Mullen & Durden, 2018). Between the two claws, there is a pad-like empodium (Mullen & Durden, 2018). The legs are covered in scales that are usually black, brown, or white and possibly arranged in certain patterns, commonly creating rings (Mullen & Durden, 2018).

Mosquito Wing
Photo Credit: Darren Blackford, USDA Forest Service, Bugwood.org

The abdomen is segmented and contains membranous areas between the dorsal (tergite) and ventral (Sternite) plates which allow for the expansion of the abdominal wall to accommodate large blood and sugar meals along with the development of egg clutches (Mullen & Durden, 2018). Abdominal segments V-VIII progressively get smaller and tapers towards the posterior end of the abdomen (Foster & Walker, 2018). Segment 9 of the abdomen contains a pair of appendages called cercus (pl., cerci) (Mullen & Durden, 2018).

The female has a post genital lobe at the terminal end of the abdomen, while the male's terminal end has claspers (Mullen & Durden, 2018). During emergence, the male genitalia is inverted (Mullen & Durden, 2018). During the first hours of the adult stage, segments 8 and 9 rotate together at 180 degrees to the mature position (Mullen & Durden, 2018). The male genitalia can provide some valuable characteristics for species identification (Mullen & Durden, 2018).



Yellowfever Mosquito *Aedes aegypti*
Photo credit: Pest and Diseases Image Library , Bugwood.org

Life History and Behavior

Mosquitoes have a holometabolous life cycle, which means they go through four different life stages: egg, larvae, pupae, and adults (Mullen & Durden, 2018). This life cycle is completed in two environments: immature stages in aquatic habitats while the adult is terrestrial (Mullen & Durden, 2018). The larvae and pupae can develop in various aquatic environments, which can depend on species (Mullen & Durden, 2018). In Wisconsin, this can include temporary surface water such as rain pools and floodwater (Mullen & Durden, 2018). Permanent surface water such as pools, streams, swamps, and lakes can be essential breeding grounds for several species (Mullen & Durden, 2018). Natural or artificial water-holding containers can also act as breeding grounds; this includes tree holes, rain barrels, leaf axils, and discarded tires (Mullen & Durden, 2018).

Both *Anopheles* and *Culex* eggs are laid directly into the water, with *Anopheles* eggs being laid singly and *Culex* eggs being oviposited in a clump that forms a floating raft (Service, 2012). Both *Anopheles* and *Culex* eggs cannot survive desiccation (Service, 2012). Additionally, *Coquilletidia* species also lay their eggs clumps forming a raft similar to *Culex* (Mullen & Durden, 2018). The eggs of *Aedes*, and *Psorophora* are also laid singly; however, the eggs are not laid directly on the water and can survive desiccation (Mullen & Durden, 2018). Instead, they are usually deposited right above the water line on damp surfaces such as mud, leaf litter, tree holes, and artificial containers (Mullen & Durden, 2018).

Culex pipiens
Photo Credit: Susan Ellis, Bugwood.org

Dependent on the species of mosquitoes and aquatic environment's specific conditions, the larvae can spend most of their time either at the water surface or at the bottom of the water column and occasionally coming up to the water surface for air (Mullen & Durden, 2018). Mosquito larvae are not buoyant, but they are suspended at the surface by special hairs and spiracular structures that adhere to the surface tension while acquiring oxygen straight from the air (Mullen & Durden, 2018). Species like *Coquilletidia perturbans* remain submerged during both their larval and pupal development with their siphons and air trumpets inserted in aquatic plant tissues where they obtain oxygen (Mullen & Durden, 2018). Culicine larvae usually migrate up and down the water column depending on food availability (Mullen & Durden,

2018). When Culicine larvae feed below the water surface, they will occasionally swim actively back to the water surface to acquire oxygen (Mullen & Durden, 2018). Larvae of the Anophelinae subfamily are found swimming horizontally at the surface film of water (Mullen & Durden, 2018). When at the water surface, *Aedes* and *Culex* are found resting at an angle (Service, 2012). *Anopheles* larvae can found resting parallel to the water surface (Service, 2012).

Culicinae Mosquito Larvae
Photo Credit: Jim Occi, BugPics, Bugwood.org

Mosquito larvae feed on a variety of organic detritus, small organisms, and suspended material (Mullen & Durden, 2018). These small organisms include bacteria, protists, fungi, and algae (Mullen & Durden, 2018). Some species of *Psorophora* and species of *Toxorhychites* are predators that feed on small macroinvertebrates or other small insects such as other mosquito larvae (Mullen & Durden, 2018). There are five different ways that larvae can collect food: filtering, gathering, shredding, scraping, and preying on macroinvertebrates (Mullen & Durden, 2018). Filtering species will generate water currents with their lateral palatal brushes located on the labrum, which will draw in suspended particles through their fine combs that are collected and directed towards the mouth (Mullen & Durden, 2018). Gatherers use their mouthparts in a similar manner to the filterer, but this occurs after stirring up the particles from solid surfaces (Mullen & Durden, 2018). Shredders will gnaw, chew, and bite off pieces of organic matter, whereas scrapers will obtain food by scraping it off solid surfaces (Mullen & Durden, 2018). Predator species will grasp prey with their large and sharp mandibles or maxillae in the case of some *Psorophora*, or use long, curved palatal brushes in *Toxorhynchites* (Mullen & Durden, 2018).

The majority of mosquito species use more than one of the techniques used for collecting food as mentioned above (Mullen & Durden, 2018). The genera *Anopheles* are primarily filter-feeding at the water surface by rotating their heads 180 degrees, allowing the oral opening to be dorsal (Mullen & Durden, 2018). The majority of *Aedes* and *Culex* species filter feed close to the surface; however, they can also gather, scrape, or shred organic matter at the bottom, depending on food availability (Mullen & Durden, 2018). For larvae anchored to submerged vegetation such as *Coquillettidia* and *Mansonia*, they use filter-feeding, gathering, and scraping methods within their immediate surroundings (Mullen & Durden, 2018).

Mosquito pupae usually remain motionless at the water surface, with their air trumpets' tips contacting the water surface (Mullen & Durden, 2018). Like the larvae, the pupae will dive when disturbed by using their caudal paddles. The pupae will extend their abdomen, then snap it back towards the cephalothorax (Mullen & Durden, 2018). The pupal stage is buoyant in water due to the ventral air space beneath the cephalothorax and can rise to the surface without swimming (Mullen & Durden, 2018). They can remain at the bottom of their aquatic environment by repeatedly swimming downwards or wedging themselves under debris (Mullen & Durden, 2018). After substantial submergence time, the pupa loses its

buoyancy as its air supply diminishes (Mullen & Durden, 2018). This air loss will require them to swim back to the surface (Mullen & Durden, 2018).

During emergence, the males usually appear earlier than the females due to their shorter larval growth periods (Mullen & Durden, 2018). With adult emergence approaching, the pupa will remain in a stationary position at the water surface, with the abdomen gradually straightening over 10-15 minutes (Mullen & Durden, 2018). The enclosed adult then ingests air which causes the cuticle of the cephalothorax to split, allowing the adult to rise out of the pupal case and stand on the water (Mullen & Durden, 2018). Adult emergence only takes a few minutes to complete, with the newlyemerged adults being capable of short flights (Mullen & Durden, 2018). The recently emerged adult will not be capable of long-term flight until after the cuticle becomes fully sclerotized or hardened (Mullen & Durden, 2018). The larval reserves of lipids and glycogen that were carried over from the larval stage will provide sufficient energy for a few days of flight and survival (Mullen & Durden, 2018). From emergence, the adult will usually seek shelter in cavities, vegetation, and other resting sites where they will remain except during periods of activity (Mullen & Durden, 2018).

Emerging Mosquito Adult
Photo Credit: John C. French Sr., Retired, Universities:Auburn, GA, Clemson and U of MO,

For dispersal, foraging, and host finding, most species will only fly 2 km in their lifetime (Mullen & Durden, 2018). Generally, these ranges are typical of domestic species that result from random factors in their repeated foraging flights for mates, hosts, sugar sources, oviposition, and resting sites (Mullen & Durden, 2018). Other species may go through a dispersal mode directed by light or assisted by the wind, which could carry them a few kilometers to even up to hundreds of kilometers from their larval habitats (Mullen & Durden, 2018). With floodwater species such as *Aedes vexans*, these flights are more evident with mass emergences in the bottomlands after a heavy rain event (Mullen & Durden, 2018).

When resting, adult mosquitoes usually position themselves with their heads up on the vertical surfaces, with their forelegs and hindlegs contacting the substrate with the hindlegs raised (Mullen & Durden, 2018). Mosquitoes in the subfamily Culicinae (*Culex, Aedes,* and *Coquillettidia* spp.) will rest or feed with their bodies more or less parallel to the surface, whereas species in the subfamily Anophelinae (*Anopheles* spp.) rest or feed at an angle to the surface (Service, 2012).

Resting *Culex pipiens*
Photo Credit: Ary Farajollahi, Bugwood.org

Anopheles punctipennis Feeding
Photo Credit: © Christopher Wirth

Usually, during the first 3-5 days of their adult life, both sexes acquire sugar from plant nectar and honeydew to obtain enough energy for sexual maturation, flight, mating, dispersal, and for females to find a host (Mullen & Durden, 2018). Typically, natural sugars are obtained repeatedly by both sexes of most species (Mullen & Durden, 2018). Females that require a blood meal for egg development feed on the following classes of vertebrates: mammals, birds, reptiles, and amphibians (Mullen & Durden, 2018). Male mosquitoes can inseminate several females before they run out of mature sperm and the accessory gland becomes depleted (Mullen & Durden, 2018). The semen supply can be refilled after a few days (Mullen & Durden, 2018).

Mating generally occurs several days after the emergence of the adult (Mullen & Durden, 2018). Males usually generate flight swarms at specific times around prominent or contrasting features in the environment, known as swarm markers (Mullen & Durden, 2018). Male mosquitoes will follow a looping flight pattern over the swarming markers (Mullen & Durden, 2018). Females may find the swarm markers using visual or host-chemical markers that males also use to locate a specific place; however, there is some evidence that swarming males potentially release a volatile pheromone to attract a female from a distance (Mullen & Durden, 2018). As a female enters the swarm, males can detect her wing beat's characteristic frequency with their Johnston's organ and plumose antennae (Mullen & Durden, 2018). A female mosquito's flight tone varies about 150-600 Hz, depending on the temperature, size, and species (Mullen & Durden, 2018). The male's flight tone is approximately 100-250 Hz higher than the females (Mullen & Durden, 2018). Both sexes can detect each other's distinct flight tone and will adjust these frequencies once they are at close range (Mullen & Durden, 2018). This ability to detect different flight tones by both sexes is critical for successful copulation (Mullen & Durden, 2018). Most of the time, swarms are species-specific, but occasionally mixed swarms can occur, with hybrid mating being rare (Mullen & Durden, 2018).

Many species of mosquitoes do feed on humans to acquire a blood meal, with a few species preferring feeding on humans compared to other animals (Mullen & Durden, 2018). Still there are many species that prefer other animals over humans and many species of mosquitoes never bite humans (Service, 2012). Species of mosquitoes that commonly feed on humans are considered anthropophagic (Mullen & Durden, 2018). Species that feed mainly on other animals are considered zoophagic in feeding habits (Mullen & Durden, 2018). Mosquito species that feed on birds are considered ornithophagic (Service, 2012).

Along with having host inclination, mosquitoes can also have a preference on where they feed (Service, 2012). The few species of mosquitoes that regularly enter homes to feed on their host are considered endophagic (Service, 2012). On the other hand, species that feed on their host outside are considered exophagic (Service, 2012). It is

important to note that many species do not fall entirely into one category but may show varying degrees of behavioral patterns (Service, 2012).

For most species, the females are anautogenous, which means the egg follicles remain in a resting stage until a bloodmeal is obtained (Service, 2012). After each blood meal, the female will develop a mature clutch of eggs (Service, 2012). Some anautogenous species will take a blood meal at the beginning of egg development and take a second and a third blood meal during egg development (Mullen & Durden, 2018). The additional bloodmeals can provide extra energy replacing sugar as an energy substitute. In some species, the female is autogenous, meaning that they can develop mature eggs without a blood meal; this can be further separated out into facultative and obligate types (Mullen & Durden, 2018). Facultatively autogenous female species will only develop the first clutch of eggs without a blood meal if she has sufficient energy reserves and a bloodmeal is not available (Mullen & Durden, 2018). After that, blood meals are required for the development of a mature cluster of eggs (Mullen & Durden, 2018).

Female mosquitoes use volatile chemicals to help locate their vertebrate host (Mullen & Durden, 2018). Chemical cues such as Carbon dioxide, lactic acid, and octenol have been the most widely documented host attractants (Mullen & Durden, 2018). Other odors from a live host's skin have been known to be more important and attractive to female mosquitoes than a combination of the three chemicals mentioned above in a warm and humid airstream (Mullen & Durden, 2018). Vision plays an essential role in host finding, specifically in diurnal or daytime species, especially in open environments at intermediate or close distances (Mullen & Durden, 2018). As the female gets within one or two meters of a possible host, not only are chemical and visual cues important, but the convective heat and humidity surrounding the body also is essential (Mullen & Durden, 2018). Odors, Carbon dioxide, and humidity are detected by sensory receptors called sensilla, located on the antennae and palps (Mullen & Durden, 2018).

If the female mosquito finds the host stimuli acceptable, she will attempt to land on the host animal, often preferring specific body parts such as the head, arms, or legs (Mullen & Durden, 2018). Once she has landed, the female mosquito will go through four phases of feeding behavior: exploration, penetration, vessel-seeking, imbibing, and withdrawal (Mullen & Durden, 2018). For the first few seconds, the female mosquito remains motionless; then, she will start exploratory movements, including probing motions if the skin surface with her proboscis (Mullen & Durden, 2018). She may leave without feeding if the host is not suitable (Mullen & Durden, 2018). Even with the right host, she may explore to find an appropriate area that is well vascularized for a moment (Mullen & Durden, 2018). Heat and moisture stimulate probing activity along with other chemical cues (Mullen & Durden, 2018). In addition to the palps and antennae, receptors on the proboscis, tarsi, and other parts of the legs are important in detecting stimuli (Mullen & Durden, 2018).

The adult female mosquito can feed on a variety of skin surfaces, which includes the moist skin of frogs and the scaly legs of birds and reptiles (Mullen & Durden, 2018). The proboscis can penetrate matted hair, a light leather of feathers, heavy cloth such as denim, and mucus provided that the given layer or layers are not thicker than the proboscis (Mullen & Durden,2018).

Once the female mosquito selects a site for feeding, the fascicle of stylets will pierce the skin as the labium will serve as a guide and bends backward

without penetrating the skin (Mullen & Durden, 2018). Once the stylets puncture the skin, the maxillae and mandibles on each side of the proboscis alternately slide by each other while creating quick stabbing or puncturing movements (Mullen & Durden, 2018). While this occurs, the backward-directed maxillary teeth grip the tissue as the stylets continue penetrating the epidermal and subepidermal tissue (Mullen & Durden, 2018). The flexible end of the fascicle will bend at sharps angles while the female probes in various directions of the subepidermal tissue in search of a small arteriole or venule (Mullen & Durden, 2018).

During this time, saliva streams from the tip of the hypopharynx. This saliva contains the enzyme apyrase and anticoagulants (Mullen & Durden, 2018). The enzyme apyrase prevents platelets from aggregating while the anticoagulants prevent blood from clotting, allowing the blood to flow freely from the vessel (Mullen & Durden, 2018). After finding a vessel, the female glides the tip of her fascicle into the vessel's interior (Mullen & Durden, 2018). She begins drawing blood up through the food canal by pumps located in the pre-oral cavity known as the cibarium, and in the pharynx (Mullen & Durden, 2018; Klowden, 2013). The blood then accumulates in the midgut, which allows the female mosquito to be fully engorged in approximately 1-4 minutes (Mullen & Durden, 2018).

Aedes vexan Obtaining Blood meal
Photo Credit: Tara Armijo-Prewitt

During blood uptake, the female will begin extracting water from the bloodmeal and possibly excrete small droplets on the host skin (Mullen & Durden, 2018). In *the Anopheles* species, this fluid is ample to an amount that comes directly from the midgut instead of from the excretory tubes known as the Malpighian tubules (Mullen & Durden, 2018; Triplehorn & Johnson, 2005). This fluid looks red due to blood cells from the blood meal (Mullen & Durden, 2018). Once the female uptakes enough blood, which is signaled by abdominal stretch receptors in the midgut, she will withdraw her stylets by pushing with her forelegs and then flies away (Mullen & Durden, 2018). Generally, she will be too heavy to fly far until a significant amount of water and salt has been excreted from the blood meal (Mullen & Durden, 2018).

During the digestion of the bloodmeal and egg development, females will find a species-specific resting site (Mullen & Durden, 2018). Species that rest inside during the digestion of the blood meal and egg development are considered endophilic (Service, 2012). Female mosquitoes that rest outside are considered exophilic (Mullen & Durden, 2018). The female's abdomen is dilated and bright red but turns dark red after several hours (Mullen & Durden, 2018). As the blood is being digested and the eggs in the ovaries start enlarging, the abdomen becomes whitish posteriorly. In contrast, the anterior end of the abdomen is dark reddish (Mullen & Durden, 2018). This condition is the mid-point in the digestion of the bloodmeal and egg development, also known as half-gravid in mosquitoes (Mullen & Durden, 2018).

The abdomen becomes fully dilated and whitish due to the maturation of the fully developed eggs (Mullen & Durden, 2018). This condition is known as fully gravid, and she will now search for a suitable egg-laying site (Mullen & Durden, 2018). Once egg laying is complete, the female will take in

another blood meal, and she will shortly have another batch of eggs fully matured and laid (Mullen & Durden, 2018). The blood-feeding and oviposition process that can occur several times during the female's lifespan and is known as the gonotrophic cycle (Service, 2012).

Mosquito species that only complete one generation per year are considered univoltine. This can occur when development time is slowed in relation to the change in season (Mullen & Durden, 2018). Other species may go through two or more generations in one season, which is known as bivoltine and multivoltine (Mullen & Durden, 2018). This is dependent on temperature, photoperiod during the season, available larval habitats, or available host (Mullen & Durden, 2018). In colder climates like Wisconsin, overwintering takes place in a state of diapause (Mullen & Durden, 2018).

Besides mosquito species living in the tropics and subtropics, all mosquito species go through dormancy, which occurs in Wisconsin due to the winter season (Mullen & Durden, 2018). For *Aedes* and *Psorophora* have quiescent eggs that require inundation no matter the season and typically hibernate as embryonated eggs (Mullen & Durden, 2018). *Anopheles* and *Culex* species will overwinter as adults in a dormant stage in well-protected harborages or hibernacula (Mullen & Durden, 2018).

Before dormancy occurs, mosquitoes will habitually enter diapause, a physiological state of arrested development and reduced metabolic rate induced or deactivated by specific environmental cues (Mullen & Durden, 2018). During diapause, growth, differentiation, and metamorphosis cease until environmental conditions become favorable (Mullen & Durden, 2018). For egg diapause, mosquitoes can either go through facultative or obligate egg diapause (Mullen & Durden, 2018). Multivoltine species go through a facultative egg diapause, which is induced when the larvae, pupae, or adult females are exposed to decreasing temperatures and short photoperiods (Mullen & Durden, 2018). The laid diapausing eggs will not hatch even in days unseasonably warm periods in autumn, winter, and early spring, where the following larvae and adults would unlikely survive (Mullen & Durden, 2018). In univoltine species, obligate egg diapause occurs regardless of preceding environments and is still maintained regardless of warm, long-day conditions (Mullen & Durden, 2018). This type of diapause is typical of snow and spring pool species of *Aedes* spp. in cold and temperate climates. Once the eggs have been subjected to winter conditions, and when favorable temperatures and long days resume, diapause ceases (Mullen & Durden, 2018). Diapause in the larval stage is parallel to facultative egg diapause in the induction and termination (Mullen & Durden, 2018). The diapausing larvae do not molt and feed and grow little or cease these functions altogether (Mullen & Durden, 2018).

With species overwintering as adult females, they will emerge in the form of reproductive diapause which was induced by larval and pupal exposure to cooler temperatures and shortening photoperiod (Mullen & Durden, 2018). In *Culex* spp. that hibernate through hard winters, they cease feeding until the beginning of spring (Mullen & Durden, 2018). Once warmer temperatures and longer photoperiods return, the lifecycle continues.

Similar Looking Species

Crane Flies (Family: Tipulidae)

Although crane flies are generally larger than mosquitoes, their long and slender body and legs have them commonly mistaken for mosquitoes (Mullen & Durden, 2018). Crane flies are also in the order Diptera (True Flies) and have halteres similar to mosquitoes (Weissling, 2020). A key feature to

determine crane flies from mosquitoes is the v-shaped external line like groove or suture in the middle of the thorax on the dorsal side (Weissling, 2020). The wings also lack the unforked vein between the two forked veins and (Weissling, 2020). Scales are also lacking on the wing and wing margin (Weissling, 2020). Crane flies do not bite people either and are not of any medical or veterinary importance (Triplehorn & Johnson, 2005).

Crane Fly
Photo Credit: Edward L. Manigault, Clemson University Donated Collection, Bugwood.org

Midges (Family: Chironomidae

Midges are another family of Diptera that look very similar to mosquitoes. Like mosquito larvae, most non-biting midge larvae live in aquatic habitats, and the adult midges share similar habitats to adult mosquitoes (Triplehorn & Johnson, 2005). Unlike the mosquito, the wings have no scales and don’t possess long proboscis (Triplehorn & Johnson, 2005). The wings also lack the unforked vein between the two forked veins and (Weissling, 2020).

Non-Biting Midge
Photo Credit: David Cappaert, Bugwood.org

Public Health and Veterinary Importance

The blood-feeding of mosquitoes on humans, domestic and wild animals is of public and veterinary health concern. The female's blood-feeding can compromise the skin, leading to hypersensitivity and the possibility of a secondary bacterial infection (Mullen & Durden, 2018). In livestock, large swarms may cause livestock to suspend feeding and search for relief (Mullen & Durden, 2019). This may result in scratching behaviors that lead to skin abrasions, hair loss, and secondary infection with bacteria at the bite site (Mullen & Durden, 2018). Additionally, this allows for the transmission of microorganisms that can cause infection in both humans and animals (Mullen & Durden, 2018). Mosquito-borne diseases are caused by three different pathogens, including viruses, protozoans, and filarial nematodes (Mullen & Durden, 2018). This manual will review mosquito-borne pathogens caused by mosquitoes in humans and domesticated animals in Wisconsin. Although there have been confirmed cases of Malaria and Zika virus in Wisconsin, all cases were reported by people who traveled outside of the

United States to a country with the pathogens (WI DHS, 2021; WI DHS, 2020).

Eastern Equine Encephalitis

Eastern Equine Encephalitis (EEE) is an *Alphavirus* in the family Togaviridae (Mullen & Durden, 2018). This *Alphavirus* was first isolated from the brains of horses that died in 1933 outbreak along the eastern coast of Virginia, Delaware, Maryland (Mullen & Durden, 2018). Eastern Equine Encephalitis infection in humans results in high morbidity and mortality with the severity of this illness depending on the individual's health and age (Mullen & Durden, 2018). Children, the elderly, immunocompromised individuals, and sometimes healthy adults can develop acute EEE (Mullen & Durden, 2018). Symptoms include high fever, drowsiness, lethargy, vomiting, convulsions, and coma (Mullen & Durden, 2018). In clinical cases, mortality rates with EEE exceed 50% (Mullen & Durden, 2018). The individuals who survive EEE will often show neurologic sequelae (Mullen & Durden, 2018). Some survivors will recover completely and show rapid improvement from being in a coma (Mullen & Durden, 2018).

This disease also is a significant cause of death in horses and other equids (Mullen & Durden, 2018). Additionally, this disease can be responsible for mortalities of caged pheasants and whooping cranes (Mullen & Durden, 2018). Horses will rapidly succumb to the infection with a short incubation period that is 2-5 days (Mullen & Durden, 2018). Horses will exhibit abnormal behavior, fever, drooping eyelids, drooping lower lip, twitching paralysis, aimless wandering, and blindness (WI DATCP, 2020). Eventually, the infected horse will drop to the ground and go into a coma before death (Mullen & Durden, 2018). In pheasant flocks, a single infected, sick bird will be pecked by other birds and transfer the virus directly to healthy birds without mosquito bites (Foster & Walker, 2018).

In Wisconsin, EEE virus is maintained in a bird-mosquito enzootic cycle in tamarack, conifer and red cedar swamps that support the mosquito vector *Culiseta melanura* (UW-Madison, 2021). *Culiseta melanura* feeds on birds in the swamp and may leave the area to find a host and return later to oviposit (Mullen & Durden, 2018). Although *C. melanura* is not attracted to mammals, the female mosquitoes are highly efficient vectors of the EEE virus and primarily transmit the disease to swamp-dwelling passeriform birds (Mullen & Durden, 2018). The overwintering mechanisms of the EEE virus is unknown (Mullen & Durden, 2018). On rare occasions in the summer, for reasons possibly related to weather patterns and densities of bird hosts and mosquitoes, the EEE virus infection rates increase with certain mosquito species functioning as bridge vectors (Mullen & Durden, 2018). In Wisconsin, the main bridge vector species is *Coquillettidia perturbans* (MCEVBD, 2021). *C. perturbans* feed on infected birds, then later obtain a blood meal from mammals (Mullen & Durden, 2018). Humans and equines are dead-end hosts for EEE and cannot further transmit the virus to others (Robertson, 2019).

Although EEE is rare in Wisconsin, there are still confirmed cases in humans and animals. Between 1964 and 2018, there have been three human EEE virus cases in Wisconsin (WI DHS, 2021). In horses the disease is still rare but occurs more frequently than in humans. According to Wisconsin Department of Agriculture, Trade and Consumer Protection (WI DATCP, 2020), there were 5 EEE cases in 2019 and 26 cases in 2020. Humans and horses are dead-end hosts for Eastern Equine Encephalitis (Robertson, 2019).

Jamestown Canyon Virus

Jamestown Canyon Virus (JCV) is an *Orthobunyavirus* in the family Bunyaviridae (Mullen & Durden, 2018). This virus was initially isolated from *Culiseta inornate* in Colorado in 1961 (WI DHS, 2020; Mullen & Durden, 2018). JCV is relatively rare in Wisconsin, but there have been significant increases in cases (WI DHS, 2020). More cases could be unaccounted for due to many infected people never developing symptoms (WI DHS, 2020). Some individuals may develop mild symptoms, including fever, headache, fatigue, chills, muscle aches, nausea, vomiting, and joint pain (WI DHS, 2020). Severe illnesses usually develop in the elderly or in individuals who have compromised immune systems (WI DHS, 2020). Severe signs and symptoms are either meningoencephalitis or meningitis (WI DHS, 2020). In meningoencephalitis, inflammation of the brain and surrounding tissue occurs, which results in increased lethargy and altered mental status (WI DHS, 2020). In meningitis, inflammation occurs around the brain and spinal cord, which causes severe headaches and neck stiffness (WI DHS, 2020). Death from JCV is rare but can occur (WI, DHS, 2020). Once you are infected with JCV, you become immune to the virus (WI DHS, 2020).

Several mosquito species, including *Anopheles quadrimaculatus* and *Anopheles punctipennis* have been considered important mosquito-borne vectors in United States (Molaei et al., 2009). Female mosquitoes become infected with JCV by feeding mammals, primarily white-tail deer (WI DHS, 2020). White-tail deer are predominating host to both *An. quadrimaculatus* and *An. punctipennis*, which supports the idea of enzootic amplification of deer-associated arboviruses such as JCV (Molaei et al., 2009). Once the female mosquito is infected with the virus, it can infect other mammals, including humans, when she takes her next blood meal (WI DHS, 2020). Transovarial transmission Jamestown Canyon Virus has been proven in several mosquito species (Foster & Walker, 2020). Between the years 2010 and 2019, there have been 111 human case of JCV in Wisconsin (CDC, 2020). This is almost half of all the cases in the entire United States during that timetable (CDC, 2020). Although James Canyon Virus has been detected in many mosquito species in the United States, the vector in Wisconsin is still unknown (WI DHS, 2020).

La Crosse Encephalitis

La Crosse Encephalitis (LAC) is closely related to Jamestown Canyon Virus and is considered the most important human pathogen within the California serogroup viruses, causing acute, febrile illness in children (Mullen & Durden, 2018). LAC is an *Orthobunyavirus* in the family Bunyaviridae (Mullen & Durden, 2018). The majority of cases are either subclinical or mild, but in rare cases, it progresses to severe encephalitis and, in rare cases, death (Mullen & Durden, 2018). The virus was first isolated in 1964 from preserved brain tissue of a child who had died of encephalitis in 1960 near La Crosse, Wisconsin (Mullen & Durden, 2018).

Most people who are infected show no apparent symptoms. Those who become ill develop a severe neuroinvasive disease, which affects the nervous systems. Symptoms may include fever, headaches, nausea, vomiting, fatigue, and lethargy (CDC, 2019). Severe cases generally lead to inflammation of the brain or encephalitis, resulting in seizures, coma, and paralysis (CDC, 2019). Severe cases often occur in children under the age of 16 (CDC, 2019). The incubation period, or the time from the infected mosquito bite to the onset of symptoms, ranges from 5 to 15 days (CDC, 2019).

The main vector of LAC virus is *Aedes triseriatus* (Mullen & Durden, 2018). The vertebrate reservoirs would primarily be white-tail deer, chipmunks, and

squirrels (Mullen & Durden, 2018; Robertson, 2019). The female can also transmit the virus to their eggs, which is another mechanism of overwintering for LAC virus and means that a vertebrate reservoir is not always needed (Mullen & Durden, 2018). This means that a female mosquito can transmit the virus during its first blood meal without any prior feedings on an infected host (Mullen & Durden, 2018). Although cases are rare, LAC virus still does occur in Wisconsin. According to the Centers for Disease Control and Prevention (CDC) (2019), there have 34 cases between 2010 and 2019 in Wisconsin. Humans are dead-end hosts for La Crosse Encephalitis (Robertson, 2019).

St. Louis Encephalitis

St. Louis Encephalitis (SLE) is a *Flavivirus* in the family Flaviviridae. Cases in Wisconsin are rare, with only a total of 6 cases from 1964 to 2018. This virus was initially identified during an encephalitis-like outbreak in Paris, Illinois, in 1932 and St. Louis, Missouri, in 1933 (Mullen & Durden, 2018). The virus generally affects the more vulnerable age groups, which includes children and the elderly (Mullen & Durden, 2018). Most people who SLE infects show no signs of illness (CDC, 2021). For those who become ill, symptoms include fever, headache, nausea, vomiting, and tiredness (CDC, 2021). In severe cases, inflammation of the brain occurs and can result in long-term disability or death (CDC, 2021).

The primary vector of the St. Louis Encephalitis virus in Wisconsin would be *Culex pipiens* (UW-Madison, 2021; CDC, 2021). The virus is maintained in a bird-mosquito cycle and is amplified periodically by peridomestic birds and *Culex* species in an urban-suburban environment (Mullen & Durden, 2018). The principal avian host reservoirs that are generally abundant in an urban-suburban environment are the house sparrow, pigeons, blue jay, and robin (Mullen & Durden, 2018). Although humans and domestic mammals can obtain SLEV, they are a dead-end host (CDC, 2021).

West Nile Virus

West Nile Virus (WN) is another *Orthobunyavirus* in the family Bunyaviridae that was first isolated in 1937 from the blood of a febrile man in Uganda (Mullen & Durden, 2018). West Nile was first introduced to the United States by unknown means into New York City, New York, and was linked to 61 confirm human encephalitis cases (Mullen & Durden, 2018). The first case of WN in Wisconsin was confirmed in 2002, and there have been 338 total cases from 2002 to 2019 (WI DHS, 2019; CDC, 2020).

Approximately 80% of individuals infected with the West Nile Virus will never develop symptoms (CDC, 2020). About 20% will experience mild symptoms, and less than 1% of cases will be severe (CDC, 2020). Mild signs and symptoms include fever, headache, muscle aches, joint pain, rash, swollen lymph nodes, sensitivity to light (photophobia), nausea, and vomiting (CDC, 2020). In severe cases, signs and symptoms include extreme muscle weakness, inflammation of the brain (encephalitis), confusion or disorientation, paralysis, and coma (CDC, 2020). Rarely, West Nile virus cases can be fatal, with higher mortality rates among the elderly and individuals with other medical conditions (CDC, 2020).

The primary vector of the West Nile virus *Culex pipiens*, with birds being the reservoir and amplifying host during epidemics (Mullen & Durden, 2018). In North America, there are approximately 200 species of birds that have suffered mortality from the West Nile virus, with crows, jays, raptors, and exotic species being more vulnerable (Mullen & Durden, 2018). Species such as the Northern Cardinal and the Mourning Dove with high seroprevalence (**>**20%) can be important

reservoirs of the virus (Loss et al., 2009). Other species that can have high seroprevalence of West Nile Virus in Wisconsin include Blue Jays, Gray Catbird, Brown-Headed Cowbird, House Sparrow, and the Baltimore Oriole (Loss et al., 2009). Many bird species vary widely in their tolerance to infection and seroprevalence (Mullen & Durden, 2018).

With equines such as horses and donkeys, approximately 40% of infected hosts die from the virus (Mullen & Durden, 2018). When initially infected, symptoms start with depression and anorexia without a fever (EDCC, 2017). About 25% of affected horses will develop a mild low-grade fever (101.5-103.5 °F) (EDCC, 2017). With the onset of the neurologic disease, symptoms include periods of hyperexcitability, apprehension, or drowsiness (EDCC, 2017). Additional symptoms in horses include facial paralysis, head tilt, droopy lip, muzzle deviation, weakness, ataxia, dysmetria (incoordination) in one or all limbs, paralysis in one or all of the limbs, colic, and inability to stand (recumbency) (EDCC, 2017). Although humans and equines can become infected with the West Nile virus, they are considered dead-end hosts (Robertson, 2019).

Dog Heartworm

Dog heartworm is a mosquito-borne filarial nematode mainly caused by *Dirofilaria immitis* (Mullen & Durden, 2018). The adult *D. immitis* occupies the right ventricle of the canine heart and pulmonary arteries (Mullen & Durden, 2018). The worms are generally 12-31 cm in length and form aggregates of up to 50 or more individuals (Mullen & Durden, 2018). When aggregations are large enough, infections can extend to the left atrium (Mullen & Durden, 2018). This high-intensity aggregation results in deleterious changes in the endothelium and the integrity of the pulmonary artery walls (Mullen & Durden, 2018). The weakness of the pulmonary arteries leads to pulmonary hypertension and right ventricular hypertrophy (Mullen & Durden, 2018). The following changes cause decreased cardiac output to the lungs, weakness, lethargy, chronic coughing, and eventually congestive heart failure which can be fatal in dogs (Mullen & Durden, 2018).

Cats can also get infected with *Dirofilaria immitis* but only 20% become transiently microfilaremic (Cornell, 2016; Robertson, 2019). Signs and symptoms in cats can be intermittent vomiting, diarrhea, rapid and difficult breathing, coughing, gagging, loss of appetite, lethargy, and weight loss (Cornell, 2016). Cats can survive for an extended period of time before succumbing to other feline disorders, but fatal cases can occur abruptly (Cornell, 2016).

Dogs get infected with heartworms by a bite of a mosquito, whose labium contains the third-stage larvae (Mullen & Durden, 2018). The larvae break out of the labium when it is bent during feeding and are deposited on the skin of the dog in addition to a small droplet of hemolymph (Mullen & Durden, 2018). Only about 10% of the third stage larvae successfully enter the skin (Mullen & Durden, 2018). The larvae remain in the same location it enters under the skin, and there it will molt into the fourth-stage larvae (Mullen & Durden, 2018). The fourth stage larvae will then migrate to other subcutaneous, fat, and muscle tissue to molt into the fifth stage larvae (Mullen & Durden, 2018). This stage enters venous circulation and becomes established in the heart and pulmonary arteries (Mullen & Durden, 2018). On average, the fifth stage larvae reach the heart within 70-90 days upon infection (Mullen & Durden, 2018). In the fifth stage, larvae then develop into sexually mature adults (Mullen & Durden, 2018). After mating, the females will release the active embryotic stage known as the microfilariae 6-7 months after (Forest

& Walker, 2018). The microfilariae have peak concentrations in peripheral blood during the evening when most mosquito species are at peak flight activity (Mullen & Durden, 2018).

The mosquito will become infected with heartworms when they obtain a blood meal from a microfilariae-infected dog (Mullen & Durden, 2018). Within 48 hours of ingestion, the microfilariae will migrate posteriorly in the mosquito's midgut to the Malpighian tubules (Mullen & Durden, 2018). Within 12-14 days after ingestion, the first-stage larvae will molt into the second and third stage larvae, with the third stage larvae migrating to the head and base of the mouthparts (Mullen & Durden, 2018). Here the lifecycle repeats, and *D. immitis* finds a new host to infect.

The life cycle of *Dirofilaria immitis* involves several mosquito species and canids, including domestic dogs, foxes, wolves, and even raccoons (Mullen & Durden, 2018, CDC). Mosquito species in Wisconsin that have been naturally infected with *D. immitis* are *Aedes trivittatus, Aedes vexans, Anopheles punctipennis, Anopheles quadrimaculatus,* and *Culex pipiens* (Ledesma & Harrignton, 2011).

Important Mosquito Species of Wisconsin

Although there are approximately 56 species of mosquitoes recorded in Wisconsin, only several species are of importance (UW-Madison, 2021). Not only are these species a nuisance to humans and domestic animals, but they are also of medical and veterinary importance (Foster & Walker, 2018). Each mosquito species also has a specific preference of host, breeding habitats, and peak flight activity which makes identification critical for proper control.

Eastern Tree-hole Mosquito: Aedes triseriatus

Aedes triseriatus is a widely distributed mosquito species in the Eastern United States (Linley, 1989). This mosquito species is generally associated with hardwood forests and natural and artificial containers (Linley, 1989).

Description of Life Stages and Biology

Eggs are dull black, broadly cigar-shaped, and have a conspicuous micropylar collar (Linley, 1989). The outer shell pattern is irregularly hexagonal and some areas pentagonal (Linely, 1989). The eggs of *Ae. triseriatus* are typically attached to the inside of tree holes, but natural and artificial containers such as road ruts and discarded tires have been used (Linley, 1989). *Ae. triseriatus* overwinters in Wisconsin as eggs with hatching occurring with the thawing of tree hole water (WRBU, 2020).

Larvae go through four instars and can inhabit various natural and artificial containers, including tires (Linley, 1989). Although they share similar traits and can form hybrids with *Aedes hendersoni*, a few key characteristics can be used to identify *Ae. triseriatus* (Grimstad et al., 1974). The anal gills or papillae are unequal in length, with the dorsal pair being longer than the ventral pair (Grimstad et al., 1974). The siphonal tuft is either one to two-branched, with the two-branched being more common (Grimstad et al., 1974). On rare occasions, the siphonal tuft can be three-branched (Grimstad et al., 1974). The pecten teeth are long and narrow, and dark in color (Grimstad et al., 1974). The thorn-like comb scales are in one uneven row (FMEL, 2019). According to Walker & Merritt (1991), 90.8% of the larva’s time is spent feeding, with 52.5% of that time spent feeding at or near the surface. The larval stage primarily feeds on bacteria, algae, and other inorganic material that is suspended in the water (Yale, 2014).

Once the fourth instar larva is fully developed, it will then transform into a pupa. Like other mosquito species, it is comma-shaped and is divided into two body sections, the cephalothorax and the abdomen, with two paddles (Service, 2012). One key characteristic that is used to identify *Ae. triseriatus* from other species is that the air trumpets are dark in color (Robertson, 2019).

After spending three to four days as a pupa, the adult emerges (Yale, 2014). The adults' vital feature is no bands on the hind legs and the edges of the dorsal portion of the thorax having silvery-white scales (Connelly, 2013). The scutum is dark, with no lines or patterns of white scales (FMEL, 2019). The palps and the adult's proboscis are dark in color with no pale or white scales (FMEL, 2019).

Female *Aedes triseriatus*
Photo Credit: Susan Ellis, Bugwood.org

The males and females both feed on nectar, with females requiring a blood meal to develop eggs. The female generally rests in heavily shaded areas close to the larval habitat, but she will fly into open spaces to feed (WRBU, 2020). The female *Ae. triseriatus* prefers to feed deer, Raccoon, red fox, opossum, rabbits, chipmunks, and grey squirrels (WRBU, 2020; Wright & DeFoliart, 1970). If mammals are rare, the female will feed on humans, birds, amphibians, and reptiles (WRBU, 2020). During the female's 30-day lifespan, she may lay four to five batches of eggs, with one female being capable of laying up to 1000 eggs during their brief lifetime (Yale, 2014).

Medical Importance

Aedes triseriatus is an important vector of the La Crosse virus (LAC) (WRBU, 2020). The female can transovarially transmit LAC to the eggs, allowing it to overwinter (WRBU, 2020). Additionally, sexual transmission of LAC between males and females can occur (WRBU, 2020). Laboratory research also found that females infected with LAC took smaller bloodmeals, which led to re-feeding several times during one egg development cycle. (WRBU, 2020).

Plains Floodwater Mosquito: *Aedes trivittatus*

The plains floodwater mosquito (*Aedes trivittatus*) is a common pest species in Wisconsin (UW-Madison, 2021). Populations of *Ae. trivittatus* can be high in the summer, especially after rainstorms (UW-Madison, 2021).

Description of Life Stages and Biology

Eggs, on average, are 0.574mm in length and 0.178mm in width (Abdel-Malek, 1949). Eggs are spindle-shaped, with the ventral side being a little flatter than the dorsal side (Abdel-Malek, 1949). The egg's whole external surface is sculptured into large and small lens-shaped bosses forming a polygonal pattern (Abdel-Malek, 1949). When the eggs are first laid, they are white and turn black after a few hours (Abdel-Malek, 1949).

Eggs are deposited in fully shaded sites in grassy margins of freshwater streams, ponds, and temporary pools in the fall and overwinter as eggs (WBRU, 2020). *Ae. trivittatus* are commonly associated with pools with bluegrass and nodding

fescue-grasses with water infusions impacting the hatching of eggs (Abdel-Malek, 1948). Overall, the temperature has the largest impact on hatching (Abdel-Malek, 1948). Under natural conditions, the minimal period from the hatching of eggs to adults' first emergence was 8 days; however, this can vary with temperatures (Adbel-Malek, 1948).

The length of the fourth instar larvae varies from 5.04 to 6.57mm, with the average length being 6.1mm (Abdel-Malek, 1949). The head capsule width is 1.10mm, and length of the anal siphon 0.81mm (Abdel-Malek, 1949). The head is broader than long, with the broadest portion of the head capsule basal to the eyes (Abdel-Malek, 1949). The larvae's antennae are cylindrical, and the anterior half being curved inwards (Adbel-Malek, 1948). The antenna is studded and contains numerous forwardly pointed spines (Abdel-Malek, 1949). The antennal tuft is inserted near the middle of the shaft and formed of three barbed hairs that don't reach the antennae's tip (Abdel-Malek, 1949).

The upper lateral abdominal hairs of the larva are in pairs of two on the first two abdominal segments (Abdel-Malek, 1949). The remaining abdominal segments having single hairs (Abdel-Malek, 1949). The lateral comb on the eighth abdominal segment is in a triangular patch containing 19-28 scales and on average comprising of 22 scales (Abdel-Malek, 1949). The anal siphon is conical in shape and about twice as long as wide with widest portion at the middle. (Abdel-Malek, 1949). The pecten is formed from 15-17 scales that are equally spaced and occupying the basal half of the anal siphon (Abdel-Malek, 1949). Each pecten is followed by tuft of 4-5 hairs called the subventral tuft (Abdel-Malek, 1949). The ninth abdominal segment is longer than wide, with the saddle enveloping three-fifths of its posterior (Abdel-Malek, 1949). The anal gills or papillae are four in number and about two times the length of the saddle (Abdel-Malek, 1949).

Generally, the smaller larvae can be observed at the surface of the water pool feeding on microorganisms (Abdel-Malek, 1948). As the larvae develop, they spend more of their time at the lower levels of the water, with the larger fourth instar being concealed by the vegetation at the bottom of the pool (Abdel-Malek, 1948).

Like other mosquito species, the pupa is comma-shaped with two body segments, the cephalothorax, and abdomen (Abdel-Malek, 1948). The air trumpets are located dorsally on the cephalothorax, and like other *Aedes* species, used to acquire air at the water surface (Abdel-Malek, 1948). The setae on the cephalothorax have similar arrangement to *Aedes aegypti,* which is a species not found in Wisconsin (Abdel-Malek, 1948; Zettel & Kaufman, 2019). There are 12 pairs of setae altogether: three on the head shield, four near the front margin of the thorax, and one on the dorsal surface of the thorax close to the air trumpets (Abdel-Malek, 1948). The paddles are fringed with minute denticles that cover their entire surface (Abdel-Malek, 1948).

The pupae of *Ae. trivittatus* congregate in large clusters along the pool's edge, hanging near the surface film of the water (Abdel-Malek, 1948). This behavior is considered a preparation for the emergence of the adult mosquito (Abdel-Malek, 1948). When the young adults emerge from the pupa, it stands on the pupal case for a short time before crawling to the edge of the pool, where it will remain for one to two hours before flying off to nearby vegetation (Abdel-Malek, 1948).

The adults are small mosquitoes with a pointed abdomen and no leg bands (MSU, 2021). A key feature in identifying this species is the two pale stripes on top of the thorax (MSU, 2021). The sides of the abdomen have a triangular-shaped pattern of pale scales (MSU, 2021).

Female *Aedes trivittatus*
Photo Credit: Betsy Betros

Both the males and females feed on nectar (Foster & Walker, 2018). The female *Ae. trivittatus* prefers to feed on cottontail rabbits (WRBU, 2020). Other species in Wisconsin that the female will feed on are humans, raccoons, red fox, opossum, woodcock, domestic chickens, turtles, and fox snakes (Wright & DeFoliart, 1970). Although *Ae. trivittatus* is more active at dusk, it can also be active during day and night and even feed with bright sunshine (WRBU, 2020). The Adults are long living, with the female being able to live 5-6 weeks while producing several batches of eggs (WRBU, 2020). The female can produce up to 100 eggs per blood meal with the average being 55 eggs (WRBU, 2020). This mosquito species stays near its breeding site and does not migrate far (MSU, 2021).

Medical Importance

Female *Aedes trivittatus* are aggressive biters that can build populations quickly after a heavy rainstorm, making it a significant pest to human populations near their breeding sites (WRBU, 2020; MSU, 2021). *Ae. trivittatus* is also a vector of dog heartworm (Ledesma & Harrington, 2011).

Inland Floodwater Mosquito: *Aedes vexans*

Aedes vexans is the most common mosquito species that people in Wisconsin will encounter in the summer and a frequent pest (MCEVBD, 2021). Although it is a common species that will readily feed on humans and mammals, it is not considered a significant vector of any mosquito-borne pathogens.

Description of Life Stages and Biology

Eggs of *Ae. vexans* are spindle-shaped and generally symmetrical with faintly reticulated shells black in color (Gjullin et al., 1950). They average 0.61 to 0.645 mm in length and 0.18 to 0.215 mm in width (Gjullin et al., 1950). Ae. vexans eggs are usually deposited in the soil in areas likely to be flooded (WRBU, 2020). Breeding sites include inundation areas such as floodplains of rivers or lakes with fluctuating water (Becker et al., 2020).

Increases in numbers can occur after rain events where ponding can occur (Becker et al., 2020). *Ae. vexans* prefer areas with temporary water bodies that have neutral to alkaline water only present only a few days to weeks after the flooding, such as willow and reed areas (Becker et al., 2020). Once flooding has occurred, larvae will hatch within minutes to hours, depending on the time the flooded water becomes stagnant and the oxygen content decreases (Becker et al., 2020). Not all the eggs will hatch after flooding, but only a portion of them will (Becker et al., 2020). If the first population of larvae does fail due to drying out, then the second population could develop following another flooding even without eggs laid (Becker et al., 2020). Even if suitable hatching conditions fail to occur, the eggs can survive for at least five years (Becker et al.,2020).

The eggs hatch into aquatic larvae that go through four instars before going into the pupal stage. The larva's antennae are less than half as long as the head and contain numerous spicules (Becker et al., 2020). The antennal seta is 5-10 branched and inserted under the middle of the antenna (Becker et al., 2020). The upper and lower portions of the head hairs are multiple branched (FMEL, 2019). The side hairs on abdominal segments III-V have 2-3 branched hairs (FMEL, 2019). The pecten has 13-18 teeth and is not evenly spaced, with the last 1-3 teeth more widely spaced (Becker et al., 2020; FMEL, 2019). The four anal papillae are equal in length and distinctly longer than the saddle (FMEL, 2019; Becker et al., 2020). There are 9-12 thorn-like comb scales that are roughly in two rows (FMEL, 2019).

Like other culicine mosquitoes, *Ae. vexans* pupae are divided into two body segments: the cephalothorax and abdomen (Service, 2012). The pupae obtain oxygen directly from the water surface from a pair of respiratory tubes that extend from the cephalothorax (Service, 2012). The pupa is undescriptive, with the larvae and adults being used for identification.

Aedes vexans Larva
Photo Credit : Thomas Palmer

With *Ae. vexans* being considered a summer species, they have an optimal temperature of 30°C for its development (Becker et al., 2020). When water temperatures are at 30°C, development from hatching of the first instar to the emergence of adults occurs in one week (Becker et al., 2020). When temperatures are at 15 °C, development time moves to three weeks (Becker et al., 2020). Generally, *Ae. vexans* becomes the dominant mosquito species during summer months that are rich in flooding (Becker et al., 2020). There are often hundreds of larvae per liter of water, which generally equates to more than 100 million larvae per hectare (Becker et al., 2020). Bick and Penn (1947) found that the pupa is more resistant to drought than the larva, and even with the lack of water, there was still a 94% emergence of adults.

The adults have several characteristics that can help with identification. A key feature of *Ae. vexans* are the white basal scales on the abdominal segments that are notched in the middle to form a v-shape pattern (Connelly, 2013). The palps and proboscis of the adult female are dark, and the pale rings on the tarsi are very narrow and usually not reaching more than ¼ the length of the tarsomeres (FMEL, 2019; Becker et al., 2020). The scutum contains short brown scales that have no apparent pattern (Murray et al., 2014). The wings of the adult have dark scales (FMEL, 2019).

Female *Aedes vexans*
Photo Credit : Tara Armijo-Prewitt

Both the females and males feed on plant nectar to obtain energy, but females also require a

bloodmeal to develop eggs (Becker et al., 2020). The female *Ae. vexans* feeds on a wide variety of host, with its preferred host being white-tail deer, raccoon, red fox, opossum, rabbits, and humans (Wright & DeFoliart, 1970). *Ae. vexans* females are not readily attracted to or feed on cold-blooded vertebrates (Wright & DeFoliart, 1970). After obtaining a blood meal, the female can lay eggs in 5-8 days at the earliest (Becker et al., 2020). She will be able to deposit more than 100 eggs after a single blood meal with occasionally being able to lay multiple batches after repeated blood meals (Becker et al., 2020). With optimal conditions, *Ae. vexans* would only need three weeks from the hatching of the first generation to the hatching of the next generation's larvae (Becker et al., 2020). The *Ae. vexvans* mosquito is a multivoltine species that overwinter as eggs (Robertson, 2019).

Medical Importance

With large population pressure after emergence, the adult mosquitoes can migrate long distances away from their breeding site to locate a host for a bloodmeal (Becker et al., 2020). Migration of up to 15 km (1km/night) or more is possible (Becker et al., 2020). It also has been speculated that only a portion of the emigrated population returns to the original breeding sites after obtaining a blood meal, which leads to the natural regulation of population densities (Becker et al., 2020). This immigration and establishment by female mosquitos into residential areas, recreational areas, parks, and areas prone to flooding can make *Ae. vexans* one the most important nuisance mosquitoes in Wisconsin (Becker et al., 2020).

With *Aedes vexans* being widely distributed, abundant during peak virus activity, and readily feeding on mammals and humans, it makes this mosquito species an ideal vector host (Becker et al., 2020). Natural infections with eastern equine encephalitis, western equine encephalitis, California encephalitis, and dog heartworm have been reported in the North America (Becker et al., 2020). It has also been found to carry the West Nile virus naturally, but its role in the pathogen's circulation is still unclear (Becker, 2020). Although females have been found to be naturally infected with these viruses, *Ae. vexans* is not considered a significant vector of any mosquito-borne pathogen in the Midwest (MCEVBD, 2021).

Woodland Malaria Mosquito: *Anopheles punctipennis*

Anopheles punctipennis is the most common species of *Anopheles* encounter in Wisconsin (UW-2021). This species is associated with swampy and boggy areas (UW-Madison-2021).

Description of Life Stages and Biology

The eggs are spindle shape in appearance and on the ventral side of the egg, the side that is completely covered by the water, is clothed with a delicate membrane know as the exochorion (Lawlor, 1940). The exochorion gives the ventral side a silvery appearance (Lawlor, 1940). The sides of the egg contain specialized portions of the exochorion know as floats, which allows the egg to stay afloat on top of the water (Lawlor, 1940). The "winter" eggs are larger than the eggs laid in the summer and have an exochorion covering the egg's entirety except for the tips (Fritz & Washino, 1992). Although there is no evidence that either type of eggs is produced seasonally, the amount "winter" eggs collected by females that took blood meals in October and oviposited such eggs were high (Fritz & Washino, 1992). The function of the "winter" variation is to have a cold-hardy egg or resistance to desiccation (Fritz & Washino, 1992). According to the study by Herms & Freeborn (1920), they observed a range of 83-321 eggs laid from 33 females, making the average egg-laying per female 203.

The larvae go through 4 instars before going into the pupal stage. Identification requires the use of a microscope to look at the numerous hairs on the head and abdominal segments (Fahmy et al., 2016). The head hairs 5, 6, and 7 are long, multibranched, and plumose (Fahmy et al., 2016). Head hairs 8 and 9 usually with five to seven branches (Fahmy et al., 2016). The lateral setae on the abdominal segments IV-VI are not plumose (Fahmy et al., 2016). The larvae lay horizontal to the water surface feeding on filamentous green algae, which they also rely on for protection (WRBU, 2020). Typical larval habitats include small open, sunlit freshwater stream pools or margins, flood-plain ground pools, and drainage ditches (WRBU, 2020).

Anopheles punctipennis Larva
Photo Credit: Tom Murray

The pupal stage is similar to other mosquito species, being comma-shaped in appearance (Service, 2012). They obtain oxygen from the water surface through their air trumpets on the dorsal side of the cephalothorax (Becker et al., 2020). This life stage does not feed, but they will "tumble" down to the bottom of their aquatic habitat when disturbed (Rio & Connelly, 2018).

Adult's wing patterns have helpful characteristics to identify this species. Wings are predominantly dark, with cream-colored scales on the anterior portion of the wings (WRBU, 2020). The palps are as long as the proboscis, with both the palps and proboscis being dark scaled (APHC, 2014). The dorsal posterior portion of the head is covered with forked scales, with those on the central part being white while the others are dark (APHC, 2014). The frontal tuft is white (APHC, 2014). The thorax contains a broad frosted strip with the sides brown, with the frosted area containing pale-yellow scales (APHC, 2014). The legs are dark scaled with the femora and tibiae tipped with pale scales (APHC, 2014).

Male *Anopheles punctipennis*
Photo Credit: Tom Murray

The female prefers to feed on mammals such as cattle, horses, mice, squirrels, chipmunks, rabbits, canines, and humans (Magnarelli, 1979). Females are exophagic, with daily peak feeding times being early evenings (Ledesma & Harrington, 2011). The females overwinter in abandoned buildings, cellars, caves, hollow trees, and similar habits to the ones mentioned above (Fahmy et al., 2016). *Anopheles punctipennis* is multivotine (Fahmy et al., 2016).

Medical Importance

Anopheles punctipennis is a potential vector of Jamestown Canyon virus, dog heartworm, and the historical vector of Malaria in Wisconsin (Mullen &

Durden, 2018; UW-Madison, 2021; Ledesma & Harrington, 2011).

Common Malaria Mosquito: *Anopheles quadrimaculatus*

Anopheles quadrimaculatus Say was the historical vector of malaria in the Eastern United States and Wisconsin (UW-Madison, 2021; Rio & Connelly, 2018). This mosquito is a common *Anopheles* species in Wisconsin (UW-Madison, 2021).

Description of Life Stages and Biology

The eggs are spindled-shaped and have floats on each side of the egg (Rio & Connelly, 2018). The female deposits the eggs individually on the water's surface (Rio & Connelly, 2018). Preferred oviposition sites are sunlit freshwater ground pools that are slightly alkaline with abundant emergent freshwater, including freshwater streams, ponds, and lakes with aquatic vegetation (Rio & Connelly, 2018). The eggs cannot survive drying or desiccation (Rio & Connelly, 2018). Eggs will hatch two to three days after being laid (Rio & Connelly, 2018).

Like other *Anopheles* mosquitoes, *An. quadrimaculatus* lies horizontally at the water surface, where they filter feeds on various plant and animal matter (Rio & Connelly, 2018). They do not have a breathing siphon for breathing and obtain oxygen through the palmate hairs along their abdomen (Rio & Connelly, 2018). The hair tufts on the antennae of the *An. quadrimaculatus* are inserted near the middle, with hairs #3 on the head being multibranched (FMEL, 2019). The palmate hairs on abdominal segments I and II are reduced while being well-developed on III-VII (FMEL, 2019). Hairs #0 on abdominal segments are exceedingly small, while hair #2 is single on segments IV and V (FMEL, 2019).

The pupal stage, also known as "tumblers," looks similar to other mosquito larvae and is comma-shaped in appearance (Rio & Connelly, 2018). They obtain oxygen from the water surface through their air trumpets located dorsally on their cephalothorax (Rio & Connelly, 2018). This stage does not feed, but when disturbed, they will "tumble" to the bottom of their aquatic habitat (Rio & Connelly, 2018). Generally, the first three aquatic stages are completed within 5-14 days depending on the ambient temperature (Rio & Connelly, 2018).

Adult males and females both have long palps that are near equal to the length of the proboscis, similar to other *Anopheles* species (Rio & Connelly, 2018). The frontal tuft on the head contains pale setae, and the palps are predominantly dark with no rings (FMEL, 2019). The knob on the halter is dark scaled, and the scales on the wings are light and dark-colored with four distinct spots (FMEL, 2019).

Female *Anopheles quadrimaculatus*
Photo Credit: Graham Montgomery

Both the males and females feed on plant sugars and nectar, with the females obtaining blood meals to provide nutrients for the developing eggs (Rio & Connelly, 2018). The *An. quadrimaculatus* females

feeds on wild and domestic mammals and humans (Rio & Connelly, 2018). The female prefers to feed primarily on ruminants, equines, lagomorphs, and canines (Rio & Connelly, 2018). Feeding on humans will vary depending on proximity to larval habitats and resting sites (Rio & Connelly, 2018). This species is both endophagic and exophagic in feeding behaviors (Ledesma & Harrington, 2011). Research has shown that feeding rates on humans can reach 93% when resting mosquitoes have been collected indoors (Rio & Connelly, 2018). The adult female flight activity peaks a short period after dark, with limited flight time used to find a host and the remaining time of the night and dusk used to find resting sites (Rio & Connelly, 2018). In laboratory conditions, the adult female can live for a little over a month; however, in nature, most generally do not survive for more than two weeks (Rio & Connelly, 2018). Overwintering occurs as fertilized females with winter shelters including barns, tree holes, and other dark, protected areas (Rio & Connelly, 2018).

Medical Significance

Although malaria has been considered eradicated from the United States since 1954, *Anopheles quadrimaculatus* is still considered an important vector of the pathogen (Rio & Connelly, 2018). The CDC still reports about 1,500 in the US annually, with Wisconsin reporting 263 cases between 2001 and 2018 (Rio & Connelly, 2018; WI DHS, 2021). All of the cases in Wisconsin were reported by individuals who have traveled outside of the country to a country where malaria is present (WI DHS, 2021). In addition to malaria, *Anopheles quadmaculatus* potentially vector of Jamestown Canyon virus and dog heartworm (Molaei et al., 2009; Rio & Connelly, 2018).

Cattail Mosquito: *Coquillettidia perturbans*

Also known as the cat-tail mosquito in North America, *Coquillettidia perturbans* is a permanent freshwater species with the larvae and pupae having an unusual behavior of attaching to roots of aquatic plants (Johnson & Cuda, 2017). The female mosquitoes of this species can be a significant nuisance to humans and domestic animals (Johnson & Cuda, 2017). They are also a vector of several important diseases that affect humans and equines (Johnson & Cuda, 2017).

Description of Life Stages and Biology

Eggs of *C. perturbans* are deposited on the water near emergent vegetation (Johnson & Cuda, 2017). The eggs are elongate and white within the first two hours of being laid and later darken (Johnson & Cuda, 2017). The female glues together the individual eggs to form a raft that floats above the water (Johnson & Cuda, 2017).

Coquillettidia Egg Raft
Photo Credit: S. L. Doggett, Department of Medical Entomology, NSW, Australia

When the larvae are mature, they are grayish white in color (Johnson & Cuda, 2017). They have a long, whip-like antenna, with each of them bearing a bristle that is profusely branched (Johnson & Cuda, 2017). The larvae's head is wider than it is long, and two short hairs inserted near the middle of the

antennae do not reach the tip (Johnson & Cuda, 2017; FMEL, 2019). The comb on the abdominal segment eight contains 8-15 thorn shape scales (FMEL, 2019). Larvae of *C. perturbans* differ from other mosquito larvae because they obtain oxygen directly from the hollow roots or submerged stems of aquatic plants instead of directly from the water surface (Johnson & Cuda, 2017). The siphon is heavily sclerotized and resembles a short, pointed saw used to pierce the stem or hollowed root (Johnson & Cuda, 2017). In the northern regions of its range which includes Wisconsin, the larvae overwinter as immature or mature larva, with the larval stage possibly lasting for nine months (Johnson & Cuda, 2017).

As mentioned previously, the pupae are separated into two different body regions, the cephalothorax and the elongated abdomen that ends in a pair of paddles (Service, 2012). Like the larvae, the pupae obtain oxygen through the hollow roots or submerged stems of aquatic plants (Johnson & Cuda, 2017). Instead of using a siphon, the pupae insert their air trumpets that project from the cephalothorax into the submerged vegetation.

Both the larval and pupal stages of *C. perturbans* prefer cattails in the family Typhaceae (Johnson & Cuda, 2017; Black & Judziewicz, 2009). In Wisconsin, this would include the narrow-leaved cattail and the broad-leaved cattail, both common and potentially invasive wetland species (Black & Judziewicz, 2009). Other than cattails, *C. perturbans* larvae and pupae are also associated with other aquatic species with submerged roots and stems (Johnson & Cuda, 2017). This includes arrowheads (*Sagittaria spp*.), pickerel-weed (*Ponderia cordata*), water lily (*Nymphaea odorata*), reeds (*Phragmites australis*), sedges (*Carex spp*.), and water arum (*Calla palustris*), which are all species native to Wisconsin (Black & Judziewicz, 2009).

Coquillettidia perturbans Pupa (Left) and Larva (Right)
Photo Credit: Nathan Burkett-Cadena, Florida Medical Entomology Laboratory

The adult mosquito is medium sized with a "salt and pepper" appearance due to the broad black, white, brown scales (Johnson & Cuda, 2017). A key characteristic of this species is the sub-apical band of white scales on the hind tibiae (Connelly, 2013). The female's proboscis is dark scaled with a median band of white scales with golden brown scales on the thorax's dorsal surface (Johnson & Cuda, 2017). The female's abdomen is bluntly rounded and covered in dark scales with patches of pale white scales on each segment (Johnson & Cuda, 2017). Both males and females' wings are covered with broad dark and light-colored scales (Johnson & Cuda, 2017).

Female *Coquillettidia perturbans*)
Photo Credit: Christine Young

The sex ratio of males to females is approximately 1:1, with the adults emerging in the spring or summer (Johnson & Cuda, 2017; Becker et al., 2020). Adults' life span is generally one to two months though females generally live longer than males (Johnson & Cuda, 2017). Females can oviposit up to 150-350 eggs with a blood meal (Johnson & Cuda, 2017). Both males and females feed on nectar, but females require a blood meal for production (Johnson & Cuda, 2017). The female *C. perturbans* have been reported to feed on a wide variety of wild and domestic animals (Johnson & Cuda, 2017). This would include deer, chickens, cattle, rabbits, raccoons, opossums, and humans (Johnson & Cuda, 2017). According to Molaei et al. (2008), the majority of the bloodmeals were mammalian, with minimal bloodmeals obtained from bird species (Johnson & Cuda, 2017). Most of the mammalian bloodmeals came from white-tail deer, while for avian species, most bloodmeals were obtained from the American Robin, both common species in Wisconsin (Molaei et al., 2008). The female will occasionally attack humans during the daylight hours in shady locations (Becker et al., 2020). The adult *C. perturbans* is a strong flyer with a flight range that is greater than 10 km (Becker et al., 2020). In northern regions such as Wisconsin, *C. perturbans* go through a univoltine (one generation) lifecycle (Johnson & Cuda, 2017).

Medical Importance

Female *C. perturbans* are aggressive biters that are capable of penetrating clothing (Johnson & Cuda, 2017). They are also important vectors of the eastern equine encephalitis and potentially can spread the West Nile virus (Becker et al., 2020).

Northern House Mosquito: *Culex pipiens*

Culex pipiens, or the northern house mosquito, is another species that is frequently encountered in Wisconsin (MCEVBD, 2021). According to various authors, it is essentially part of the *Culex pipiens* complex, consisting of several species, subspecies, forms, races, and biotypes (Becker et al., 2020).

Description of Life Stages and Biology

The eggs of *Cx. pipiens* are ovoid with a cup-shaped corolla at one end to allow them to sit vertically on the water surface in a raft (Service, 2012; Becker et al., 2020). The egg raft floats on top of the water surface and is held together by the surface water tension and the apical droplets on the upper end of the eggs (Service, 2012; Mullen & Durden, 2018). The egg rafts consist of approximately 150-240 eggs, with the larvae hatching within one to two days (Becker et al., 2021).

The organic material in the water plays a crucial role in attracting female *Cx. pipiens* to an oviposition site (Becker et al., 2020). Gaseous substances such as ammonia, methane, or carbon dioxide are released as the organic matter decomposes and draws the female to the site (Becker et al., 2020). Breeding sites usually consist of permanent polluted waters (Robertson, 2019). Breeding sites also include many artificial water bodies such flooded cellars, constructions sites, water barrels, metal tanks, and ornamentation ponds and containers in urban and suburban settings (Becker et al., 2020).

The larvae have a wider head than it is long, with the antennae being shorter than the head (Becker et al., 2020). The number of comb scales is approximately 40, with each individual scale short and widened at the apex and evenly fringed (Becker et al., 2020). Pecten contains 10-18 spines that are evenly spaced up to seta 1a-S (WRBU, 2020; Becker et al., 2020). Each pecten spine has a long-pointed tip with three lateral denticles (Becker et al., 2020). The siphonal tuft consists of four widely spaced 2-branched pairs of setae distal to the pecten in an irregular row (Becker et al., 2020). The anal papillae are elongated, with the dorsal pair being twice as long as the saddle (Becker et al.,2020). The larvae diets consist of microorganisms, algae, and detritus (Becker et al., 2020). The larvae of *Cx. pipiens* can successfully develop in temperatures ranging from 10-30 °C (Becker et al., 2020). The larvae complete their development to adults in one to several weeks, depending on temperature (Becker et al., 2020).

Culex pipiens Larva
Photo Credit: Omar Fahmy

Like other mosquito species, the pupa are comma-shaped in appearance with a cephalothorax and abdomen with a pair of paddles at the end (Service, 2012). They obtain oxygen from the water surface through their air trumpets on the dorsal side of the cephalothorax (Becker et al., 2020).

The adult is a medium-sized mosquito with a yellowish-brown to dark brown integument (Becker et al., 2020). The antennae are dark, with the pedicel and flagellomere 1 having a few small white scales (Becker et al., 2020). The palps mostly consist of black scales, with the proboscis having creamed color scales ventrally (Becker et al., 2020). The head retains dark forked scales and includes some paler scales laterally (Becker et al., 2020). The scutum consists of delicate golden-brown scales that are lighter laterally, and the abdomen has curved white bands across the base of each abdominal segment (Becker et al., 2020; Robertson et al., 2019). The hind femur has mostly white scales, with the hind tibia lacking a longitudinal pale stripe (Becker et al., 2020).

Resting *Culex pipiens*
Photo Credit: Ary Farajollahi, Bugwood.org

Adult females generally overwinter in diapause and without feeding (Becker et al., 2020). The female *Culex pipiens* biotype *pipiens* is anautogenous, meaning that they will need a bloodmeal prior to laying eggs (Becker et al., 2020). The female *Cx. pipiens pipiens* form primarily feeds on birds (ornithophilic) such as woodcock and domestic chicken (Wright & DeFoliart, 1970) The *pipiens* biotype will occasionally feed on mammals, which in Wisconsin includes rabbits, woodchucks, grey and ground squirrels, raccoon, and red fox (Wright & Defoliart, 1970). The *pipiens* biotype also mainly feeds exophagic and exophilic (ECDC, 2020). However, the *Culex pipiens* (biotype) *molestus* mainly feeds on humans and mammals and endophagic and exophilic and endophilic (ECDC, 2020). The biotype *molestus* can lay fewer eggs without taking a bloodmeal (autogenous) (Becker et al., 2020). Mortality of the *molestus* biotype in winter shelters is very high due to lack of body fat reserves obtained as a larvae, and death by entomopathogenic fungi and spiders (Becker et al., 2020). Both biotypes are multivoltine (Crans, 2016).

Medical Importance

Both biotypes are the principal bridge vectors of the West Nile virus and St. Louis encephalitis (Becker et al., 2020; Robertson, 2019). With the biotype *pipiens* being primarily ornithophilic, it can keep both the West Nile virus and St. Louis encephalitis in circulation (Robertson, 2019). When both mammals and humans are readily available, and birds are rare, the infected *Cx pipiens* female biotypes can then transfer these pathogens to humans.

Culex tarsalis

Although *Culex tarsalis* is an uncommon mosquito found in Wisconsin, it is a painful and persistent biter and an important vector of several diseases (UW-Madison, 2021; WRBU, 2020).

Description of Life Stages and Biology

The eggs of *Culex tarsalis* are ovoid with a cup-shaped corolla at one end to allow them to sit vertically on the water surface in a raft (Service, 2012; Becker et al, 2020). The egg raft floats on top of the water surface and is held together by the surface water tension and the apical droplets on the upper end of the eggs (Service, 2012; Mullen & Durden, 2018). The raft consists of 190 eggs on average (Robertson, 2019).

The larvae of *Cx tarsalis* have antennae that are 2/3 as long as the head and distinctly constricted beyond the antennal seta's insertion point (Becker et al., 2020). The antennae are dark near the base, with the remainder being pale in color (Becker et al., 2020). The comb consists of approximately 50 small scales arranged in an irregular triangular patch (Becker et al., 2020). The siphon is slender and evenly tapers towards the apex (Becker et al., 2020). The pecten is comprised of approximately 10-15 spines that extend from the basal 1/3 of the siphon (Becker et al., 2020). The anal papillae vary in length and are generally 1.0-1.5 times the saddle's length (Becker et al., 2020).

Like other mosquito species, the pupae are comma-shaped in appearance with a cephalothorax and

abdomen with a pair of paddles at the end (Service, 2012). They obtain oxygen from the water surface through their air trumpets on the dorsal side of the cephalothorax (Becker et al., 2020).

The larvae can be found in permanent and semi-permanent water bodies and can tolerate a wide range of water conditions except for excessive organic pollution (Becker et al., 2020; MSU, 2021). These habitats include ditches, irrigation systems, ground pools, marshes, pools in stream beds, rain barrels, hoof prints, and ornamental ponds (Becker et al., 2020). Larvae can also tolerate saline and secondary treated sewage effluent (MSU,2021). Larval development usually occurs in the late spring and continues into late autumn (Becker et al., 2020). Larval and pupal development can range from seven days to little less than four weeks depending on water temperature and food availability (Montana).

The adult female *Cx. tarsalis* is a medium sized species with primarily dark scaled proboscis (Becker et al., 2020). The palps have a few white scales on the tip and dark scales basally (Becker et al., 2020). The posterior dorsal portion of the head, or occiput, has narrow white scales in a median triangular patch (Becker et al., 2020). There are narrow white scales around the eye margins (Becker et al., 2020). There are pale bandings on the apical and basal ends of hind tarsomeres, with the abdominal terga having pales bands along the basal border (MSU,2021).

The females generally prefer mammals as hosts compared to domestic and wild birds (Becker et al., 2020). They will readily feed on cattle, horses and humans in agricultural areas (WRBU, 2020). They usually feed at dusk and preferably at dark, but they also have been known to look for a host during the day and will readily enter dwellings to get a blood meal (Becker et al., 2020; WRBU).

Adult *Culex tarsalis*
Photo Credit: Joseph Berger, Bugwood.org

The host-seeking females are also strong flyers that can cover a distance of up to 4 kilometers in the search for a bloodmeal (Becker et al., 2020). During the winter months, the adult female will seek shelter in cellars, basements, caves, and rodent burrows (Becker et al., 2020).

Medical Importance

Culex tarsalis females are persistent and painful biters and a severe pest of livestock, poultry, and humans (Becker et al.,2020). It is also a vector of St. Louis encephalitis and the West Nile Virus (Becker et al., 2020). This mosquito species is also considered the to be the main vector of western equine encephalitis, which is extremely rare in Wisconsin (Becker et al., 2020).

Prevention and Control

The prevention and control goals are to prevent mosquito bites, keep mosquito populations at acceptable densities, minimize mosquito and vertebrate contact, and reduce female mosquitoes' longevity (Mullen & Durden, 2018). By combining these different strategies, we can mitigate the

annoying and harmful effects of mosquito bites and interrupt pathogen transmission (Mullen & Durden, 2018). Eradication of mosquitoes and their associated pathogens is no longer considered a feasible objective except in small, isolated regions (Mullen & Durden, 2018). A more realistic objective in modern mosquito control is integrated pest management to reduce mosquito populations and disease prevalence while using a combination of practices (Mullen & Durden, 2018).

Integrated Pest Management

Integrated pest management (IPM), or sometimes known as integrated vector management (IVM), is a science-based, common-sense approach to managing pests like mosquitoes (EPA, 2016). IPM or IVM uses various pest management practices to prevent, reduce, and remove favorable conditions for the development of a pest (EPA, 2016). This program relies significantly on public education and pest monitoring (EPA, 2016). An IPM program can be successful while using pesticides, but this control strategy is based on surveillance and keeping track of mosquito populations in an area (EPA, 2016). Surveillance is critical in any successful IPM/IVM program, for it will determine the appropriate response to an infestation (EPA, 2016). Control other than chemical can include physical, biological, and cultural.

Surveillance and Sampling

Surveillance

Surveillance is critical for planning, implementing, and evaluating an IPM/IVM program focused on mosquito control, whether the goal is to prevent mosquito-borne illnesses or reduce mosquito population levels to allow for normal activities to occur without discomfort (Dame & Fasulo, 2002). Information gathered from surveillance and sampling can identify the mosquito species present and determine what control practices will be used (Dame & Fasulo, 2002). This information can be used to assess life cycles, female feeding preferences, adult resting areas, larval habitats, flight ranges and determine a preliminary recommendation (Dame & Fasulo, 2002).

The first step is a formal surveillance program where routine monitoring for mosquito presence is performed (Dame & Fasulo, 2002). This inspection should include inspection for adult and larval populations, breeding site location, and monitoring rainfall and flooding events (Dame & Fasulo, 2002). These inspections will not determine mosquitoes' total populations in the area, but they can show fluctuations in relative mosquito abundance and diversity over time in various habitats (Dame & Fasulo, 2002).

With Geographic Information System (GIS) tools and technology being more readily available to the local municipalities, private contractors, and the public, these mapping tools can be essential in a successful mosquito IPM program (Dame & Fasulo, 2002). Comprehensive mapping can assist in orientating known breeding sites, lakes, rivers, streams, ponds, elevation determination, and other features such as flooded areas (Dame & Fasulo, 2002). Maps can also be used to determine priority areas and points of entry to a site that may be difficult to access (Dame & Fasulo, 2002). When large areas are being accessed for control, a master map may be needed to plan field operations (Dame & Fasulo, 2002). A master map can indicate treatment areas, potential flight ranges of adults from breeding sites, and degree of penetration into populated areas (Dame & Fasulo, 2002). Adult and larvae sampling stations can be indicated by symbols or a numbering system (Dame & Fasulo, 2002). Counts made by these stations can then be used to determine areas of high or low population densities, disease potential and assist in

determining appropriate control measures (Dame & Fasulo, 2002).

Along with mapping, record keeping is also an essential practice. This can help inspectors avoid comparing dissimilar parameters and keep inspections consistent both in the method and locations (Dame & Fasulo, 2002). Additionally, maintaining clear, accurate records can help determine priority areas and whether the control program succeeded or needs adjustment (Dame & Fasulo, 2002). Records should include the inspector's name, date of inspection, and exact location the data was located (Dame & Fasulo, 2002). Data-recording forms and devices promote uniformity during inspections, which allows the data collected to be easily read, interpreted, and summarized (Dame & Fasulo, 2002). Organized record forms are also helpful reminders to record all relevant information.

Sampling

There are a variety of sampling procedures that can be used to determine the presence of mosquitoes in a given area. Sampling is usually focused on eggs, larvae, and adult stages of mosquitoes (Dame & Fasulo, 2002). Equipment can include fundamental tools such as plastic dippers, light traps, and sweep nets to truck trap collections depending on budgets and accessibility (Dame & Fasulo, 2002).

Egg Collection

The collection of eggs in oviposition traps are used to detect and monitor container-breeding species of mosquitoes such as *Aedes triseriatus* (Dame & Fasulo, 2002). Oviposition traps can be easily made with food cans such as a three-pound coffee can or pint jars painted black inside and out (Dame & Fasulo, 2002). Oviposition substrate made of various materials like seed germination paper, muslin, Formica, and balsa wood can place vertically inside the container with water covering half the substrate (Dame & Fasulo, 2002). Female mosquitoes will use the substrate to lay eggs just above the waterline (Dame & Fasulo, 2002). Traps should be inspected every 10 or 14 days to prevent them from becoming potential breeding sites (Dame & Fasulo, 2002). If larvae are present in the trap, collect the larvae for identification and dump the water and reset (Dame & Fasulo, 2002). The substrate should be collected periodically and taken to a laboratory in a plastic bag (Dame & Fasulo, 2002). Samples should be kept cool and moist, but too much moisture should be avoided to prevent early hatching (Dame & Fasulo, 2002). The resulting fourth instar larvae can be used for identification (Dame & Fasulo, 2002).

Larvae and Pupae Sampling

For larval and pupal surveillance, general information on the suspected mosquito species' breeding behaviors and habitats should be reviewed prior (Dame & Fasulo, 2002). Sampling areas should be marked and numbered on a map or with a GPS device (Dame & Fasulo, 2002). Specific breeding sites that will become permanent larval sampling sites require further inspection beforehand (Dame & Fasulo, 2002). Surveys conducted for mosquitoes' breeding sites can be of significant value to a mosquito IPM program (Dame & Fasulo, 2002).

Equipment used for larval inspections is a white enamel or plastic dipper that is about 4 inches in diameter and has about 1 pint or 350 milliliters of capacity (Dame & Fasulo, 2002). If needed, the handle of the dipper can be extended by using a wooden dowel or PVC pipe (Dame & Fasulo, 2002). Mosquito larvae are usually found at the water surface near vegetation and debris (Dame & Fasulo, 2002). In larger water bodies such as ponds, larvae are generally found near the shoreline (Dame & Fasulo, 2002). When sampling larger water bodies,

proceed slowly and carefully for the disturbance of water or shadows may disturb mosquito larvae (Dame & Fasulo, 2002). For *Anopheles* species, larvae should be collected by skimming the dipper to one side and pressed just below the water surface with the stroke ending before filling the dipper to prevent any larvae loss (Dame & Fasulo, 2002). For culicine species such as *Aedes vexans,* a quicker chopping motion will be required because they are most likely to dive below when disturbed (Dame and Fasulo, 2002). If clumps of erect vegetation are present in the sampling location, the best practice is to press the dipper into the debris with one edge depressed to allow water to flow into the dipper (Dame & Fasulo, 2002).

The larvae and pupae of *Coquillettidia perturbans* do not need to breathe at the water surface, making them difficult to collect in the field (Becker et al., 2020). When scouting for the larvae and pupae of *C. perturbans*, pull up aquatic plants of cattails, arrowheads, pickerel-weed, water lily (Nymphaea odorata), reeds, sedges, and water arum (Johnson & Cuda, 2017). Wash the aquatic vegetation over a white pan and check the debris for larvae and pupae (Johnson & Cuda, 2017).

When looking for *Aedes triseriatus,* looking for breeding sites will require searching tree holes and natural and artificial containers such as tires, muddy ruts filled with water, and tree holes (Dame & Fasulo, 2002).

Adult Mosquito Monitoring

Adult mosquito surveillance allows the inspector the ability to evaluate the incidence of mosquitoes within residential areas where they could potentially feed on people and their abundance within a given area (Dame & Fasulo, 2002). Identifying the adult can help determine oviposition and flight behavior in relation to the distance from the breeding site (Dame & Fasulo, 2002). This can assist the inspector in locating larval breeding grounds and determining the necessary level of control (Dame & Fasulo, 2002).

Insect sweep net collection is a valuable in collecting mosquito species that rest in the grass and other vegetation during the day like *Aedes vexans* (Dame & Fasulo, 2002). Power vacuums collectors and aspirators can also be used similarly (Dame & Fasulo, 2002).

Daytime resting collection can be helpful in collecting anopheline mosquitoes and some culicine species. This kind of surveillance is commonly used for *Anopheles quadrimaculatus* and can be valuable when estimating populations of *Culex pipiens* (Dame & Fasulo, 2002). Most adult mosquitoes are inactive during the day and rest in dark, cool, humid locations (Dame & Fasulo, 2002). Although this kind of surveillance can be labor-intensive, a careful inspection can be effective in identifying the population densities of these mosquitoes in a given area (Dame & Fasulo, 2002). Resting sites include houses, stables, chicken houses, outhouses, culverts, bridges, caves, hollow trees, and overhang banks along freshwater streams (Dame & Fasulo, 2002).

Light traps can attract many mosquito species and can be an efficient sampling tool between dusk and dawn (Dame & Fasulo, 2002). When placed in remote locations away from other light sources, light traps can attract adult mosquitoes from a considerable distance away (Dame & Fasulo, 2002). The light trap can be complemented with a two-pound block of dry ice wrapped in newspaper place above the light trap (Dame & Fasulo, 2002). The trap should be placed in open areas near trees and shrubs (Dame & Fasulo, 2002). Traps should be placed at least 30 ft from buildings, away from lights, areas open to strong winds, and industrial plants that give off strong smoke or gas (Dame Fasulo, 2002).

Several models of mosquito light traps are available such as the New Jersey, CDC, and Encephalitis Vector Survey (EVS) traps (Dame & Fasulo, 2002). These battery-operated light traps were developed for portability to conduct live mosquito catches in remote areas where electric power is not available for use (Dame & Fasulo, 2002). The adult female mosquitoes are attracted to the carbon dioxide produced by dry ice or UV light and are blown downward by a fan into a kill jar or mesh bag hanging below the trap (Dame & fasulo, 2002).

These traps are often used to complement other practices sampling mosquito populations (Dame & Fasulo, 2002). When these kinds of traps contain a light attractant, they can be instrumental in collecting mosquito species such as *Aedes vexans* and *Coquillettidia perturbans* (Dame & Fasulo, 2002). Mosquito species such as *Anopheles quadrimaculatus* and *Culex pipiens* are seldom collected by these traps (Dame & Fasulo, 2002). Light trap collections can vary in results depending on the dark and bright phases of the moon, with a higher collection of mosquitoes during the dark phase (Dame & Fasulo, 2002).

Gravid traps can be valuable in capturing large numbers of undamaged gravid *Culex* females (Dame & Fasulo, 2002). They are easy to build, lightweight, portable, and can run on one 6-volt battery (Dame & Fasulo, 2002). One gallon of attractant such as infusions of grass and rabbit pellets place in a black tray can attract gravid female mosquitoes towards the trap (Dame & Fasulo, 2002). The gravid female is drawn to the collection chamber by an air current produced by the motorized fan, and the mosquitoes are captured live for identification (Dame & Fasulo, 2002).

Control Practices

Mosquito IPM/IVM programs can vary depending on mosquito species present, area of concern, and public perceptions (i.e. environmental concerns of chemical use) (EPA, 2016). An IPM program uses multiple control strategies instead of relying on a single control strategy, with education and surveillance being critical for success (Dame & Fasulo, 2002). Control strategies include habitat modification, sanitation, personal protection, biological control, and chemical control (EPA, 2016).

Habitat Modification and Sanitation

Many artificial containers and structures can act as breeding grounds for mosquitoes (Dame & Fasulo, 2002). Tires, buckets, trash bins, rain barrels, and other artificial containers that hold water can act as a breeding site for mosquitoes (Dame & Fasulo, 2002). Additionally, plugged roof gutters and wrinkle tarps that accumulate water and organic debris can also act as breeding sites (Dame & Fasulo, 2002). In construction and agricultural sites, water storage containers, flooded basements, excavations, tire ruts, and drainage systems can act as potential breeding sites (Becker et al., 2020). Cemeteries can be significant and unexpected breeding sites for mosquitoes (Becker et al., 2020). Mosquito species such as *Culex pipiens* be found breeding in flower vases in cemeteries (Becker et al., 2020).

Tipping over containers and preventing rainwater and debris from accumulating within them can prevent mosquitoes from breeding (Dame & Fasulo, 2002). Fixing plugged roof gutters, removing organic debris and trash will prevent water from pooling near homes and structures (Becker et al., 2020). Inspection for discarded containers in woodlots should be conducted during the early spring and late fall when both the vegetation and snow are minimal to increase

detection and prevent breeding from occurring in the active seasons (Dame & Fasulo, 2002). Tires should be recycled if not used or placed in storage where they will not collect water (Dame & Fasulo, 2002). On dairy or beef farms that use tires to hold down plastic to cover silage, tires can be cut in half to reduce the amount of water they hold. Lids should be placed on water storage containers to prevent female mosquitoes from accessing water within (Becker et al., 2020). Excavations and tire ruts should be graded and leveled, with high traffic areas being covered with gravel, concrete, or blacktop to prevent future tire ruts (Becker et al., 2020).

Tires Used to Hold Down Silage Covers on Dairy Farm
Photo Credit: Steve Schank

In cemeteries, removing flower vases from the site is a viable solution; however, in certain cases, this may not be possible due to cultural views (Becker et al., 2020). Cemetery staff may be paid to replace flowers (Beckers et al., 2020). Filing vases with sand or soil can limit water pooling in vases (Becker et al., 2020). Replacing real flowers with artificial flowers in vases with a perforated base could be another option (Becker et al., 2020).

With both species of cattails being potentially invasive in Wisconsin, the best long-term control of C. perturbans would be the excessive removal off cattail growth (Johnson & Cuda, 2017). With common reed being an invasive species in Wisconsin, control should be taken to not only control mosquito populations but common reed as well (WI DNR). It is important to note that some species of arrowheads and sedges are important ecologically, so make sure to identify aquatic plant species before doing any removal or contact a local university extension agent to assist with identification (Black & Judziewicz, 2009). With any management within wetlands, contact the Wisconsin Department of Natural Resources to learn more about possible restrictions or permits required before implementing control practices.

Education

Education is an essential tool that should be implemented with any IPM/IVM program. If the mosquito species are known, educating the public about what they can do in their community to reduce mosquito populations can be a long-term control tactic. Education can be conducted by local municipality, agency, or University of Wisconsin-Madison Division of Extension. Education materials can be in the form of binders, pamphlets, and mailings. Meetings and webinars can be set up if an adequate amount of people within the community are interested in assisting.

Prevention and Protection

Both the public and professionals conducting the mosquito control must take additional steps to protect themselves from the bite of the female mosquito and the potential pathogens they vector (Dame & Fasulo, 2002). This can be done using personal protection or excluding mosquitoes from homes and facilities (Dame & Fasulo, 2002). Knowing the behaviors and breeding habitats of specific mosquito species can reduce your chances of being bitten (Dame & Fasulo, 2002).

In residential areas, installing appropriate screenings on windows, doors, porches, and other opens leading into the structure will exclude female mosquitoes from entering (Dame & Fasulo, 2002). Staying indoors during mosquito's peak flight activity and avoiding known mosquito habitats can reduce people's exposure to female mosquitoes (Dame & Fasulo, 2002). Although it is not always possible or practical to stay indoors or avoid mosquito habitats, personal protective gear can be used to decrease your chance of being bitten by a female (Dame & Fasulo, 2002). Wearing light color clothing, long-sleeved shirts, pants while applying repellents can help reduced the chances of being bitten. The most common repellent used is *N, N-diethyl-meta*-toluamide, also known as DEET. Always read instructions of any repellents prior to use (Dame & Fasulo, 2002).

Vaccinations and Treatments for Mosquito-Borne Pathogens

To prevent eastern equine encephalitis and West Nile virus in horses, keep them up to date on vaccinations with the initial vaccination followed by a booster in 4 to 6 weeks (EDCC, 2017). Dogs should be tested when they are seven months old before starting heartworm protection (FDA, 2019). Detecting heartworm in cats is more difficult than in dogs with two types of blood tests being used (FDA, 2019). Various FDA-Approved products are available in topical liquid form that is applied to the skin or oral tablets that prevent heartworm infection in both cats and dogs (FDA, 2019). Annual testing of all dogs on heartworm prevention is recommended (FDA, 2019). There are no vaccines for West Nile Virus available for humans (Johnson & Cuda, 2017). There is an experimental vaccine created for humans for eastern equine encephalitis (Mullen & Durden, 2018).

Horse Receiving Vaccination
Photo Credit: Steve Schank

Synthetic and Microbial Insecticides

Insecticides are any formulations or substances used to control insects (Tomasko & Nice, 2020). Not all insecticides kill the pest; some inhibit their growth, repel it, or reduce their ability to reproduce (Tomasko & Nice, 2020). Insecticides can vary in their selectivity (Tomasko & Nice, 2020). Insecticides that target several different kinds of insects are known as broad-spectrum, while insecticides that target only a few, usually related species without harming others, are known as narrow-spectrum (Tomasko & Nice, 2020). They can also be classified as organic or inorganic pesticides (Tomasko & Nice, 2020). Organic pesticides mean that the pesticide contains the element carbon (Tomasko & Nice, 2020). Most pesticides used today are derived from organic compounds (Tomasko & Nice, 2020). Example of organic pesticides include, organophosphate,

pyrethrin, and pyrethroids (Tomasko & Nice, 2020). Inorganic pesticides are generally derived from minerals that occur in nature (Tomasko & Nice, 2020). Common inorganic pesticides silica aerogels, boric acids, borates, diatomaceous earth, copper, and sulfur (Tomasko & Nice, 2020). Insecticides can also differ in modes of action or how they kill or harm an insect (Tomasko & Nice, 2020). Most insecticides focus on attacking the nervous system, but some focus on attacking the growth and development, metabolism, and midgut (IRAC, 2021).

Larvacides

For larval control, oils, insect growth regulators, and microbial insecticides are commonly used (Tomasko & Nice, 2020). Most mosquito species mentioned above need to obtain oxygen from the water surface (Tomasko & Nice, 2020). A variety of oils and surface films can be used to prevent mosquito larvae from getting oxygen from the water surface, or they can reduce the surface tension of the water, which causes the larvae to drown (Tomasko & Nice, 2020). This application will not work with *Coquillettidia perturbans* larvae, for they do not need to obtain oxygen from the water surface (Johnson & Cuda, 2017).

Insect Growth Regulators (IGR) is another group synthetic chemical that are used to control the larval stage (Tomasko & Nice, 2020). Insect growth regulators work by preventing insects' development by mimicking hormone activity (Tomasko & Nice, 2020). Growth regulators are similar to the natural juvenile hormone of the mosquito larvae (Tomasko & Nice, 2020). When growth regulators are applied to breeding sites and taken up by the mosquito larvae, the hormone prevents the larvae from developing into an adult (Tomasko & Nice, 2020). The mosquito larvae are not immediately killed, but their development is stopped, and the malformed changes that occur from exposure eventually kill the immature form (Tomasko & Nice, 2020). The common and widely available IGR is Methoprene, specific to mosquitoes and related flies (Tomasko & Nice, 2020).

Insect pathogens like bacteria have been developed into biological insecticides (Tomasko & Nice, 2020). A commonly used, commercially available bacteria that is used for mosquito control is *Bacillus thuringiensis israeliensis* (Bti) (Tomasko & Nice, 2020). *Bti* is applied to larvae's water breeding sites, and the larvae will take in the Bti spores when they feed (Tomasko & Nice, 2020). Within 1-4 hours of ingestion, the spores' crystals break down the gut wall, and within 2-12 hours, the spores infect the body cavity and kill the mosquito larvae (Tomako & Nice, 2020). One advantage of *Bti* is that it is selective for mosquitoes, black flies, and fungus gnats (Tomako & Nice, 2020). *Bti* is inactive in mammals due to the lack of having an alkaline gut, which is required for the solubilization of crystals (Yuen, 2020).

Another bacterium used for mosquito larvae control is *Bacillus sphaericus*, which has the benefit of reproduction (Tomasko & Nice, 2020). As it kills the mosquito larvae, it replicates itself and more spores spread through the water column (Tomasko & Nice, 2020). This gives *B. sphaericus* residual control, and there is no need to increase application rates in water that is high in organic matter (Tomask & Nice, 2020). *B. sphaericus* is effective against *Culex* species, but less effective against other mosquito species (Tomask & Nice, 2020).

Adulticides

During an outbreak of mosquito-borne diseases, local government departments and mosquito control districts will use insecticides targeting adults, known as adulticides, to knock down their populations (CDC, 2020). Adulticides are applied as

liquid applications that are commonly applied as ultra-low volume (ULV) or residual sprays (CDC, 2020). ULV applications turn the liquid into small droplets that float in the air and kill flying mosquitoes on contact (CDC, 2020). Residual sprays are sprayed on vegetation and buildings and are allowed to dry (CDC, 2020). This kind of application is used on sites where adult mosquitoes are likely to rest and be exposed to the insecticide (CDC, 2020). Residual applications are more often used by pest control professionals than local government departments and mosquito control districts (CDC, 2020). The main type of EPA-approved adulticides are organophosphates, natural pyrethrins, and synthetic pyrethroids (CDC, 2020).

Organophosphates contain the element phosphorus, with most being broad-spectrum and relatively non-residual insecticide (Tomasko & Nice, 2020). Organophosphates work by interfering with the passage of impulses in the mosquito nervous system (Yu, 2015). Commonly used organophosphates in controlling mosquitoes are malathion and naled (CDC, 2020).

Pyrethrins are collected from chrysanthemum flowers with mosquito control professionals using ULV formulation to kill adult mosquitoes (CDC, 2020). Like pyrethrins, synthetic pyrethroids are generally used in a ULV formulation, with common synthetic pyrethroids including permethrin, d-pheothrin, and deltamethrin (CDC, 2020). Both synthetic pyrethroids and natural pyrethrins kill mosquitoes by preventing their nervous system from functioning correctly (CDC, 2020).

When considering what kind of insecticides to use, targeting the most vulnerable stages will lead to higher success (Dame & Fasulo, 2002). In this case, the larval stage would be the most susceptible because it is generally immobile and concentrated into a small area, and minimal acreage needs to be covered compared to the adult stage that can rapidly disperse into large areas (Robertson, 2019; Dame & Fasulo, 2002).

If targeting adults, applications should be under plants, in dense brush or tall grasses, and on the underside of leaves on trees and bushes (CDC, 2016). Female adult mosquitoes rest under the eaves on buildings, under decks, porches, and in moist, shady areas (CDC, 2016). Avoid applying insecticides on blooming flowers or plants that bees, butterflies, and other pollinators visit (CDC, 2016). After application, keep people and pets out of the area you treat and follow the label instructions that determine when it is safe to return to the treated area (CDC, 2016). Appropriate signage bordering the application site can help prevent people from entering. Do not apply directly to fruits and vegetables in gardens or agricultural areas (CDC, 2016). Maintaining good public relations during the application of insecticides is critical. With that, well thought out public education program can go a long way in creating a good relationship with the public (Dame & Fasulo, 2002). Notifying the community that you are applying pesticides before a planned application can be crucial in discussing issues residents may have with their use (Dame & Fasul, 2002). Letters, pamphlets, meetings, and other forms of media can be used for educational purposes and discussion on concerns with pesticides (Dame & Fasulo, 2002).

Whenever using insecticides, appropriate personal protection equipment (PPE) should be worn (Tomasko & Nice, 2020). At a minimum, you should be wearing long sleeve shirts, long pants, shoes, socks, and all clothing should be dry and free of holes and tears (Tomasko & Nice, 2020). Gloves should be unlined, chemical-resistant gloves and never cotton, leather, or canvas gloves (Tomasko & Nice, 2020). Eyewear should be tightly

fitting, non-fogging protective eyewear when you are using pressurized equipment that has the chance to produce mist, dust, or splashes (Tomasko & Nice, 2020). The pesticide label should be read before use, and it will provide information on what is required for PPE (Tomasko & Nice, 2020).

Before using any pesticides, the appropriate training should be completed (Tomasko & Nice, 2020). In Wisconsin, anyone that will be using restricted-use pesticides (RUP) would be required to complete pesticide applicators training and exam (Tomasko & Nice, 2020). Even without using restricted-use pesticides, appropriate training should be done prior to use to familiarize yourself with equipment and the pesticide being considered for control. Additionally, understanding how to calibrate equipment, measuring the right amount of product for a given area, and knowing how to properly clean and store equipment is essential to prevent unnecessary health and environmental risk. The pesticide label is the law and should be followed (Tomasko & Nice, 2020). If you are uncertain about specific rules and regulations around the use of pesticides in Wisconsin, you can contact the Department of Agriculture, Trade and Consumer Protection at datcppesticideinfo@wi.gov or (608) 224-4548 (DATCP, 2021).

In addition to environmental and health concerns, resistance to insecticides should be accounted for in any mosquito IPM/IVM program. Resistance occurs with the overuse of pesticides to control a pest in addition to the use of a single mode of action instead of using multiple. Rotating different modes of insecticides' actions during an IPM/IVM program can delay mosquito larvae and adults' resistance. As mentioned previously, insecticide use should be a temporary practice to implement long-term control strategies to help preserve the efficacy of insecticides (Tomasko & Nice, 2020).

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