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Pyrethroid Insecticides in Urban Underground Storm Drain Systems: Occurrence, Distribution, and Potential Contribution to Mosquito Resistance

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ABSTRACT OF THE DISSERTATION

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by

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Pyrethroid insecticides frequently contaminate urban runoff and are transported to downstream waterways. Due to their high hydrophobicity, these insecticides have a propensity to partition from surface water into sediment, where they may be slow to degrade. Past studies have shown adverse impacts to aquatic ecosystems, where many fish and invertebrates are especially susceptible to pyrethroids. Apart from non-target toxicity, pyrethroid pollution may lead to larval mosquito (*Culex quinquefasciatus*) exposure. These insects can be nuisance pests and vectors for disease across the world. Urban species of mosquitoes often lay their eggs in underground storm drain systems due to their tendency to accumulate standing water and detritus. In this exposure scenario, selection pressure may be exerted on populations of larvae, increasing their resistance to pyrethroids. Pyrethroids are one of the few types of insecticides used for adult mosquito control in the United States, and a reduction of pesticide efficacy imposes costs on pest management operations. Though pyrethroid contamination of urban water bodies, like

streams, is well-documented, specific information on their occurrence within storm drain systems along with their influence on current mosquito populations is lacking. In the first study of this dissertation, the prevalence of pyrethroid residues in water from urban catch basins, which are storm drain structures that accept runoff, was determined. Results showed that pyrethroids were present in nearly every sample, with the pyrethroid bifenthrin being the most frequently detected analyte. In the second study, pyrethroid distribution across catch basins, open channels, and stormwater outfalls in a storm drain system was assessed. Pyrethroids were detected in every sediment, algae, and biofilm sample, and bifenthrin concentrations in catch basin water were notably elevated compared to those in other structures. In the third study, larval mosquitoes of strains established from field-collected populations were exposed to bifenthrin at environmentally relevant concentrations for multiple generations, and parameters associated with permethrin resistance in adults were measured. It was found that mosquito strains were already highly resistant to permethrin and that the treatment increased survival after exposure to permethrin for one of the strains, which may indicate that exposure effects may differ significantly by strain. The results of this dissertation research highlight the ubiquity of pyrethroids in urban storm drains and suggest that they may play a role in maintaining, or even encouraging the development of, pyrethroid resistance in mosquitoes.

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Chapter 1 Introduction

1.1 Control of Urban Mosquitoes in the United States

Mosquitoes are a top priority of global pest control due to the numerous diseases they can transmit to humans. Malaria alone caused over 400,000 deaths in 2019, and other mosquito-borne illnesses, including chikungunya, dengue, Japanese encephalitis, West Nile fever, and yellow fever, contribute to hundreds of thousands of deaths and hospitalizations annually (Lee et al., 2018). Advancements in technology have reduced the impacts of or even eradicated some of these diseases, but future factors like population growth, increased urbanization, globalization, and climate change are projected to increase the prevalence and severity of others (Ferguson, 2018). As a result, billions of dollars are invested into efforts such as limiting mosquito spread, preventing humans from being bitten, and developing new treatments for those infected. However, care must be taken at the same time so that current methods remain effective for the foreseeable future.

In the United States, West Nile virus (WNV) is the most common mosquito-transmitted pathogen, confirmed in 971 cases and 60 deaths in 2019 (Soto et al., 2022). WNV is predominantly spread by multiple species in the genus *Culex*, some of which are also vectors for St. Louis encephalitis, filariasis, and avian malaria (Farajollahi et al., 2011). The prominence of these species varies geographically; for example, *Cx. quinquefasciatus* Say is found in all south-bordering states, while *Cx. tarsalis* mostly resides west of the Mississippi River (Rochlin et al., 2019). There are numerous other

mosquitoes that harbor vector-borne diseases in the U.S., including *Aedes aegypti*, *Ae. Albopictus*, and *Anopheles freeborni*.

Apart from containing over 80% of the U.S. population, urban settings as an environment for mosquitoes are important to distinguish from the others due to multiple characteristics (Balk et al., 2018). Artificial containers, plentiful shade, and urbanized vegetation are known to facilitate mosquito breeding (Gleiser and Zalazar, 2010). Additionally, while mosquitoes mainly threaten public health, residential regions can have a dense, broad range of pests causing health, nuisance, and structural concerns. Treatments intended for a single pest may inadvertently have implications on others, including mosquitoes. This is particularly true for many of the modern insecticides that exhibit a broad spectrum of activity.

1.1.1 Pyrethroid Insecticides

Synthetic pyrethroids, structurally analogous to the pyrethrins in *Chrysanthemum* flowers, have been used in pest control for decades (Elliott, 1989). Vector control agencies employ pyrethroids such as permethrin, resmethrin, and sumithrin when targeting adult mosquitoes. Pyrethroids, along with pyrethrins and organophosphates, are one of the few classes of pesticides registered for adult mosquito control in the continental United States (McGregor and Connelly, 2021). Adult susceptibility to pyrethroids is vital for the efficacy of current mosquito management.

The mode of action for pyrethroids involves persistent opening of voltage-sensitive sodium channels (*Vssc*), causing paralysis (called “knockdown”) and eventual

death. They share multiple similarities in function with dichlorodiphenyltrichloroethane (DDT), including proposed receptor sites, though pyrethroids act on both the central and peripheral nervous system while DDT mainly acts on the latter (Davies et al., 2007; Zhorov and Dong, 2017). Mammals, whose sodium channels do not facilitate efficient binding of pyrethroids, are much less sensitive than insects. The relatively low mammalian toxicity from pyrethroids has contributed to their widespread use as insecticides.

Used in combination with other methods, the application of pyrethrins and pyrethroids is an effective tool for urban mosquito management. Ultra-low volume sprays are used to spread pyrethroids in aerosols that kill adult mosquitoes after contact. Larvae are also susceptible, but, in the U.S., they are primarily treated with biological or chemical larvicides that kill or inhibit growth (Floore, 2006). Extensive work is also put into monitoring the status of mosquito populations and their pathogens throughout the country, as this is an important component of integrative management for vector-borne diseases.

Due to their potency to a wide spectrum of insects and relatively low mammalian toxicity, pyrethroids are among the main insecticides used in agricultural and urban settings. For example, in California, large quantities of synthetic pyrethroids are used annually for pest control in residential areas, including outdoor use for ant and spider control along with indoor use for controlling ants, cockroaches, ticks, and fleas. According to the California Department of Pesticide Regulation Pesticide Use Reporting database, approximately 92,324 kg of permethrin, as an active ingredient in various

products, was applied in California in 2021. Of this, 45,034 kg was used for agriculture, and 47,290 kg was used non-agriculturally (primarily landscape maintenance and structural pest control). Most reported urban applications are performed by private operations for structural pest control or landscape maintenance (CDPR, 2023).

Many monitoring studies in California have shown the presence of pyrethroid residues in the water column and sediment of urban waterways, often at levels exceeding acute and chronic toxicity thresholds for sensitive aquatic species (Davis et al., 2007; Delgado-Moreno et al., 2011; Domagalski et al., 2010; Holmes et al., 2008; Sayeed et al., 2003; Siegler et al., 2015; Weston and Lydy, 2012). Holmes et al. (2008) analyzed 8 pyrethroids in sediments from urban waterways across California and found concentrations ranging from 3.78 to 333.6 ng/g (Holmes et al., 2008). In the same study, several creeks were found to contain concentrations of bifenthrin near a previously derived LC_{50} to *Hyallela azteca*, a freshwater amphipod frequently found in California (Amweg et al., 2005). Studies have further established that uses around resident homes for structural pest control are primary sources of pyrethroid contamination in urban streams (Jiang et al., 2012, 2011; Jiang and Gan, 2012; Richards et al., 2016; Weston et al., 2009, 2005; Weston and Lydy, 2010).

1.1.2 Pyrethroid Resistance in Mosquitoes

Resistance development is a major concern for mosquito management. Pyrethroid resistance has already been detected in many mosquito populations globally. Larval resistance to permethrin in *Culex pipiens* was observed as early as 1984 in Saudi Arabia.

In California, pyrethroid resistance was first documented in *Cx. pipiens* in 2001; since then, resistant populations have become widespread across the state (McAbee et al., 2004; Yoshimizu et al., 2020). Such observations have raised concerns that pyrethroids may become ineffective as they are currently used (Amin and Hemingway, 1989). However, this resistance is highly heterogeneous across regions, varying in level and underlying mechanisms (Scott et al., 2015; Smith et al., 2016).

Most research on mosquito pyrethroid resistance has focused on changes in the target-site (i.e., the sodium channel) or metabolism. Target-site mutations limit the sodium channel's sensitivity to pyrethroids, with the most well-known form being termed "knockdown resistance" (*kdr*). This phenotype, first observed in flies (*Musca domestica*) resistant to both DDT and pyrethrins, is most frequently associated in *Culex* spp. with a point mutation L1014F in the *Vssc* gene (Busvine, 1951; Scott et al., 2015). Another mutation at the same codon, L1014S, has been detected in multiple *Culex* strains worldwide, and many sodium channel mutations in other mosquitoes and insects have been linked to resistance. The in-depth mechanism by which *kdr* and similar genotypes affect pyrethroid effectiveness has yet to be determined (Rinkevich et al., 2013).

Metabolic changes in mosquito populations can alter the function or increase the expression of certain enzymes that may inhibit pyrethroids. Cytochrome P450 monooxygenases can metabolize pyrethroids in a variety of ways, reducing concentrations and facilitating elimination from the body. For example, permethrin can be transformed into products such as 4'-hydroxypermethrin, 3-phenoxy-benzoic acid, and 3-phenoxy-benzoic alcohol (Kasai et al., 1998). The specific P450(s) that are responsible

depend on the strain of mosquito and the pyrethroid(s) rendered ineffective. Zou et al. (2019) recently identified a cluster of *CYP6* genes that were more expressed in deltamethrin-resistant *Culex pipiens pallen*s compared to a susceptible strain (Zou et al., 2019). Many pesticide products include piperonyl butoxide (PBO), a P450-inhibitor, to reduce the ability of pests to metabolize the active ingredient. Other enzymes in mosquitoes that may be important include carboxylesterases and glutathione-*S*-transferases (known to be relevant for certain insecticides), as some resistant strains have shown elevated expression. However, any direct interaction of these enzymes with pyrethroids in mosquitoes has yet to be verified (Scott et al., 2015; Smith et al., 2016).

There are also other areas of research on mosquito resistance. While not coding for enzymes, G protein-coupled receptor genes are implicated since they regulate P450 gene expression and are overexpressed in some resistant strains (Liu et al., 2007). Resistance in mosquitoes may also come in other forms; changes in the cuticle may reduce absorption and/or accumulation of insecticides, whereas changes in behavior may lead to mosquitoes avoiding contact with treated areas (Chareonviriyaphap et al., 1997; Noh et al., 2015; Sun et al., 2017).

Though some mosquito strains may be more naturally resistant to pyrethroids, selection pressure can encourage the development of higher levels of resistance in the environment. This can be brought about by faults in the application regimen; while the desired result may be complete eradication of a population, any surviving mosquitoes may be resistant and continue to proliferate with limited competition. Selection can also

be caused by exposure to insecticides reaching unintended areas or remaining longer than expected.

1.2 Unintended Pyrethroid Exposure in Urban Environments

1.2.1 Environmental Behavior of Pyrethroids

Synthetic pyrethroids share similar physicochemical properties in that they are strongly hydrophobic (log K_{ow} ranging from 4.5 to 7.0) and poorly water soluble (Katagi, 2012; Laskowski, 2002). Consequently, pyrethroids favor partitioning into the organic carbon component of soil and sediment phases as compared to water. While sorption to sediment can reduce their bioavailability to aquatic organisms living in the water column, exposure to sediment-dwelling invertebrates and other fauna may occur as a result.

In the environment, pyrethroids can be transformed in multiple ways. Abiotically, pyrethroids are susceptible to degradation via hydrolysis and photolysis, but factors like pH and sorption to organic matter can significantly affect these processes. Biotically, microbes are capable of mediating degradation of pyrethroids. However, most synthetic pyrethroids are relatively persistent in the environment (Gan et al., 2005; Katagi, 2012; Meyer et al., 2013; Qin et al., 2006). For example, Meyer et al. (2013) studied laboratory degradation rates of pyrethroids in sediments from California, and pyrethroid half-lives at 20 °C were found to range from 53 to 630 days (Meyer et al., 2013).

1.2.2 Potential Sources of Exposure

While pyrethroids may be present in different compartments of urban features, some are more relevant than others when considering mosquito exposure. Adult mosquitoes may come in contact with low doses in the plants they feed on and in the air (Foster, 1995). Mosquito breeding sources warrant special attention, as larvae and pupae are aquatic and may be exposed to any contaminants in these habitats. Therefore, mosquitoes may be exposed to pyrethroid residues present in breeding sources, continuously selecting for more resistant mosquitoes over multiple generations.

Many urban structures can serve as breeding sites for mosquitoes, as the primary requirement for mosquito breeding is a sufficient depth of standing water. Uncovered containers, tires, and planters are often neglected and collect water after precipitation or irrigation (Wilke et al., 2019). Certain urban vegetation may also collect water and serve as a breeding source. For example, ornamental bromeliads are known to harbor *Ae. aegypti* in parts of the U.S., such as Los Angeles County, CA, and Miami-Dade County, FL (Wilke et al., 2018). Some building features, like gutters, can pool water and act as mosquito habitats.

Urban underground storm drain systems (USDS), designed to transport and discharge runoff water, are often a target for larviciding efforts, as they are a common habitat for *Culex* and other mosquitoes (Justin E Harbison et al., 2014). In the U.S., these are known as municipal separate storm sewer systems (MS4s), and regulated MS4 area represents 4% of U.S. land area along with over 80% of its population (“Stormwater Discharges from Municipal Sources,” 2020). In larger cities, these systems may consist

of thousands of miles of horizontal drain channels and thousands of catch basins and manhole chambers. For example, the MS4 associated with the Los Angeles County Flood Control District includes "approximately 500 miles of open channel, 3,500 miles of underground drains, and an estimated 88,000 catch basins" (Kluh et al., 2001). With standing water and moist, cool conditions (relative to aboveground), USDSs are considered the main breeding area of WNV-spreading mosquitoes such as *Culex quinquefasciatus* Say (Kluh et al., 2006).

1.2.3 Pyrethroids in USDSs

Previous studies have shown ubiquitous occurrence of urban-use insecticides at urban storm drainage outfalls, suggesting prevalent contamination of urban runoff passing through USDSs (Amweg et al., 2006; Carpenter et al., 2016; Gorgoglione et al., 2018; Holmes et al., 2008; Weston et al., 2009, 2005). Weston et al. (2009) analyzed 7 pyrethroids in storm drain outfalls around Sacramento, CA, and found total pyrethroid concentrations in the range of 31 to 154.8 ng/L in drain discharge (unfiltered), 1149 to 3393 ng/g in suspended sediments, 391.1 to 1649.6 ng/g in sediment from drain mouths, and 11.3 to 30.9 ng/g in streambed sediment downstream from the outfalls (Weston et al., 2009). Mosquitoes may also breed downstream from outfalls, as some areas are constructed to attenuate incoming discharge and may hold water (Metzger et al., 2011).

Pyrethroids may be introduced to USDSs in multiple ways. For example, perimeter spraying is a common practice for applying insecticides to control ants and spiders around homes in California. Following application, pesticide residues from these

surfaces can be mobilized via surface runoff induced by rainfall or irrigation events. Lawns and gardens, containing soil and vegetation, can act as sinks for pyrethroids. Impervious surfaces, like concrete pavement, can effectively retain pyrethroids and release trace levels of pyrethroid residues over an extended period (Jiang et al., 2012, 2011, 2010; Jiang and Gan, 2012; Jorgenson and Young, 2010; Trask et al., 2014). Pesticide residues in bare soil or vegetated areas may also be transported with wind or traffic (Richards et al., 2016). As a result, pyrethroids may be distributed throughout different compartments in the urban environment, which contributes to diffused and prolonged contamination to USDSs, including catch basins that may serve as mosquito breeding sites.

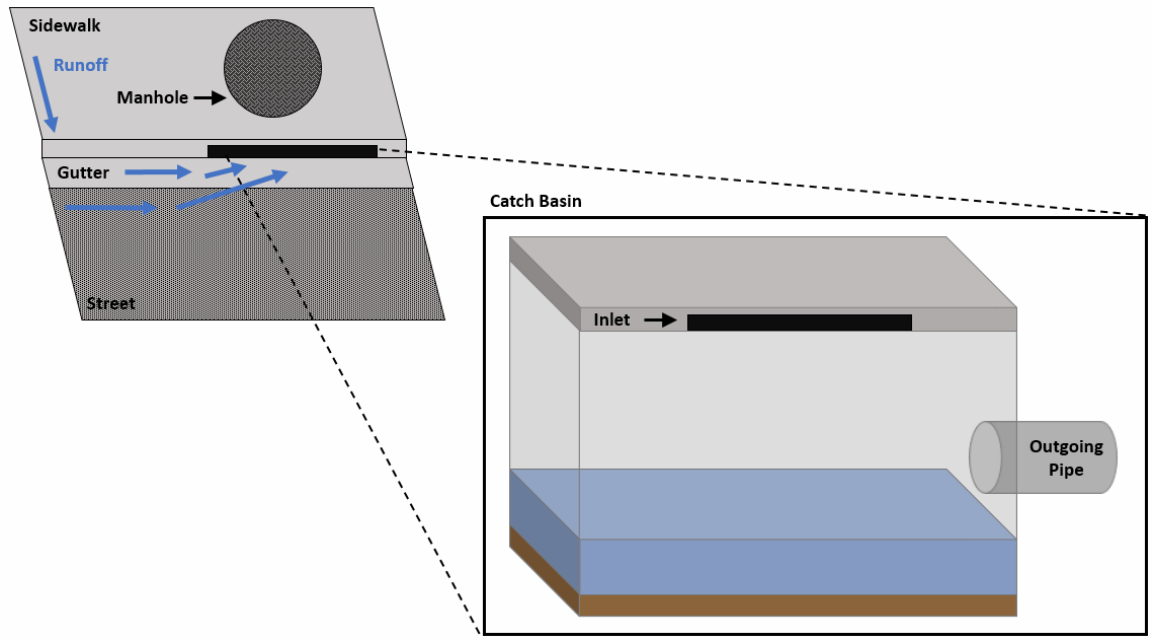

Figure 1.1 Diagram of a roadside catch basin accepting surface runoff through a road-facing inlet (if there is a sump, runoff may accumulate before flowing out through a pipe)

Generally located on the roadside, catch basins consist of a circular or rectangular sump that receives surface debris and runoff, which may be transferred out of the catch basin after reaching a certain level (Figure 1.1). To prevent clogging by large debris, their inlets are often kept small or covered by a grate. Basin dimensions and placements vary across municipalities. For example, the urban catch basins used in a larviciding study in Stratford, CT, measured 82.3 cm × 100.6 cm and had reservoir depths ranging from 2.5 cm to 30.5 cm (Anderson et al., 2011). Two studies of *Cx.* spp. productivity in different residential sites in Chicago, IL, found densities of approximately 500 basins/km^2 and 288 basins/km^2 (Hamer et al., 2011; Harbison et al., 2014).

Catch basins can often provide suitable conditions for mosquitoes to breed. Since catch basins may hold their contents for a prolonged period, they may provide cues for gravid mosquitoes looking to lay eggs and provide water, shade, and plentiful food for larvae as they develop. Environmental factors which may increase larval productivity in catch basins include higher ambient temperature, lower precipitation, higher nutrient abundance, and the presence of certain vegetation (Arana-Guardia et al., 2014; Gardner et al., 2013, 2012; Harbison et al., 2017; Murrell et al., 2011).

As acceptors of urban runoff, urban USDSs, including their catch basins, warrant further research as places where pyrethroids can accumulate and select for resistant mosquitoes. Larvicides, designed to remain for long periods of time, are applied to these structures, but their use is not universal and may not be as effective as intended. To date, there has not been direct research on the presence and distribution of urban-use insecticides such as pyrethroids in stormwater catch basins. Moreover, there has not been

any study attempting to understand this potential exposure setting, including how conditions in USDSs may result in mosquitoes being exposed to pyrethroids and selected for resistance.

1.3 Knowledge Gaps

1.3.1 Pyrethroid Residues in Catch Basins

In previous studies, pesticide residues in various urban compartments, including around residential homes, paved surfaces, and urban streams, have been evaluated (Jiang et al., 2016; Richards et al., 2016; Weston et al., 2009). Other previous work has considered water quality, mosquito ecology, and larvicide efficacy in catch basins (Alam et al., 2018; Gardner et al., 2013; Harbison et al., 2014). However, there is little information on the occurrence of pyrethroid residues in USDSs, including catch basins that are prevalent throughout most urban areas. Spatial and temporal data on pyrethroid residues in catch basin media, such as water and suspended solids, would improve our understanding of what is retained in catch basins and whether larval mosquitoes in basins may be ambiently exposed to pyrethroids. Pyrethroid contamination in USDSs also has risk implications for downstream aquatic ecosystems receiving drainage water.

1.3.2 Pyrethroid Distribution in Larval Food Sources

Various urban environmental media may serve as food sources for larval mosquitoes, including organic matter-rich detritus, biofilms, and algae, among other items. These food sources may be present within stormwater catch basins and/or around

stormwater drainage outfalls. Residues of pyrethroids are expected to adsorb to such organic matter-rich matrices due to their strong hydrophobicity. Monitoring of pyrethroid residues in the potential food sources for mosquitoes in and around catch basins under field conditions will further establish whether they may act as a conduit to promote insecticide resistance development in urban-breeding mosquitoes.

1.3.3 Potential Contribution of USDSs to Pyrethroid Selection Pressure

The frequency of mosquito pyrethroid resistance in areas such as California has increased in recent years. (Yoshimizu et al., 2020) Previous work has also shown that the level of pyrethroid resistance in mosquitoes may be correlated with selection pressure (Machani et al., 2020; Nazni et al., 1998; Sarkar et al., 2009; Shi et al., 2015). However, these selection studies used increasing concentrations with each generation of mosquito or focused on exposure and resistance in a single stage. To date there has been minimal research on the effects of larval exposure at environmental concentrations on resistance levels in adult mosquitoes. Using environmentally derived concentrations may more closely reflect natural selection pressures taking place from pyrethroid exposure in mosquito breeding habitats, such as those in a USDS.

1.4 Objectives

The overall objective of this interdisciplinary project was to assess pyrethroid contamination in storm drains and the potential to contribute to pyrethroid resistance in adult mosquitoes. The specific objectives were to:

1) Determine the occurrence of pyrethroid residues in catch basins;

2) Understand the distribution of residues along the storm drain flow path; and

3) Evaluate the effects of larval exposure to environmentally relevant concentrations of pyrethroids on pyrethroid resistance in adults.

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Chapter 2 Influence Pyrethroid insecticides in urban catch basins: A potential secondary contamination source for urban aquatic systems

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2.1 Introduction

Pesticides are man-made chemicals with biological activity. Off-site transport commonly occurs due to widespread use and various environmental processes, leading to contamination in sensitive ecosystems. Compared to agricultural fields, relatively little is known about the urban fate and movement of pesticides, although a wide range of pesticides are routinely used for pest management of structures and landscapes. Pyrethroids, one of the most applied types of insecticides in urban settings, are used broadly for managing nuisance pests and vectors such as ants, spiders, cockroaches, and mosquitoes. In California alone, reported use of seven pyrethroids for structural pest control and landscape maintenance was over 70,000 kg (as active ingredient) in 2017 (Budd et al., 2020; "California pesticide information portal application," 2022). This was likely a significant underestimate, as products containing pyrethroids are readily available to homeowners through retail stores and online sites.

Urban areas differ characteristically from agricultural fields in that there is a prevalence of impervious surfaces on which pesticide residues may accumulate (Jiang et al., 2016; Richards et al., 2016). Surface runoff water after rainfall or irrigation events moves swiftly over impervious surfaces and may mobilize the deposited residues. For example, a study of simulated runoff from concrete slabs showed detectable levels of pyrethroids in runoff water 112 d after the treatment (Jiang et al., 2010). Studies over recent years has implicated surface runoff for contamination of pyrethroids and other pesticides in urban water bodies (Weston et al., 2009). Many studies have shown occurrence of pyrethroids in creeks and rivers in urban watersheds, with concentrations in the water column or bed sediment frequently exceeding toxicity benchmarks for aquatic invertebrates (Ensminger et al., 2013; Weston et al., 2009; Weston and Lydy, 2012). More recently, Budd et al. (2020) reported that 78% of surface water samples from urban watersheds across 8 California counties contained one or more pyrethroids, with bifenthrin being the predominant compound. Pyrethroid contamination has also been reported for urban sites in other states, including Texas and Illinois, and other countries, including China, Vietnam, and Australia (Allinson et al., 2015; Ding et al., 2010; Duong et al., 2014; Hintzen et al., 2009; Kuivila et al., 2012; Tang et al., 2018).

Pyrethroid exposure poses a risk to many non-target species, such as crustaceans, aquatic insects, amphibians, and fish, and their habitats may suffer a loss of biodiversity and ecosystem functions (Haya, 1989; Siegfried, 1993; Vanzetto et al., 2019). For example, a study of urban streams and associated outfalls in Oregon, U.S.A., showed the presence of over 30 pesticides, of which bifenthrin was found at the highest levels in the

sediment and the contamination was associated with impairment of invertebrate communities (Carpenter et al., 2016). The strong hydrophobic nature of pyrethroids results in their accumulation in sediment, where pyrethroids may exhibit a prolonged persistence due to slow degradation, especially under anaerobic conditions (Gan et al., 2005; Laskowski, 2002; Meyer et al., 2013).

Before entering surface waters, urban runoff drainage generally passes through an underground storm drain system (USDS). These systems, designed to limit flooding and stormwater pollution, consist of underground catch basins, pipes, and channels. The USDS can be extremely extensive in highly urbanized areas. For example, the USDS of the City of Los Angeles, CA, comprises of over 38,000 catch basins, 2,414 km of pipes, and 161 km of open channels, with estimated annual dry weather and wet weather runoffs of 189,250 m^3 and 38 million m^3, respectively (Sadeghi et al., 2017). On a broader scale, Los Angeles County has over 70,000 catch basins, 7,400 km of sewer pipes, and 1,340 km of surface channels (Kwan et al., 2010; Porse, 2018). Catch basins, usually located curbside, accept runoff from the paved surface. Most catch basins contain a sump that can temporarily hold water and debris and a connecting pipe allowing water to enter the USDS. Catch basins are usually concrete-lined and rectangular in shape with a depth of up to several meters (Anderson et al., 2011; Harbison et al., 2018). There have been a few studies on stormwater structures concerning nutrients, such as nitrate and phosphorus, and conventional contaminants, such as PAHs and metals (Azah et al., 2017; Eriksson et al., 2007; Lundy et al., 2012). Modeling studies have also considered sediment capture dynamics and contaminant removal (Alam et al., 2017, 2018b; Jang et al., 2010; Morgan

et al., 2005; Yang et al., 2018). Despite the magnitude of urban USDS systems and their significance in transporting urban runoff, there is a dearth of information on contaminant (including pesticides) movement and fate through these underground systems before discharge into the open environment.

The current study evaluated the occurrence of the commonly used pyrethroid insecticides in urban catch basins to improve understanding of the role of USDSs in the overall contamination of urban watersheds. Their large number and broad distribution, along with their unique environmental settings (e.g., underground, dark, lower temperature) imply that catch basins may significantly affect contaminant movement and distribution by first intercepting and then serving a secondary source for contaminant emission. Insights into pyrethroid environmental behavior may also be useful for predicting that of other strongly hydrophobic compounds in the urban environment.

2.2 Materials and Methods

2.2.1 Chemicals and Materials

Analytical standards of pyrethroids were obtained from various pesticide manufacturers. Tefluthrin (94.0% purity) and lambda-cyhalothrin (89.0%) were obtained from Syngenta Crop Protection (Greensboro, NC). Bifenthrin (98.4%), permethrin (98.5%), and cypermethrin (98.5%) were obtained from FMC Corporation (Philadelphia, PA). Fenpropathrin (99.7%) and esfenvalerate (99.9%) were obtained from Valent (Dublin, CA). Cyfluthrin (86.4%) and deltamethrin (99.4%) were obtained from Bayer

Crop Science (Research Triangle Park, NC). Physicochemical properties for select pyrethroids are listed in Table 2.1. Deuterated bifenthrin-d_5 used to determine surrogate recovery was purchased from MilliporeSigma (St. Louis, MO).

Table 2.1 Physicochemical properties of selected pyrethroid analytes. Log Kow, water solubility, and Koc are from Katagi, (2012).

Pyrethroid	**Molecular weight**	**log K_{ow}**	**Water solubility[a] (ppm)**	**K_{oc} x 10^4 (L/kg)**
Bifenthrin	422.9	8.15	0.100	23.30
Fenpropathrin	349.4	6.00	0.330	4.23
Permethrin	391.3	6.50	0.006	3.24
Cypermethrin	416.3	6.05	0.004	4.57
Esfenvalerate	419.9	6.20	0.024	5.60

[a] Water solubility at 25 ˚C.

Dippers and glass bottles for water sample collection were purchased from John W. Hock Company and J.G. Finneran Associates, respectively (Gainesville, FL; Vineland, NJ). pH buffer solutions for calibration were purchased from Thermo Fisher Scientific (Waltham, MA). Total dissolved solid standards were purchased from Oakton Instruments (Vernon Hills, IL). Glass fiber filters were purchased from Advantec MFS (Dublin, CA). Sodium sulfate, sodium chloride, and GC/MS grade solvents (dichloromethane, acetone, and hexane) were purchased from Thermo Fisher Scientific (Waltham, MA). Florisil was purchased from Spectrum Chemical (New Brunswick, NJ). Before use, Florisil was activated via baking at 130 °C for 4 h, and sodium sulfate was dehydrated via baking at 400 °C for 4 h. All glassware used for sample processing was baked at 400 °C for 4 h beforehand to prevent cross contamination.

2.2.2 Site Selection and Sampling

Much of California, including the regions where catch basins were sampled, falls under a Mediterranean climate, characterized by dry summers and infrequent storm events during the winter (Polade et al., 2017). The majority of the state's rainfall occurs from November through March, with very little rainfall observed May through September (Fierro, 2014). Thus, it is assumed that water in urban catch basins over the summer is predominantly, if not exclusively, from irrigation runoff.

Figure 2.1 Counties in California where catch basins were sampled. Red dots represent areas where catch basins were sampled.

The catch basins were selected from locations in 5 northern California counties (Sacramento, Yolo, Alameda, Madera, and Tulare) and 3 southern California counties (Los Angeles, Orange, and San Bernardino), therefore representing a large portion of the urbanized areas in California (Figure 2.1). Site selection and sampling was performed in collaboration with regional agencies, including Sacramento-Yolo Mosquito and Vector Control District (MVCD), Alameda County Mosquito Abatement District, Madera County MVCD, Delta MVCD, Greater Los Angeles County Vector Control District, Orange County MVCD, and West Valley MVCD. Vector control agencies in California regularly access catch basins for surveillance and management of mosquitoes. Sampling sites consisted of 8-13 urban catch basins in each sampling region (see Table 2.2 for site details). When possible, additional catch basins were sampled to make up for any sites that were dry at the time of sample collection.

Table 2.2 Catch basin locations and sampling instance.

County	**Number**	**Latitude**	**Longitude**	**Sampling Month (in 2020)**		
				July	**Aug**	**Sept**
Sacramento / Yolo	1	38.47848	-121.52909	x	x	x
	2	38.47936	-121.53275	x	x	x
	3	38.48352	-121.15282	x	x	x
	4	38.54584	-121.67953	x	x	x
	5	38.54782	-121.68006	x	x	x
	6	38.54651	-121.68764	x	x	x
	7	38.71343	-121.28495	x	x	x

	8	38.71335	-121.28352	x	x	x
	9	38.71345	-121.28415	x	x	x
	10	38.54449	-121.50356	x	x	x
	11	38.54336	-121.50379	x	x	
	12	38.54123	-121.50461	x	x	x
	13	38.54142	-121.50355			x
Alameda	1	37.66382	-122.09674	x	x	x
	2	37.63895	-122.10771			x
	3	37.64828	-122.10483	x	x	
	4	37.66943	-122.14267	x	x	x
	5	37.67010	-122.14921	x	x	x
	6	37.67181	-122.14854	x	x	x
	7	37.67301	-122.14991	x		
	8	37.66480	-122.14087	x	x	x
	9	37.66360	-122.13947	x		
	10	37.66512	-122.13517	x	x	
	11	37.67444	-122.14708		x	x
	12	37.66690	-122.13976		x	x
	13	37.68201	-122.13582			x
Madera	1	36.96214	-120.09311	x	x	x
	2	36.95611	-120.09116	x	x	x
	3	36.95462	-120.08099	x	x	x
	4	36.95825	-120.07770	x	x	
	5	36.96256	-120.06783	x	x	
	6	36.95783	-120.06582	x	x	x
	7	36.95376	-120.06165	x	x	x
	8	36.94924	-120.05827	x	x	x
	9	36.96002	-120.05220	x	x	x

	10	36.96709	-120.05418	x	x	x
	11	36.97723	-120.05379	x	x	x
	12	36.97912	-120.05381	x	x	x
Tulare	1	36.32468	-119.31156	x	x	x
	2	36.32569	-119.30994	x	x	x
	3	36.32555	-119.30799	x	x	x
	4	36.32389	-119.30675	x	x	x
	5	36.32199	-119.30659	x	x	x
	6	36.32100	-119.32247	x	x	x
	7	36.32187	-119.30994	x	x	x
	8	36.32288	-119.30673	x		
	9	36.32326	-119.30423	x	x	
	10	36.32551	-119.30218	x	x	x
	11	36.32384	-119.30791		x	x
Los Angeles	1	33.83836	-118.05840	x	x	x
	2	33.86828	-118.05754	x	x	x
	3	33.85544	-118.07288	x	x	x
	4	33.85738	-118.07460	x	x	x
	5	33.85704	-118.08742	x	x	x
	6	33.86462	-118.08646	x	x	x
	7	33.87008	-118.09925	x	x	x
	8	33.87695	-118.06540	x	x	x
Orange	1	33.93627	-117.97171	x	x	x
	2	33.91039	-117.89093	x	x	x
	3	33.87378	-117.95073	x	x	x
	4	33.80853	-118.04003	x	x	x
	5	33.79976	-118.06912	x	x	x
	6	33.78390	-117.88877	x	x	x

	7	33.75962	-117.97196	x	x	x
	8	33.75242	-117.80447	x	x	x
	9	33.74494	-118.10976	x	x	x
	10	33.68422	-117.95199	x	x	x
	11	33.68119	-117.89147	x	x	x
	12	33.58577	-117.63709	x	x	x
San Bernardino	1	34.02662	-117.61525	x	x	x
	2	34.02697	-117.61525	x	x	x
	3	34.02594	-117.63283	x	x	x
	4	34.03911	-117.66348	x	x	
	5	34.04109	-117.66344	x	x	x
	6	34.04109	-117.66332	x		
	7	34.04087	-117.66345		x	
	8	34.04104	-117.66328		x	
	9	34.03385	-117.66337			x
	10	34.04903	-117.66994			x

Each catch basin with water was sampled once a month from July to September 2020. Telescoping dippers with coated stainless-steel cups were used to transfer approximately 500 mL of water into 16 oz amber glass bottles. Care was taken to collect from near the surface of the water to avoid disturbing bottom sediment or debris. Samples were immediately chilled and transported to the University of California, Riverside. Upon receipt at the laboratory, samples were stored at 4 °C in the dark prior to processing and analysis.

2.2.3 Sample Preparation

A Fisherbrand AE150 pH meter was used to measure sample pH before filtration (Waltham, MA). To separate suspended solids, each whole water sample was vacuum filtered through a 0.4 μm pore size glass fiber membrane (Advantec MFS, Dublin, CA). After filtration, a 20 mL aliquot of water was taken to measure total dissolved solids (TDS) and dissolved organic carbon (DOC) with a YSI EcoSense EC30A conductivity pen (Yellow Springs, OH) and a Shimadzu TOC-V analyzer, respectively (Columbia, MD). Filters with suspended solids were covered with aluminum foil and stored in a refrigerator at 4 °C until extraction.

After weighing, water samples were extracted using liquid-liquid extraction. Briefly, in a 2 L glass separatory funnel, 30 g of sodium chloride was added to the 500 mL water sample and then shaken until dissolved. Bifenthrin-d_5 (100 ng in 100 μl acetone) was added to each sample as a recovery surrogate. For extraction, 60 mL dichloromethane was added to each sample, and then the separatory funnel was mixed vigorously by hand for 3 min and left to rest for 1 h. After phase separation, the solvent extract was drained into a round-bottom glass flask through a glass funnel packed with anhydrous sodium sulfate (30 g) to remove residual water. This extraction was repeated for a total of three consecutive times, and the extracts were combined for each sample. The combined extract was then condensed on a Buchi Rotovapor (New Castle, DE) at 40 °C and under vacuum to approximately 5 mL. The sample container was rinsed with 9:1 hexane:acetone (v/v) and the extract was transferred to a glass vial. The final sample was evaporated to near dryness at 40 °C under a gentle stream of nitrogen and then

reconstituted in 1.0 mL hexane for analysis via gas chromatography/mass spectrometry (GC/MS).

To extract pyrethroids from the suspended solids retained on the filter paper, the sample filter membranes were first dried in an oven at 105 °C for 24 h. Total suspended solids (TSS) were measured gravimetrically by weighing the dried filters. Afterwards, samples were placed in a 40 mL glass vial and spiked with bifenthrin-d_5 (100 ng in 100 µl acetone). For extraction, 10 mL of 1:1 hexane:acetone (v/v) was added to each sample, and then the samples were sonicated for 30 minutes in a sonication water bath. For cleanup, the extract was loaded onto a 1.0 g Florisil cartridge preconditioned with 6 mL hexane, and the cartridge was eluted with 10 mL of 9:1 hexane:acetone (v/v). The cleaned extract was evaporated to near dryness at 40 °C under a gentle stream of nitrogen and then reconstituted in 1.0 mL hexane for GC/MS analysis.

2.2.4 Chemical Analysis and Quality Control

Sample extracts were analyzed for tefluthrin, bifenthrin, fenpropathrin, lambda-cyhalothrin, permethrin, cyfluthrin, cypermethrin, esfenvalerate, and deltamethrin on an Agilent 6890N/5973B GC/MSD operated in electron ionization mode with a 30 m × 0.25 mm × 0.25 µm DB-5MS column. The GC oven temperature program was as follows: initial temperature 50 °C held for 1 min; heated to 220 °C at 30 °C/min; heated to 300 °C at 10 °C/min and held for 2 min; and post run hold at 310 °C for 5 min. Injection volume was 1.0 µL and carrier gas (helium) flow rate was set to 1.0 mL/min. Temperatures for the injector, transfer line, MS source, and MS quadrupole were 250 °C, 280 °C, 230 °C,

and 150 °C, respectively. A solvent delay of 6 min was set for protection of the MS filament. Quantitation was performed with an 8-point calibration curve of concentrations from 1 to 500 μg/L.

For quality control and assurance, a calibration standard was run for every 10 samples to check for potential instrumental drift. Relative standard deviations were found to range from 6% to 14%. Bifenthrin-d_5 was added to each sample as a surrogate to measure recovery. The average recovery was 99 ± 18% and ranged from 72% to 127%. Method extraction efficiency was determined from the analysis of triplicate samples of water or sediment spiked with the target analytes. Method blanks simultaneously extracted with samples showed negligible matrix effects. Reagent blanks, analyzed after every 10 samples, showed no presence of the target analytes.

Method detection limit were determined following EPA method 40 CFR, Part 136, Appendix B. Reporting limits were determined using the calibration curve concentrations closest to 3 times the MDLs. Detailed analyte MS parameters, reporting limits, and method extraction efficiencies are given in Table 2.3. Detection frequencies were calculated using the number of samples in which residues were found above the reporting limit in either the aqueous phase or suspended solids. Non-detects and concentrations below the reporting limit were considered 0 when calculating values from all sample data. All concentrations in suspended solids were calculated and used in other calculations on a dry weight basis. The partitioning coefficient K_d was calculated using the formula C_s/C_w, where C_s and C_w are the concentrations in the solid and aqueous phases, respectively. Using quantified pyrethroid concentrations and other environmental

data, linear regressions were performed to determine whether variables, including pyrethroid concentrations, pyrethroid partitioning across media, TSS, and DOC, were correlated with one another.

Table 2.3 MS parameters, reporting limits, and method extraction efficiencies for each pyrethroid analyte. MS ions used for quantification are bolded; other ions were used for qualification.

Pyrethroid	**MS target ions (m/z)**	**Retention time (min)**	**Water reporting limit (ng/L)**	**Suspended solids reporting limit[a] (ng/g)**	**Extraction efficiency (%)**
Bifenthrin-d_5[b]	**186**, 170	16.0	4.0	1.0	96 ± 23
Tefluthrin	**177**, 197, 199	12.3	2.0	1.0	113 ± 15
Bifenthrin	**181**, 165	16.1	2.0	1.0	91 ± 14
Fenpropathrin	**181**, 152, 125	16.6	4.0	1.0	82 ± 12
Lambda-cyhalothrin	**181**, 197, 152	18.1	10.0	1.0	106 ± 16
Permethrin	**183**, 153	19.8	4.0	1.0	104 ± 21
Cyfluthrin	**163**, 127	20.8	10.0	1.0	97 ± 18
Cypermethrin	**163**, 181, 127	21.5	10.0	1.0	84 ± 15
Esfenvalerate	**167**, 152, 125	23.2	10.0	1.0	85 ± 21
Deltamethrin	**181**, 253, 152	24.2	20.0	1.0	79 ± 20

[a] Derived from matrix spike tests using sediment.
[b] Used as a recovery surrogate.

2.3 Results and Discussion

2.3.1 Detection Frequencies and Concentrations

A total of 79 urban catch basins in California were sampled from July to September 2020, months during which runoff would have been driven by irrigation only. Of these basins, 58 were sampled for all 3 months, showing consistent retention of water. The results showed that 98% of catch basin water samples contained at least one pyrethroid compound at a concentration above the reporting limit (Table 2.4). Bifenthrin consistently exhibited the highest frequency of detection, being detected in 97% of the samples. In previous studies on dust around residential homes, urban runoff, and urban surface waters receiving urban drainage in California, bifenthrin was often found at the highest frequency among urban-use pesticides (Budd et al., 2020; Carpenter et al., 2016; Ensminger et al., 2013; Holmes et al., 2008; Jiang et al., 2016; Richards et al., 2016). This may be attributed to the widespread use of bifenthrin-containing products in urban environments and also its relatively long persistence in the environment (Gan et al., 2005; Meyer et al., 2013). According to the California Department of Pesticide Regulation's (CDPR's) pesticide use databases, bifenthrin was the most applied pyrethroid for non-agricultural landscape maintenance and structural pest control in many regions in the state ("California pesticide information portal application," 2022).

Table 2.4 Detection frequencies of pyrethroids in catch basins.

County	Period	n	Detection Frequency[a] (%)							
			Bifenthrin	Lambda-cyhalothrin	Permethrin	Cyfluthrin	Cypermethrin	Esfenvalerate	Deltamethrin	Any Pyrethroid
Sacramento / Yolo	July 2020	12	100	58	0	67	0	0	0	100
	August 2020	12	100	17	67	17	0	0	0	100
	September 2020	12	100	0	17	17	0	0	0	100
Alameda	July 2020	9	100	67	0	0	0	0	0	100
	August 2020	9	100	22	0	0	0	0	0	100
	September 2020	9	100	0	22	22	11	0	0	100
Madera	July 2020	12	100	83	0	0	0	0	0	100
	August 2020	12	83	33	0	42	0	0	0	92
	September 2020	10	90	0	0	20	0	0	0	90
Tulare	July 2020	10	100	50	0	30	70	0	0	100
	August 2020	10	100	40	0	0	0	0	0	100
	September 2020	9	100	0	0	22	0	22	0	100
Los Angeles	July 2020	8	100	100	0	0	0	100	13	100
	August 2020	8	100	25	0	0	0	0	0	100
	September 2020	8	100	0	0	25	0	0	0	100
Orange	July 2020	12	100	83	0	0	0	33	0	100
	August 2020	12	100	17	0	0	0	0	0	100
	September 2020	12	83	0	0	17	0	0	8	83
San Bernardino	July 2020	6	100	0	0	0	0	0	0	100
	August 2020	7	100	0	0	43	0	0	0	100
	September 2020	6	67	0	83	0	0	0	0	100
All Regions	July 2020	69	100	67	0	16	10	17	1	100
	August 2020	70	97	23	11	14	0	0	0	99
	September 2020	66	92	0	14	18	2	3	2	95
	Total	205	97	30	8	16	4	7	1	98

[a] Defined as frequency of samples with concentration above analyte reporting limit.

Lower detection frequencies were observed for other pyrethroids (Table 2.4). The second most frequently detected pyrethroid was lambda-cyhalothrin, which was found in about 30% of the water samples. Cyfluthrin, permethrin, esfenvalerate, cypermethrin, and deltamethrin had overall frequencies of detection of 16%, 8%, 7%, 4%, and 1%, respectively. In previous studies, permethrin and cyfluthrin were associated with toxicity in several impaired waters, though not as frequently as bifenthrin (Budd et al., 2020; Holmes et al., 2008; Jiang et al., 2016; Richards et al., 2016; Weston et al., 2009). Fenpropathrin and tefluthrin were not detected in any of the samples in this study. Frequencies of detection between the aqueous and suspended solid phases were nearly identical, although there were a few exceptions. For example, bifenthrin was detected in the filtered water (i.e., aqueous phase) in the Sacramento-Yolo region at 86%, but at 97% in the suspended solids (n=36). This small discrepancy was likely due to the different reporting limits for the aqueous and solid phase samples.

Table 2.5 Summary of pyrethroid concentrations in aqueous phase of catch basin water (n=205).

Pyrethroid	Detection frequency (%)	Median (ng/L)	Max. (ng/L)	75th percentile (ng/L)
Tefluthrin	0	<RL	<RL	<RL
Bifenthrin	90	20	324	31
Fenpropathrin	0	<RL	<RL	<RL
Lambda-cyhalothrin	25	<RL	626	<RL

Permethrin[a]	1	<RL	20	<RL
Cyfluthrin[a]	12	<RL	689	<RL
Cypermethrin[a]	3	<RL	253	<RL
Esfenvalerate[a]	4	<RL	127	<RL
Deltamethrin	1	<RL	85	<RL
All pyrethroids	92	32	726	87

RL = reporting limit.
[a] Quantified as the sum of isomers.

Table 2.6 Summary of pyrethroid concentrations in suspended solids from catch basin water (n=205). Concentrations are expressed on a dry weight basis.

Pyrethroid	**Detection frequency (%)**	**Median (ng/g)**	**Max. (ng/g)**	**75th percentile (ng/g)**
Tefluthrin	0	<RL	<RL	<RL
Bifenthrin	97	1440	75400	3190
Fenpropathrin	0	<RL	<RL	<RL
Lambda-cyhalothrin	30	<RL	18200	<RL
Permethrin[a]	8	<RL	76100	<RL
Cyfluthrin[a]	16	<RL	39800	<RL
Cypermethrin[a]	4	<RL	5690	<RL
Esfenvalerate	7	<RL	8430	<RL
Deltamethrin	1	<RL	7230	<RL

All pyrethroids	98	2350	93600	5910

RL = reporting limit.
[a] Quantified as the sum of isomers.

Pyrethroid concentrations in catch basin water (C_w) and suspended solids (C_s) are summarized in Tables 2.5 and 2.6. Except for bifenthrin, median concentrations for pyrethroids were below reporting limits in both aqueous phase and suspended solids. Total concentrations of the target pyrethroids in water ranged from 3 to 726 ng/L, with a median of 87 ng/L. For suspended solids, they ranged from 42 to 93,600 ng/g, with a median of 2,350 ng/L. Bifenthrin was detected at up to 324 ng/L in water and 75,400 ng/g in suspended solids. The maximum concentration of bifenthrin found on the suspended solids was significantly higher than those seen in previous studies. For example, in Weston et al., the total concentrations of pyrethroids in suspended solids ranged from 1,150 to 3,390 ng/g in water samples collected at stormwater outfalls in northern California (Weston et al., 2009). It must be noted that the high concentrations observed in the current study were only from a few samples, and the same catch basins showing high concentrations in a single sampling event did not show elevated levels at the other sampling time points. This may indicate a recent pesticide application in the vicinity of the specific catch basins, causing transient high pesticide loadings.

While bifenthrin was the most frequently detected pyrethroid in each region or month, other pyrethroids often made up a greater proportion of a region's total pesticide concentrations (Figure 2.2). For example, in the August samples collected from Los Angeles County and the September samples from the West Valley region, lambda-

cyhalothrin accounted for 88% of concentrations and permethrin accounted for 91% of concentrations, respectively. When considering the entire sampling period, the only region where the bifenthrin concentration was dominant was Madera County in northern/central California. Overall, bifenthrin represented 44% of average total pyrethroid concentrations, which was smaller compared to that (72%) observed in Budd et al. (2020) for surface waters across California.

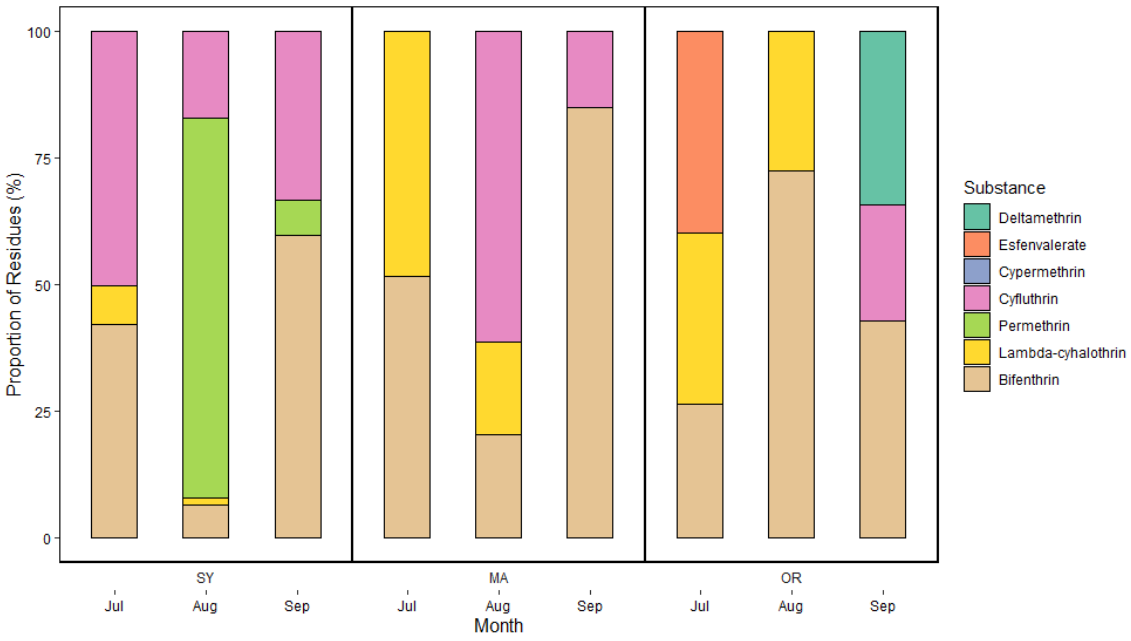

Figure 2.2 Fractions of individual pyrethroids in whole water samples for three sampling sites (Sacramento/Yolo Counties = SY; Madera County = MA; Orange County = OR).

2.3.2 Patterns and Influencing Factors

Median total pyrethroid concentrations among the different sampling regions ranged from 20 ng/L to 64 ng/L in the aqueous phase and 946 ng/g to 3,890 ng/g in the solid phase (Figure 2.3). Though differences can be observed in concentrations among

different regions, these trends cannot be extrapolated broadly given the limited number of sampling locations in each region. In California, reported use of pyrethroid insecticides differs widely by county, as counties have different populations, population densities, climate conditions, and land use patterns. Previous studies have reported higher detection frequencies in Southern California (e.g., Orange County) than in Northern California for pyrethroids and other pesticides (e.g., fipronil), but this trend was not observed in the catch basins surveyed in this study (Ensminger et al., 2013; Gan et al., 2012). (Budd et al., 2020) noted that flood control channels in Southern California are more commonly lined, increasing water-associated contaminant transport; this may partially explain why downstream detections and concentrations are more disparate between regions as compared to those from roadside catch basins. Other regional differences, such as local pest types and pressures (which may be influenced in turn by factors like climate and vegetation), management practices, and regulations, can significantly affect pesticide use and thus their offsite transport.

Median concentrations in both water and suspended solids decreased from July to September (Figure 2.4). The median total pyrethroid concentrations in July, August, and September for water were 85 ng/L, 24 ng/L, and 6 ng/L, respectively, and those for suspended solids were 2,780 ng/g, 2,480 ng/g, and 1,260 ng/g, respectively. Much of this trend was driven by decreases in bifenthrin concentrations, with median concentrations in the aqueous phase decreasing from 28 ng/L in July to 5 ng/L in September and concentrations in suspended solids from 2,180 ng/g in July to 750 ng/g in September.

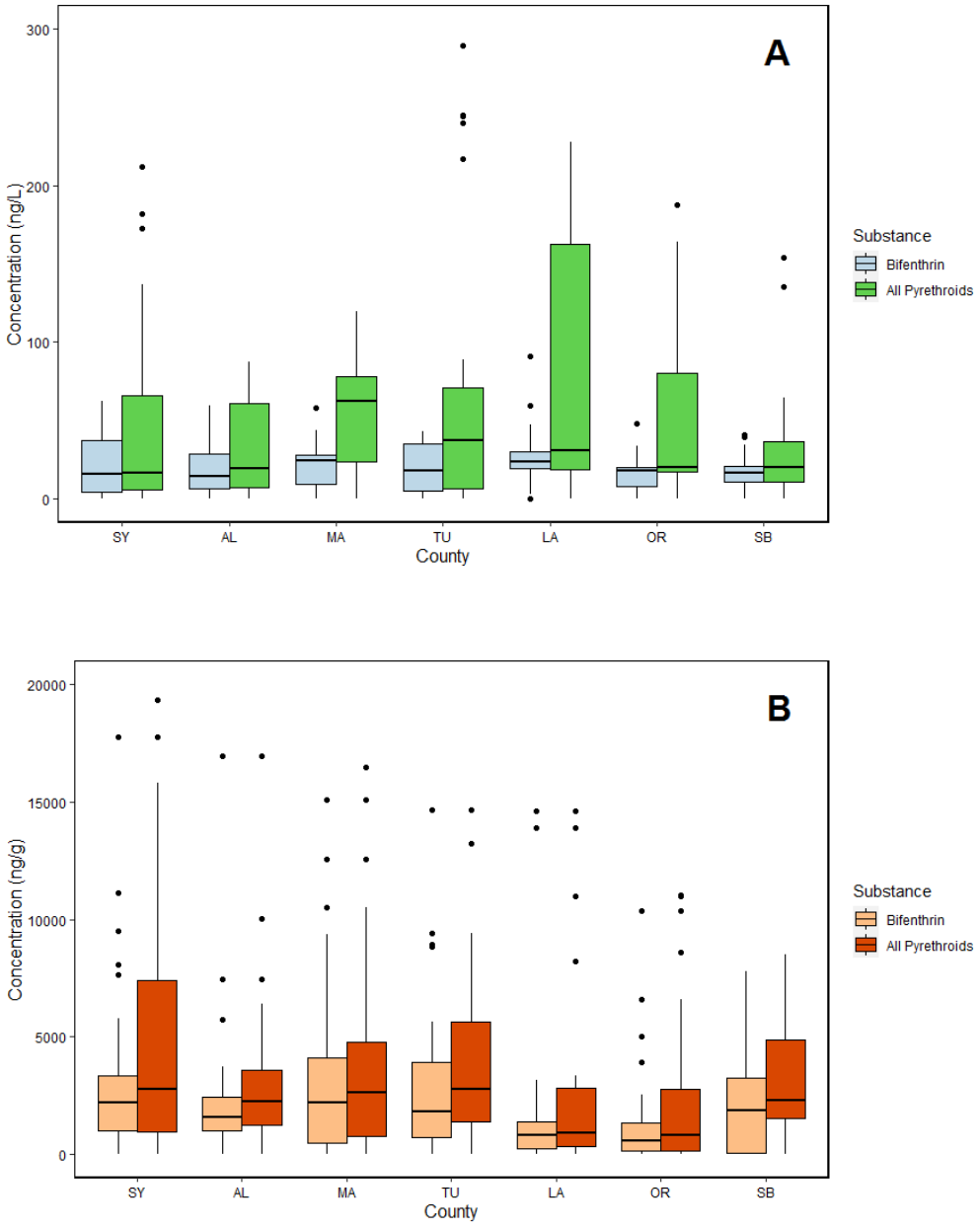

Figure 2.3 Bifenthrin and total pyrethroid concentrations in A) aqueous phase and B) suspended solids; grouped by sampling region. Sacramento/Yolo Counties = SY; Alameda County = AL; Madera County = MA; Tulare County = TU; Los Angeles County = LA;, Orange County = OR; and San Bernardino County = SB.

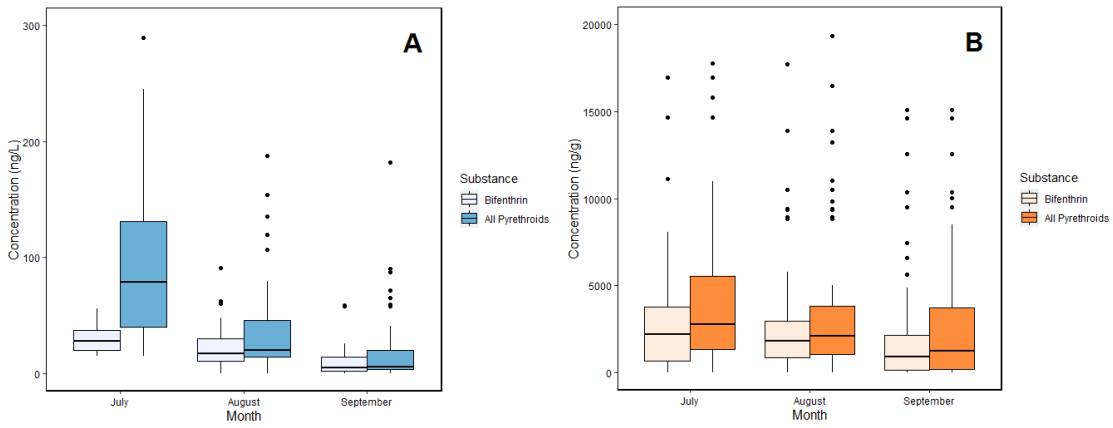

Figure 2.4 Bifenthrin and total pyrethroid concentrations in A) aqueous phase and B) suspended solids (grouped by sampling month).

Pesticide use data from CDPR's Pesticide Information Portal were taken into consideration to understand the trends of pyrethroids seen in the catch basins ("California pesticide information portal application," 2022). The trend in monthly reported urban use of pyrethroid insecticides varies among counties. For example, Sacramento County use of the pyrethroids in this study was highest in January, November, and December, Yolo County use was highest in the summer months (with a peak in July), and San Bernardino County use remained relatively consistent throughout the year. In a few counties, use in one month was much higher than in others; for example, in Tulare County, use in April was 423 kg, which was almost three times the monthly average of 146 kg for the year. In addition, winter rain events likely wash off a large amount of pyrethroid residues (relative to irrigation-induced runoff) into the catch basins, and the concentrations decrease with time during the dry season, which further complicates the response of pesticide occurrence in the catch basins to pesticide use. The suitability of predicting and understanding trends in the environmental presence of pyrethroid residues from pesticide

use data is further impacted by the difference in persistence of individual compounds. Pyrethroids are known to be extremely hydrophobic, and once entering a catch basin, are quickly partitioned into the bottom sediment. The redistribution from the sediment phase to water may serve as a continuous secondary source discharging pyrethroid residues into downstream surface water systems on a prolonged time scale (Gan et al., 2005; Meyer et al., 2013).

Table 2.7 Mean adsorption coefficient (K_d) values and standard deviations for bifenthrin, along with mean % of pyrethroid residues associated with the suspended solids (SS) phase, total suspended solids (TSS), and dissolved organic carbon (DOC) for bifenthrin in each sampling region.

County	**n**	**Bifenthrin K_d (× 10^5 L/kg)**	**% in SS**	**TSS (mg/L)**	**DOC (mg/L)**
Sacramento/Yolo	36	2.4 ± 4.2	58.3	28.6	51.2
Alameda	27	1.9 ± 1.9	55.6	16.8	35.8
Madera	34	1.7 ± 1.8	37.9	35.4	30.5
Tulare	29	1.6 ± 1.7	48.3	31.8	51.6
Los Angeles	24	1.4 ± 2.6	48.9	54.5	59.3
Orange	36	3.9 ± 12.4	44.3	71.5	54.3
San Bernardino	19	1.6 ± 1.1	36.7	22.2	78.1
All regions	205	2.1 ± 5.6	47.7	38.6	49.8

Water chemistry variables such as TSS and DOC are known to affect the behavior of stormwater contaminants, especially strongly hydrophobic compounds like the synthetic pyrethroids. Mean TSS in water samples was 39 ± 80 mg/L (Table 2.7) and the median TSS was 11 mg/L, showing wide variance in TSS levels among different catch basins as well as the same basins at different sampling times. This mean value is similar to those in urban runoff from outfalls in Aliso Viejo, CA, during the dry season (41

mg/L) and from a stormwater channel flowing into the San Francisco Bay during low flow conditions (68 mg/L) (Gilbreath and McKee, 2015; Pitton et al., 2016). However, TSS differs significantly across previous literature and may be influenced by a combination of seasonal and geographic variables. Mean TSS from some outfalls during the dry season was as low as 0.55 mg/L, while the Environmental Protection Agency's National Urban Runoff Program reported higher TSS ranging from 141 to 224 mg/L for stormwater in a "median urban site" (Morgan et al., 2005; Pitton et al., 2016; USEPA, 1983). In this study, the mean DOC level in catch basin water was 50 ± 42 mg/L and the median was 44 mg/L. This mean is similar to that from sediment pore water from San Diego Creek Watershed samples (42 mg/L), suggesting a strong contribution of sediment and organic debris to the elevated levels of DOM in the catch basin water (Budd et al., 2007). Generally, a higher TSS results in increased pyrethroid partitioning to the solid phase, and a higher DOC results in higher partitioning to dissolved organic matter in the aqueous phase (Liu et al., 2004). Suspended solids tend to settle to the bottom of a catch basin under low flow conditions, while DOM and the associated contaminants may travel over a longer distance and contaminate downstream surface water bodies. However, neither TSS nor DOC were found to be associated with pyrethroid detection or concentrations, indicating the influence of other factors on occurrence.

To better understand pyrethroid partitioning between the solid and aqueous phases, the partition coefficient K_d (L/kg) was calculated for bifenthrin residues. The average K_d for bifenthrin across all samples was 2.15×10^5 L/kg, with individual regions averaging from $1.42 \pm 2.6 \times 10^5$ L/kg in Madera County to $3.85 \pm 12.4 \times 10^5$ L/kg in

Orange County (Table 2.7). These values are on a similar order of magnitude to those observed in a previous study (2.4×10^3 to 1.1×10^5 L/kg) where pyrethroids in runoff water from concrete surfaces were characterized following simulated precipitation events (Jiang and Gan, 2012). While pyrethroids are highly hydrophobic in general (log K_{ow} ranging from 4.5 to 7.0), previous studies using soils or sediments at equilibrium conditions have often found smaller K_d values, suggesting that suspended solids, likely due to their larger specific surface areas and high organic carbon content, have a stronger affinity for pyrethroid residues (Delgado-Moreno et al., 2010; Fojut and Young, 2011; Gan et al., 2005; Laskowski, 2002; Yang et al., 2006).

Though the high apparent K_d values would imply enrichment of pyrethroid residues on the suspended solids, a significant portion of the whole-water pyrethroids was still associated with the aqueous phase. An average of 47.7% of the pyrethroid residues was associated with suspended solids, leaving more than 50% in the aqueous phase (Table 2.7). Pyrethroid interactions with DOM may decrease distribution to the solid phase, in turn enhancing off-site transport. In a study of DOC from various media, the mean dissolved organic carbon-water partition coefficient K_{DOC} (L/kg) for bifenthrin ranged from 4.8×10^4 to 25.0×10^4 L/kg, indicating a strong affinity of pyrethroids for DOM (Delgado-Moreno et al., 2010). Findings from this and previous studies together suggest that, for strongly hydrophobic contaminants like pyrethroids, DOM facilitates short-term offsite transport, while solid particles, particularly after settling, may constitute a source for sustained emission in urban environments containing both

artificial (e..g., USDS) and natural compartments (Jiang et al., 2016, 2010; Jiang and Gan, 2012; Richards et al., 2016).

Depending on the sedimentation rate in each catch basin, which may be influenced by factors like catch basin structure, particle size, water flow rate, much of the suspended solids may settle at the bottom of catch basins and act as a reservoir for pyrethroid residues. Runoff may result in scouring, where sediment is resuspended, which has the potential to carry the contaminant-laden solids downstream and eventually into urban aquatic ecosystems. For flow rates less than 0.03 m^3/s, which is likely the case for the most of the dry season, a high proportion of the sediment may be captured by catch basins (Yang et al., 2018). A study using a physical catch basin model showed that, under its highest experimental flow rate of 0.01 m^3/s, the sediment was resuspended at a rate of 128 g/min when there was an overlaying water depth of 0.01 m (Avila et al., 2011). Under this scenario, assuming pyrethroid concentrations in the settled sediment were similar to those in the suspended solids, a median catch basin would have approximately 300 μg/min of residues being re-suspended and available for offsite transport. However, increased flows from rain-induced runoff may pose a high risk of scouring. In California, this problem may be exacerbated by pollutant buildup over a long dry period, resulting in a more significant first flush when the rainy season begins (Lee et al., 2004). Climate models predict an increase in extreme precipitations for California; thus, instances of high flow and scouring may become more frequent, further highlighting the significance of catch basins as a substantial secondary source for contaminant redistribution in urban environments (Polade et al., 2017).

2.3.3 Potential Effects on Non-Target Aquatic Organisms

The potential toxicity to freshwater invertebrates from whole water bifenthrin concentrations was estimated by calculating toxic units (TUs) based on a previously reported median 96-h EC_{50} (3.3 ng/L) and LC_{50} (7.7 ng/L) for *Hyalella azteca*, a freshwater amphipod commonly used for toxicity assessments (Table 2.8) (Weston and Jackson, 2009). Based on the EC_{50}, 89% of samples (all samples with concentrations above the reporting limit) showed TU >1. Based on LC_{50}, 72% of samples contained bifenthrin at a level with TU >1. Among all regions, the lowest average TU based on LC_{50} was 1.1, suggesting a threat to sensitive invertebrates from drainage water originating from the catch basins. It must be noted that the reporting limit for bifenthrin in the aqueous phase (2.0 ng/L) is somewhat close to this LC_{50} and may contribute to the low number of samples with TU <1 for bifenthrin. Previous research has shown sediment from multiple sites in California impacted by urban runoff to be toxic to *H. azteca*, with bifenthrin being the primary driver of toxicity; though other pyrethroids, including permethrin, cyfluthrin, and cypermethrin, were also implicated (Amweg et al., 2006; Budd et al., 2020; Weston et al., 2005). While these TU estimates for catch basins are not representative of toxicity at stormwater outfalls or further downstream water bodies, they nevertheless highlight that sediments and water in the numerous catch basins, along with the USDS connecting these catch basins, may act as a significant source contributing to aquatic toxicity in urban watersheds.

Table 2.8 Toxic units (TUs) for Hyalella azteca from mean bifenthrin concentrations in whole water samples, grouped by county. SY = Sacramento / Yolo, AL = Alameda, MA = Madera, TU = Tulare, LA = Los Angeles, OR = Orange, SB = San Bernardino.

Month	**TU endpoint**	**Region**						
		SY	**AL**	**MA**	**TU**	**LA**	**OR**	**SB**
July	EC_{50}[a,b]	36.5	19.2	16.6	19.8	15.3	17.8	12.9
	LC_{50}[a]	15.6	8.2	7.1	8.5	6.6	7.6	5.5
August	EC_{50}	20.8	6.1	6.9	22.3	41.9	13.2	7.4
	LC_{50}	8.9	2.6	2.9	9.6	18.0	5.7	3.2
September	EC_{50}	7.4	12.0	15.2	5.8	110.5	2.7	5.6
	LC_{50}	3.2	5.2	6.5	2.5	47.3	1.1	2.4

[a] Values for EC_{50} and LC_{50} derived from (Weston and Jackson, 2009).

[b] Sublethal end point for EC_{50} defined as impaired swimming.

In addition to toxicity to non-target organisms, inadvertent exposure to pyrethroids may select for pesticide resistance in populations of pests. Many pests that pose threats to public health and agriculture breed in habitats that receive runoff, and resistance development in these species may hamper the efficacy of pest management practices. Resistant organisms also have the potential to bioaccumulate greater amounts of contaminants, increasing the risk of trophic transfer along the food chain (Johanif et al., 2021; Muggelberg et al., 2017). Though it is difficult to directly associate pesticide use with resistance, high agricultural use of pyrethroids has been linked to increased incidence of resistant pests, like mites and beetles, that can cause significant damages (Heimbach and Müller, 2013; Umina, 2007). For example, Hien et al. found resistant populations of the mosquito *Anopheles gambiae* and water contaminated with deltamethrin and lambda-cyhalothrin at sites of Burkina Faso in West Africa, suggesting selection pressure from environmental contamination as a result of cotton insecticide applications (Hien et al., 2017). Orondo et al. found increased phenotypic resistance in

the mosquito *An. arabiensis* in irrigated areas of Kenya, where pyrethroids are the primary insecticides used for crop protection (Orondo et al., 2021). In California, the prevalence of a single mutation conferring pyrethroid resistance in *Culex pipiens* and *Cx. quinquefasciatus* was found to be nearly 1.5 times greater in populations from 2014-2016 as compared to those collected before 2012 (Yoshimizu et al., 2020).

Catch basins themselves are common breeding habitats for several mosquito species. USDSs, frequently containing standing water and food sources like algae and detritus, are a significant source of mosquitoes depending on the region, such as *Cx. quinquefasciatus* in southern California (Harbison et al., 2009; Kluh et al., 2006, 2001). Catch basins and other storm drain structures often contain stagnant water rich in organic matter, and are isolated from wind and sunlight, providing favorable conditions for mosquito eggs and larvae. Exposure to sublethal pyrethroid concentrations results in increased resistance in later generations of larvae, and larval resistance has been associated with adult resistance (Kawada et al., 2009; Shi et al., 2015). Therefore, the importance of urban catch basins in facilitating insecticide resistance in vector insects may constitute another impetus for better understanding the transport and fate of pyrethroids and other pesticides in USDSs and catch basins.

2.3.4 Mitigation of Pyrethroid Occurrence in Catch Basins

The ubiquitous presence of pyrethroid residues in urban catch basins, and the sheer number of catch basins in many highly urbanized areas, necessitate mitigation practices to minimize this overlooked source of contamination. Apart from directly

reducing the use of pyrethroid insecticides, there are a number of practices that may be considered for reducing pyrethroid contamination in catch basins and storm drain systems. For example, smart irrigation systems would limit the generation of runoff from lawns and gardens. Many basin mouths that accept water from street gutters are completely open, meaning that the majority of sediments and small debris that can carry contaminants may enter freely (Azah et al., 2017; Jang et al., 2010). Covers and other amendments which are designed to prevent and remove large debris may also help reducing loading of contaminants that have a high affinity for solids, including pyrethroids (Alam et al., 2018a, 2018b, 2017; Morgan et al., 2005). Regular servicing of catch basins and other storm drain structures to remove the accumulated sediment and other debris, especially before the onset of the rainy season, is expected to greatly reduce the chemical loads from the first flush of rainfall-induced runoff. Physical catch basin dimensions vary widely and likely influence the fate of hydrophobic contaminants. Characteristics like height, sump depth, and outgoing channel diameter, may affect sediment capture and release into the USDS (Yang et al., 2018). For example, higher outlets, which may increase the maximum depth of water above the sediment, may reduce the amount of sediment that is resuspended during high flow (Avila et al., 2011). Storm drain system design that maximizes sediment capture would in turn limit the release of contaminants downstream.

The use of pesticides in urban areas is made by licensed operators as well as homeowners (Xie et al., 2021). While pesticide products have detailed instructions on the rate and method of application, there are often overuses with the intention to ensure

complete pest eradications. In addition, pesticide misuses, including applications in non-infested areas like walkways and driveways, as well as spills from careless handling, may contribute greatly to contamination in surface runoff. Education on environmentally responsible pesticide use and handling should not target pesticide application professionals, but also the general public, to achieve meaningful reductions in offsite contamination of pesticides. The adoption of low-risk control methods, such as enclosed baits, as well as non-chemical methods, should be promoted to further minimize pesticide input in the environment.

2.4 Conclusion

The present study sought to determine the occurrence and patterns of the widely used pyrethroid insecticides in urban catch basins that receive runoff from irrigation and rainfall-induced runoff. From analysis of water from 79 catch basins across 8 counties in California during the dry season, nearly every sample contained one or more pyrethroids at a concentration above the reporting limit. Bifenthrin was a pyrethroid compound with the highest detection frequency as well as concentrations eliciting toxicity to sensitive aquatic invertebrates, although several other pyrethroids also contributed significantly to the chemical profile in catch basin water. Pyrethroid concentrations in catch basins generally decreased over time in the dry season, but high levels of residues remained in the aqueous and suspended solid phases at the end of summer, suggesting that residues in catch basins could serve as a significant secondary source for downstream contamination of urban surface streams and estuaries; the risk is expected to be the greatest during the

initial phase of the rainy season, when rainfall-induced runoff may resuspend and mobilize pyrethroid residues retained in the catch basins. Pyrethroid pollution in urban regions adversely affect sensitive aquatic organisms and may also encourage resistance development in urban pests such as mosquitoes. To improve understanding of pesticide behaviors in urban catch basins and USDSs, further research should characterize runoff before and after it passes through a catch basin, the hydraulic retention of both water and solids in catch basins, and release of water and suspended solids from catch basins into the rest of the USDS. Efforts to design catch basins with reduced contaminant accumulation potential, and pest management practices to prevent the transport of pesticide residues from entering USDSs and catch basins, should be further explored.

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Chapter 3 Distribution of pyrethroid insecticides in urban storm drain structures: Catch basins, open channels, and outfalls

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3.1 Introduction

Pyrethroids, owing to their broad-spectrum insecticidal activity and relatively low mammalian toxicity, are among the most widely used pesticides globally for both agricultural and non-agricultural purposes. In 2015, they comprised approximately 17% of the worldwide market share of insecticides; the most common of these are cypermethrin, lambda-cyhalothrin, deltamethrin, bifenthrin, and permethrin (Matsuo, 2019). According to 2012 use data, pyrethroids were the third most used insecticide class in the non-agricultural market sector in the United States (Atwood and Paisley-Jones, 2017). In California, approximately 94% of permethrin was used for structural pest control in non-agricultural applications in 2021 (California Pesticide Information Portal Application, 2023). The dominance of these pesticides has resulted in their contamination of terrestrial and aquatic ecosystems, posing ecotoxicological risks to non-target organisms, especially invertebrates and fish in surface aquatic systems (Giddings et al., 2019).

In aquatic environments, pyrethroids partition strongly into sediments due to their high K_{ow} values and strong affinity for organic matter (Laskowski, 2002). Once in the sediment phase, pyrethroid residues may persist for a prolonged time; for example, experimentally derived half-lives were up to 17 months for bifenthrin (Gan et al., 2005). Monitoring of bed sediments showed total pyrethroid concentrations as high as 1,408 ng/g in the United States, 4,960 ng/g in China, 5,580 ng/g in Pakistan, and 59,741 ng/g in Vietnam (Chinen et al., 2016; Duong et al., 2014; Mahboob et al., 2015; Sun et al., 2015). Because of their strong hydrophobicity, pyrethroids often bioaccumulate in organisms via sediment or diet (Katagi, 2010). Very low toxicity benchmarks were established for pyrethroids in water, sediments, and biofilms for sensitive aquatic species like the amphipod *Hyalella azteca* (Amweg et al., 2006; Li et al., 2019; Mahler et al., 2020).

Runoff from residential and commercial areas is generally responsible for contamination and toxicity in downstream waterways in urban regions (Hoffman et al., 1984; Weston et al., 2009). In urbanized areas, surface runoff typically flows through storm drain systems before being discharged at an outfall. A typical urban storm drain system (USDS) consists of curbside gutters, catch basins as inlets of surface runoff, underground sewer pipes and open channels to transport the runoff water to outfall points (Figure 3.1). For example, the highly urbanized Los Angeles County (over 9,000,000 residents) has approximately 70,000 catch basins, 7,400 km of sewer pipes, and 1,340 km of surface channels (Kwan et al., 2010; Porse, 2018).

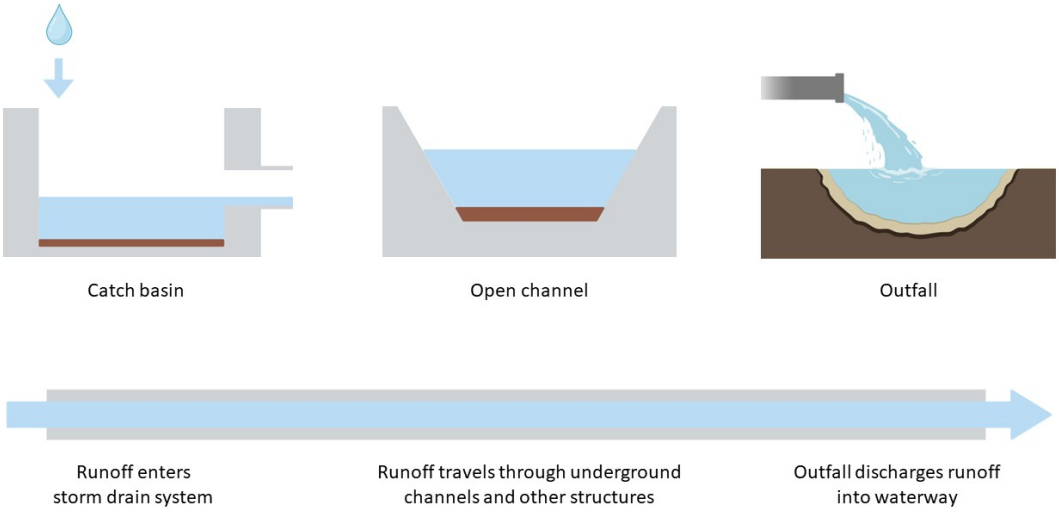

Figure 3.1 Overview of storm drain structures sampled.

Various contaminants in urban runoff transported through USDS may impact urban ecosystems (Lundy et al., 2012). Many substances, such as nutrients, metals, PAHs, and pesticides have been frequently found in urban runoff. Emerging contaminants, such as pharmaceuticals and personal care products (PPCPs) and plasticizers, are also being identified (Buffleben et al., 2002; Ellis, 2006; Hoffman et al., 1984; Lee and Bang, 2000; Regnery and Püttmann, 2010).

Previous studies have shown ubiquitous occurrence of pyrethroids in urban environments, including in storm drains. For example, dust samples collected from pavement in southern California neighborhoods, where insecticides are applied around homes for nuisance insect control, had median concentrations up to 46 ng/g for permethrin and 32 ng/g for bifenthrin (Jiang et al., 2016; Richards et al., 2016). A recent

study of catch basins across California during the summer found median total pyrethroid concentrations of 32 ng/L in the aqueous phase and 2,350 ng/g on suspended solids in water samples (Sy et al., 2022). A study of urban stream sediments from 7 metropolitan areas in the United States yielded a median total pyrethroid concentration of 7.7 ng/g (Kuivila et al., 2012). Whole, unfiltered water samples from drainage outfall points in Roseville, CA, and Elk Grove, CA, showed median total pyrethroid concentrations of 4.6 ng/L and 12.5 ng/L, respectively, during the dry season (July-September) and 51.3 ng/L and 31.9 ng/L during the wet season (November-April) (Weston et al., 2009).

To date no research has focused on understanding the occurrence and distribution of urban contaminants such as pyrethroids across different compartments of USDS. The primary objective of this work was to evaluate pyrethroid occurrence and distribution within USDS structures. Sampling was conducted in June and August 2021 to coincide with the late spring and summer, where pesticide use is often the highest in urban areas. Information on pyrethroid occurrence within USDS improves our understanding of transport and fate of contaminants via urban runoff. The findings are expected to help guide development of management and mitigation strategies to minimize impact of urban pesticide use on the integrity and functions of urban surface streams.

3.2 Materials and Methods

3.2.1 Chemicals and Materials

Analytical standards of pyrethroids were obtained from different pesticide manufacturers. Lambda-cyhalothrin (89.0%) was obtained from Syngenta Crop Protection (Greensboro, NC). Bifenthrin (98.4%), permethrin (98.5%), and cypermethrin (98.5%) were obtained from FMC (Philadelphia, PA). Fenpropathrin (99.7%) and esfenvalerate (99.9%) were obtained from Valent (Dublin, CA). Cyfluthrin (86.4%) and deltamethrin (99.4%) were provided by Bayer Crop Science (Research Triangle Park, NC). Deuterated bifenthrin-d_5 used to determine surrogate recovery was purchased from MilliporeSigma (St. Louis, MO).

Unglazed ceramic tiles (30 cm × 30 cm) were purchased from a local retail store and used for *in situ* colonizing biofilms. Dippers and glass containers for sample collection were purchased from John W. Hock (Gainesville, FL) and J.G. Finneran Associates (Vineland, NJ), respectively. The pH buffer solutions for calibration were purchased from Thermo Fisher Scientific (Waltham, MA). Total dissolved solid standards were purchased from Oakton Instruments (Vernon Hills, IL). Glass fiber filters were purchased from Advantec MFS (Dublin, CA). Sodium sulfate, sodium chloride, and GC/MS grade solvents (dichloromethane, acetone, and hexane) were purchased from Thermo Fisher Scientific (Waltham, MA). Florisil was purchased from Spectrum Chemical (New Brunswick, NJ). Before use, Florisil was activated via baking at 130 °C for 4 h, and sodium sulfate was dehydrated via baking at 400 °C for 4 h. All glassware

used for sample processing was baked at 400 °C for 4 h beforehand to prevent cross contamination. Syringe filters were purchased from Simsii (Issaquah, WA).

3.2.2 Site Description and Sampling

Catch basins, open channels, and outfalls were selected from cities in Los Angeles County based on consistent availability of drainage water according to Greater Los Angeles County Vector Control District's records (Figure 3.2). A total of 3 catch basins, 7 open channels, and 7 outfalls were sampled. All sampled structures contained flowing or standing water at least 1 cm in depth. All structures were lined with concrete, with the outfalls draining into small ponds surrounded by grassy vegetation. Site locations, sampling dates, and samples collected at each site are given in Table 3.1.

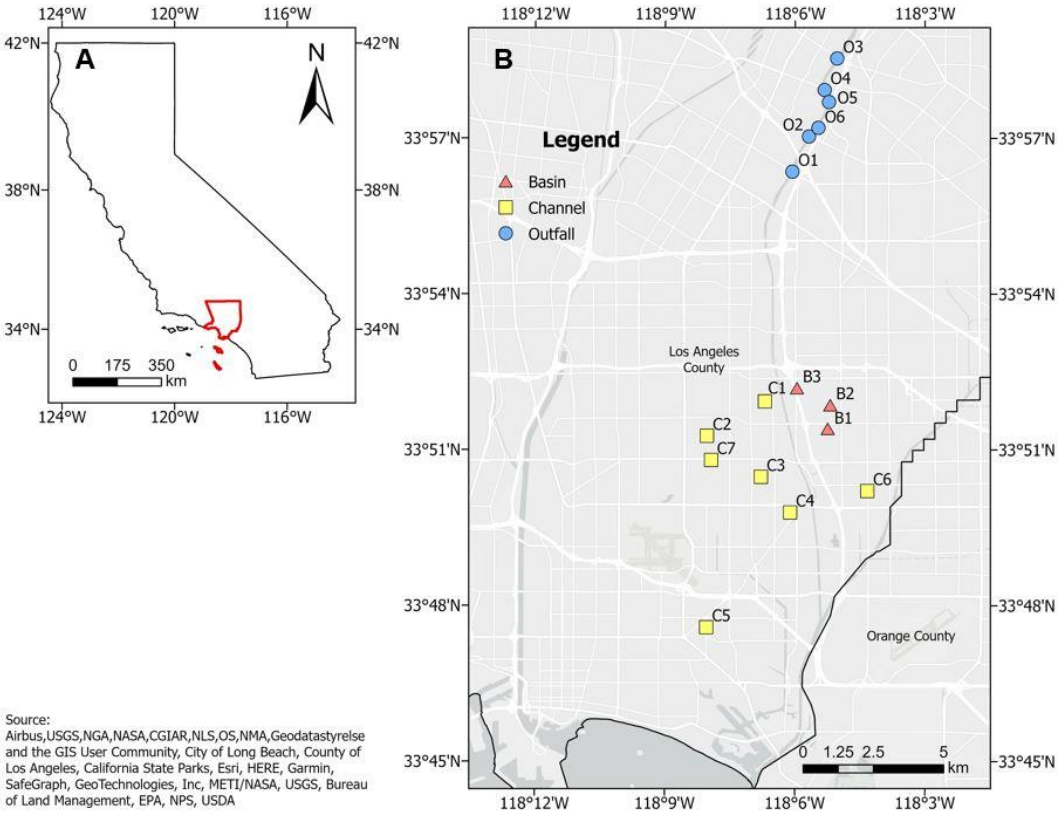

Figure 3.2 A) Location of Los Angeles County (red) within California, USA; B) Map of sampling sites in Los Angeles County.

Table 3.1 Site information, including locations and samples collected. O = outfall, C = open channel, B = catch basin.

			June 2021 samples collected				August 2021 samples collected			
Site	**Latitude**	**Longitude**	**Water**	**Sediment**	**Algae**	**Biofilm**	**Water**	**Sediment**	**Algae**	**Biofilm**
B1	33.857039	-118.087417	x				x			x
B2	33.864618	-118.086463	x				x			
B3	33.870078	-118.099245	x				x			x
C1	33.865571	-118.111568	x		x		x		x	x
C2	33.854501	-118.133836	x				x			
C3	33.841345	-118.113075	x				x			
C4	33.82992	-118.10183	x		x					
C5	33.79307	-118.13406	x				x	x	x	x
C6	33.836815	-118.072155					x		x	
C7	33.846751	-118.132239					x	x	x	
O1	33.9392745	-118.1010586	x				x	x		
O2	33.9506113	-118.0946749	x				x	x		
O3	33.975614	-118.083851	x				x		x	
O4	33.9655016	-118.0887151	x				x		x	
O5	33.9616664	-118.0869985					x			
O6	33.9533369	-118.0910325					x			

In June and August 2021, samples of water, sediment, and algae were collected from each site where available. Samples from open channels were collected within 10 m of the access point, and samples from outfalls were collected at the end of the drain mouth. Triplicates of 500 mL of water were collected into amber glass bottles using a stainless-steel dipper. Sediment, algae, and biofilm samples were collected into small amber glass jars using a stainless-steel trowel or spatula. For biofilm sampling, unglazed ceramic tiles (30 cm × 30 cm) were placed in triplicate at each site in June to allow for biofilm colonization. Tiles were checked in August (after 60 d), and biofilm was collected when available. A gentle stream of deionized water was used onsite to wash any settled sediment particles on the tiles, after which a stainless-steel spatula was used to scrape biofilms into an amber glass jar. All samples were placed on ice packs in coolers

and transported to the laboratory within 8 h, where they were stored at 4 °C in the dark before analysis. Water samples were processed within 14 days of collection, and all other samples were processed within 30 days of collection. In total, 28 water samples, 4 sediment samples, 8 algae samples, and 4 biofilm samples were collected.

3.2.3 Sample Preparation

A Fisherbrand AE150 pH meter was used to measure water sample pH before filtration (Waltham, MA). To separate suspended solids, the whole water sample was filtered under vacuum through a 0.4-μm pore size glass fiber membrane (Advantec MFS, Dublin, CA). After filtration, a 20-mL aliquot of the filtered water was taken to measure total dissolved solids (TDS) and dissolved organic carbon (DOC) with a YSI EcoSense EC30A conductivity pen (Yellow Springs, OH) and a Shimadzu TOC-V analyzer, respectively (Columbia, MD). Water pH, TDS, and DOC are detailed in Table 3.2. Filters with suspended solids were wrapped in aluminum foil and stored in a refrigerator at 4 °C until analysis. Sample filter membranes were dried at 105 °C for 24 h and the amount of total suspended solids (TSS) was measured gravimetrically by weighing the dried filters prior to extraction. Sediment, algae, and biofilm samples were freeze-dried for 72 h prior to extraction. The TOC content of dried sediments was measured using a Thermo-Finnegan Flash EA1112 nitrogen/carbon analyzer.

Table 3.2 pH, total dissolved solids (TDS), and dissolved organic carbon (DOC) concentrations for water from each site.

Site	pH		TDS (mg/L)			DOC (mg/L)		
					Jun-21			
B1	9.05	± 0.70	685	±	43.16	52.40	±	2.20
B2	7.61	± 0.69	350	±	55.65	47.42	±	5.26
B3	7.61	± 1.38	315	±	21.11	106.50	±	18.74
C1	8.01	± 0.97	325	±	59.15	76.41	±	11.08
C2	7.13	± 0.65	1200	±	52.80	137.50	±	20.63
C3	7.61	± 1.02	375	±	49.50	77.43	±	12.00
C4	8.19	± 1.24	895	±	113.67	92.76	±	5.19
C5	8.36	± 0.79	295	±	52.22	64.36	±	11.71
O1	7.65	± 0.63	1300	±	133.90	65.79	±	9.21
O2	7.72	± 0.69	725	±	74.68	63.52	±	10.86
O3	9.19	± 0.35	565	±	65.54	61.21	±	5.75
O4	9.23	± 1.22	570	±	45.60	113.90	±	15.49
O5	7.74	± 0.34	380	±	28.88	70.44	±	7.82
					Aug-21			
B1	7.85	± 0.06	930	±	31.62	318.60	±	49.06
B2	7.16	± 0.10	430	±	33.54	63.95	±	11.32
B3	7.20	± 0.11	300	±	52.20	45.23	±	1.99
C1	8.81	± 0.04	380	±	72.58	62.88	±	5.16
C2	6.79	± 0.12	395	±	56.09	146.20	±	23.25
C3	8.84	± 0.30	575	±	75.90	73.88	±	2.96
C5	9.43	± 0.02	410	±	14.76	54.74	±	10.78
C6	6.94	± 0.26	280	±	24.92	52.50	±	5.41
C7	9.38	± 0.06	320	±	62.08	94.89	±	10.34
O1	8.09	± 0.12	565	±	113.00	77.77	±	7.15
O2	8.90	± 0.06	645	±	87.08	163.00	±	25.27
O3	8.16	± 0.05	450	±	48.60	60.08	±	3.60
O4	7.49	± 0.01	700	±	29.40	80.03	±	12.32
O5	7.77	± 0.05	640	±	110.72	158.40	±	11.25
O6	7.12	± 0.00	620	±	71.30	75.31	±	3.09

Water samples were extracted using liquid-liquid extraction. Briefly, in a 2-L glass separatory funnel, 30 g of sodium chloride was added to 500 mL water sample and then shaken vigorously until dissolved. Bifenthrin-d_5 (100 ng in 100 µl acetone) was added to each sample as a recovery surrogate. For extraction, 60 mL dichloromethane

was added to each sample, and then the separatory funnel was mixed vigorously by hand for 3 min and left to rest for 1 h. After phase separation, the solvent extract was drained into a round-bottom glass flask through a glass funnel packed with anhydrous sodium sulfate (30 g) to remove residual water. This extraction was repeated for a total of three consecutive times, and the extracts were combined for each sample. The combined extract was then condensed on a Buchi Rotovapor (New Castle, DE) at 40 °C and under vacuum to approximately 5 mL. The sample container was rinsed with 9:1 hexane:acetone (v/v) and the extract was transferred to a glass vial. The final sample was evaporated to near dryness at 40 °C under a gentle stream of nitrogen and then reconstituted in 1.0 mL hexane for analysis on gas chromatography/mass spectrometry (GC/MS).

To extract pyrethroids from suspended solids, sediment, algae, and biofilm, approximately 1.0 g (dry wt.) of each sample was placed in a 40 mL glass vial and spiked with bifenthrin-d_5 (100 ng in 100 µl acetone). For extraction, 10 mL of 1:1 hexane:acetone (v/v) was added to each sample, and then the samples were sonicated for 30 min in a sonication water bath. For cleanup, the extract was loaded onto a 1.0 g Florisil cartridge preconditioned with 6 mL hexane, and the cartridge was eluted with 10 mL of 9:1 hexane:acetone (v/v). The cleaned extract was evaporated to near dryness at 40 °C under a gentle stream of nitrogen and then reconstituted in 1.0 mL hexane for GC/MS analysis.

3.2.4 Chemical Analysis and Quality Control

Sample extracts were analyzed for bifenthrin, fenpropathrin, lambda-cyhalothrin, permethrin, cyfluthrin, cypermethrin, esfenvalerate, and deltamethrin on an Agilent 6890N/5973B GC/MSD in electron ionization mode with a 30 m × 0.25 mm × 0.25 µm DB-5MS column. More detailed analyte information is contained in Table 3.3. The GC oven temperature program was as follows: initial temperature 50 °C held for 1 min; heated to 220 °C at 30 °C/min; heated to 300 °C at 10 °C/min and held for 2 min; and post run hold at 310 °C for 5 min. Injection volume was 1.0 µL and carrier gas (helium) flow rate was 1.0 mL/min. Temperatures for the injector, transfer line, MS source, and MS quadrupole were 250, 280, 230, and 150 °C, respectively. A solvent delay of 6 min was set for protection of the MS filament. Quantitation was performed with an 8-point calibration curve of concentrations ranging from 1 to 1,000 µg/L.

Table 3.3 Physicochemical properties of selected pyrethroid analytes. Log K_{ow}, water solubility, and K_{oc} are from Katagi, (2012).

Pyrethroid	**Molecular weight**	**log K_{ow}**	**Water solubility[a] (ppm)**	**K_{oc} x 10^4 (L/kg)**
Bifenthrin	422.9	8.15	0.1	23.3
Fenpropathrin	349.4	6	0.33	4.23
Permethrin	391.3	6.5	0.006	3.24
Cypermethrin	416.3	6.05	0.004	4.57
Esfenvalerate	419.9	6.2	0.024	5.6

[a] Water solubility at 25 °C.

For quality control and assurance, a calibration standard was run for every 10 samples to check for potential instrumental drift. Relative standard deviations were found to range from 5% to 16%. Bifenthrin-d_5 was added to each sample as a surrogate to measure recovery. The mean recovery was 96 ± 16% and ranged from 79% to 121%.

Method extraction efficiency was determined from the analysis of triplicate samples of water or sediment spiked with the target analytes. Method blanks simultaneously extracted with samples showed negligible matrix effects. Reagent blanks, analyzed after every 10 samples, showed no presence of the target analytes. Method detection limits were determined following EPA Method 40 CFR, Part 136, Appendix B. Reporting limits were determined using the calibration curve concentrations closest to 3 times the MDLs. Detailed analyte MS parameters, method reporting limits, and method extraction efficiencies are given in Table 3.4.

Table 3.4 MS parameters, reporting limits, and method extraction efficiencies. Quantification ions are bolded.

Pyrethroid	**MS target ions (m/z)**	**Retention time (min)**	**Water reporting limit (ng/L)**	**Sediment/algae/biofilm reporting limit[a] (ng/g)**	**Extraction efficiency (%)**
Bifenthrin-d_5[b]	**186**, 170	16.2	4	1	93 ± 13
Bifenthrin	**181**, 165	16.3	2	1	97 ± 16
Fenpropathrin	**181**, 152, 125	16.8	4	1	101 ± 14
Lambda-cyhalothrin	**181**, 197, 152	18.3	10	1	102 ± 11
Permethrin	**183**, 153	20	4	1	96 ± 14
Cyfluthrin	**163**, 127	20.9	10	1	84 ± 21
Cypermethrin	**163**, 181, 127	21.6	10	1	93 ± 24
Esfenvalerate	**167**, 152, 125	23.3	10	1	80 ± 17
Deltamethrin	**181**, 253, 152	24.3	20	1	77 ± 19

[a] Derived from matrix spike tests using sediment.
[b] Used as a recovery surrogate.

3.2.5 Data Analysis

Detection frequencies were calculated using the number of samples in which residues were found above the reporting limit. All concentrations in suspended solids, sediments, algae, and biofilms, were calculated on a dry weight basis. The partitioning coefficient K_d was calculated using $K_d = C_{ss}/C_{dis}$, where C_{ss} and C_{dis} are the concentrations in suspended solids and dissolved in water, respectively. The whole water concentration C_w was defined as the sum of concentration associated with suspended solids (C_{ss}) and that dissolved in water (C_{dis}).

The Mann-Whitney test was used to check for statistically significant differences in concentrations between different storm drain structures, environmental phases, and sampling periods. To evaluate whether environmental data, including TSS, DOC, and K_d, were associated with one another, Spearman's correlation coefficient ρ was calculated for each pair of data types. To estimate potential toxic risks from pyrethroids, toxic units (TUs) were calculated for water and sediment samples using TU = analyte concentration / reference analyte toxicity value. In the case of sediment samples, analyte concentrations were normalized for organic carbon content. Water sample TUs were derived using C_{dis} and previously-determined 96 h toxicity benchmarks for *H. azteca*; median EC50s for impairment of swimming were 3.3, 1.9, and 1.7 ng/L for bifenthrin, cyfluthrin, and cypermethrin, respectively, and median LC50s were 7.7, 2.3, and 2.3 ng/L, respectively (Weston and Jackson, 2009). Sediment sample TUs were derived using pyrethroid concentrations in sediment (C_s) that were organic carbon-normalized (OC-normalized) and previously-determined average 10-day LC_{50}s for *H. azteca*, which were 0.18 μg/g OC

for bifenthrin, 4.87 μg/g OC for permethrin, 1.08 μg/g OC for cyfluthrin, and 0.89 μg/g OC for deltamethrin (Amweg et al., 2005).

3.3 Results and Discussion

3.3.1 Detection Frequencies and Concentrations

In water from storm drain structures, 89% of samples contained pyrethroid concentrations above the reporting limit, and 85% of them contained bifenthrin, the most frequently detected pyrethroid compound. Cyfluthrin was the next most frequently detected, with a detection frequency of 43%. Permethrin, esfenvalerate, and deltamethrin were detected in 7%, 7%, and 4% of the water samples, respectively, while fenpropathrin and lamba-cyhalothrin were not detected. According to pesticide use records from California Department of Pesticide Regulation (California Pesticide Information Portal Application, 2023), in 2021 non-agricultural applications of pyrethroid insecticides in Los Angeles County, bifenthrin was the most dominant in the quantity of active ingredients used, with 3,758 kg applied. Usage of the other pyrethroids ranged from 2,343 kg for deltamethrin to only 2 kg for fenpropathrin (California Pesticide Information Portal Application, 2023). Cyfluthrin was the second least used pyrethroid, with only 206 kg applied in 2021, even though it was detected at a relatively higher frequency. The reported usages of pesticides in California do not count homeowners' use, and pyrethroid-containing products are widely available at retail stores for personal purchases and uses. The under-reporting may have contributed to the discrepancy between pyrethroid detection frequencies and their reported usages in this study. Sampling of

Southern California urban storm drain discharge points and receiving waters performed by California Department of Pesticide Regulation from 2008 to 2019 also consistently showed bifenthrin and cyfluthrin to be among the most frequently detected pyrethroid compounds in water samples (78% and 43%, respectively), with permethrin as another commonly found pyrethroid (Budd et al., 2020). Other studies of urban drainage outfalls and downstream waters in California have consistently identified bifenthrin as the pyrethroid with the highest detection frequency in both water and sediment phases (Ensminger et al., 2013; Hall et al., 2016; Holmes et al., 2008; Weston et al., 2009; Weston and Lydy, 2012). The prevalence of bifenthrin in sediment has also been observed in studies from several other states in the United Sates, including Texas, Illinois, Washington, and Oregon (Ding et al., 2010; Hintzen et al., 2009; Hladik and Kuivila, 2012; Kuivila et al., 2012; Weston et al., 2011). However, pyrethroids were not found in any sediment from 12 urban creeks in Tennessee, and only 2 out of 7 pyrethroids were detected in individual samples (Amweg et al., 2006), suggesting that region-specific pest pressures, and types and quantities of pyrethroids used, influence their occurrence in urban environments.

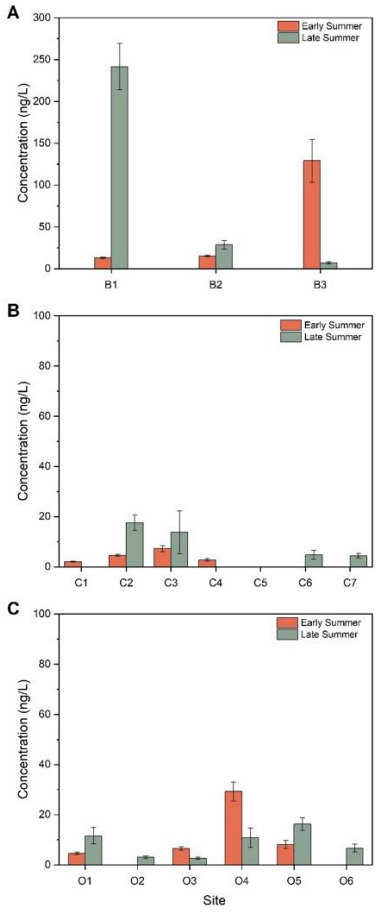

Figure 3.3 Whole water bifenthrin concentrations in A) catch basins, B) open channels, and C) outfalls. Error bars indicate standard deviation.

Figure 3.3 shows bifenthrin concentrations in water samples from different sites. Bifenthrin concentrations at individual sites ranged from <RL to 242 ng/L, while the median concentration was 7 ng/L. Bifenthrin's prevalence at detectable concentrations may be attributed to its heavy use in the Los Angeles County as well its relatively long environmental persistence. The half-lives of bifenthrin in aerobic and anaerobic sediments were found to be longer than those of other pyrethroids. For example, in a laboratory incubation study of 11 pyrethroids, bifenthrin did not reach its half-life even

after 100 days of incubation under anaerobic conditions (Gan et al., 2005; Meyer et al., 2013). Under outdoor conditions, factors such as aging could further prolong the persistence of pyrethroids in the sediment, contributing to their sustained release into the passing water. The concentrations observed for bifenthrin in this study were comparable, albeit with a higher maximum concentration, to those seen in other studies of urban runoff during the dry season in California. A study of urban drain discharges from 2006 to 2007 in Roseville, CA, and Elk Grove, CA, reported dry season median bifenthrin concentrations 5 ng/L and 9 ng/L, respectively, along with maxima of 14 ng/L and 73 ng/L , respectively (Weston et al., 2009). Ensminger et al. (2013) collected water samples from 2008 to 2011 at urban drain outfalls and streams across three urban areas of California and found a dry-season median below the reporting limit and a maximum of 50 ng/L for bifenthrin. Sampling of an engineered conveyance flowing into the San Francisco Bay from 2007 to 2010 showed a maximum bifenthrin concentration of 12 ng/L during low flow conditions (Gilbreath and McKee, 2015).

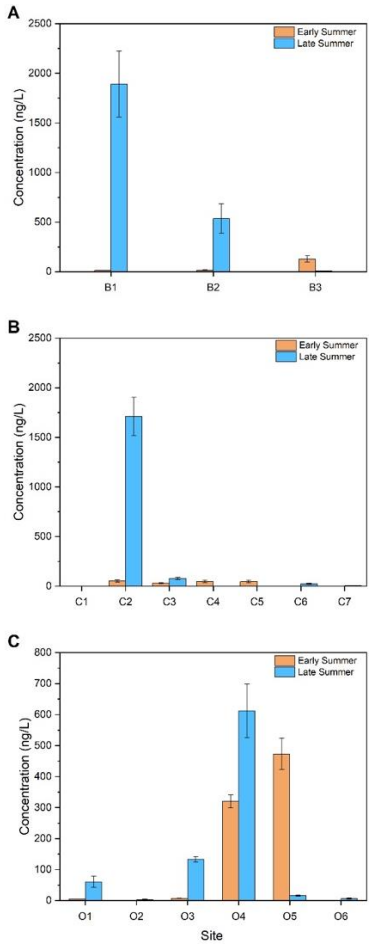

Figure 3.4 Whole water total pyrethroid concentrations in A) catch basins, B) open channels, and C) outfalls. Error bars indicate standard deviation.

Figure 3.4 shows total pyrethroid concentrations in water samples from individual sampling sites. The range of concentrations and median for total pyrethroids were <RL – 1,890 ng/L and 27 ng/L, respectively. Concentrations of cyfluthrin, detected second most frequently, ranged from <RL – 1,540 ng/L. Detections for other pyrethroids were less frequent. Earlier dry-season urban runoff studies in California showed median concentrations for most individual pyrethroids to be below their detection or reporting limits (Weston et al., 2009; Weston and Lydy, 2010). The median concentration of all

pyrethroids observed in the present study was closer to those reported in wet season urban surface waters, which were usually in the 10-100 ng/L range (Budd et al., 2020; Weston et al., 2009; Weston and Lydy, 2012, 2010). The maximum concentration in the present study appeared to differ from those found in other urban stormwater study samples; a few samples collected from urbanized sections of the Sacramento River in CA contained pyrethroid concentrations above 100 ng/L, while most maxima from other studies were in the same order of magnitude as their median concentrations.

Changes in sediment accumulation and the presence of duckweed impacted the availability of samples other than water at each site. Consequently, a total of 4 sediment, 8 algae, and 4 biofilm samples were obtained in this study. Sediment was only present in sufficient quantities for sampling in open channels and outfalls, suggesting that irrigation during the dry season may only introduce small amounts of sediment into certain catch basins. Low amounts of sunlight penetrating into catch basins may explain why biofilms could be sampled but algae were absent. During the sampling period, outfalls became inundated with duckweed, likely preventing biofilm colonization on tiles. C5 was the only site where sediment, algae, and biofilm were available for simultaneous sampling.

In all sediment, algae, and biofilm samples, bifenthrin was detected at concentrations above its reporting limit. In these non-water samples, cyfluthrin, permethrin, esfenvalerate, lambda-cyhalothrin, deltamethrin, and fenpropathrin were detected at 50%, 13%, 13%, 6%, 6%, and 0%, respectively. Bifenthrin concentration ranges and medians were 24-271 ng/g and 83 ng/g for sediment, 10-57 ng/g and 31 ng/g for algae, and 18-3743 ng/g and 72 ng/g for biofilms. For total pyrethroid concentrations,

the ranges and medians were 26-271 ng/g and 88 ng/g for sediments, 14-2,290 ng/L and 356 ng/g for algae, and 18-102,000 ng/g and 3,556 ng/g for biofilms. A recent multi-year evaluation of urban sediments in southern California showed median concentrations of 44 ng/g for bifenthrin and 106 ng/g for total pyrethroids (Budd et al., 2020). In a study comparing pesticides in sediments and biofilms (colonized on unglazed ceramic tiles) from a mixed-use watershed in California, median concentrations for individual pyrethroids in both sample types were below method reporting limits, while the maximum concentrations detected were 14 ng/g for bifenthrin in sediment, 118 ng/g for total pyrethroids in sediment, 30 ng/g for bifenthrin in biofilms, and 150 ng/g for total pyrethroids in biofilms (Mahler et al., 2020).

3.3.2 Distribution and Trends

The Mann-Whitney non-parametric test was used to determine if there were significant differences in concentrations between water samples from different structures, between water samples from different sampling periods, and between sediments, algae, and biofilms. Three types of storm drain structures, i.e., catch basins, open channels, and outfalls, were considered to evaluate whether pyrethroid residues were concentrated in certain parts of the storm drain system. Median concentrations for bifenthrin and total pyrethroids were 22 ng/L and 72 ng/L for catch basins, 4 ng/L and 32 ng/L for open channels, and 7 ng/L and 16 ng/L for outfalls. Bifenthrin concentrations in water from catch basins were significantly higher than those from channels ($p < 0.05$) or outfalls ($p < 0.05$), but significant differences in total pyrethroid concentrations were not observed

between the structures. Underground catch basins, with the trapped sediment, also acted as a secondary source for supplying pyrethroids to the flowing-through water. In comparison, above-ground open channels and outfalls, likely due to the input of other water sources and their transient nature, would usually have lower concentrations than in the underground catch basins. However, it must be noted that during the dry season, urban drainage is often intermittent, and the lack of hydrological connectivity between sampled sites limits the general extrapolation of our findings to other scenarios. Local patterns in land use and pesticide applications may differ significantly and explain the variance in site concentrations. During the wet season or in areas where urban drainage is continuous, it may be expected that the concentrations along the entire urban drain path may be more homogeneous, with the overall discharge at outfalls to be greater due to the decreased role of catch basins in retaining debris and sediments. Likewise, as underground storm catch basins are numerous in numbers and large in dimensions in regions such as Los Angeles, it may be expected that the first few significant rainfall events following the dry season would play a role to remobilize the accumulated sediment and the associated pyrethroid residues, contributing to substantial "first-flush" loadins of these and other contaminants to downstream aquatic systems.

Pyrethroid concentrations in water collected during tile deployment (June 25-27, 2021) were similar to those in water collected later in the summer (August 24-26, 2021). No statistically significant differences were observed for bifenthrin or total pyrethroids. Median bifenthrin concentrations in June (7 ng/L) and August (7 ng/L) were similar, though median total concentrations in June (31 ng/L) were higher than in August (22

ng/L). According to the pesticide use reporting data for urban sites in Los Angeles County in 2021, about 379 kg of bifenthrin was applied in June, and 263 kg was applied in August. For the eight pyrethroids targeted for analysis in this study, a total of 887 kg was applied in June, and 702 kg was applied in August (California Pesticide Information Portal Application, 2023). Though permethrin, cypermethrin, esfenvalerate, and deltamethrin were only detected in water collected in August, reported use in Los Angeles County for these compounds was similar between June and August. Overall, there were no clear correlations between county-level pyrethroid usage and observed concentrations. It is possible that use data on a finer scale may be more closely correlated, however, the persistence of pyrethroids in sediments, along with the lag time associated with offsite movement of pesticide residues, may further complicate the association.

Differences in detection frequencies and concentrations between environmental phases may improve our understanding of pyrethroid partitioning among different compartments. The median number of pyrethroid compounds detected at levels above reporting limits was 1.5 for sediments, 2 for algae, and 2.5 for biofilms. Mahler et al. (2020) measured 96 pesticides and found a more pronounced difference in median numbers of pesticides detected between biofilms (2) and sediments (0.5). However, the median number of pyrethroids detected in that study was 0 for both sample types. In the present study, concentrations in sediment, algae, and biofilms were not significantly different. Biofilm samples from basins 5 and 7 and channel 1, along with algae from channels 1 and 7, all had total pyrethroid concentrations above 1,000 ng/g. The biofilm

sample from basin 7 had the highest bifenthrin concentration (3,743 ng/g), whereas biofilm from basin 5 contained exceptionally high levels of esfenvalerate (32,079 ng/g) and deltamethrin (69,705 ng/g). Cyfluthrin was the major contributor to residues in biofilm and algae samples from channel 1 (1,839 ng/g and 1,585 ng/g, respectively) and algae from channel 7 (2,263 ng/g). These elevated detection frequencies and maximum concentrations in biofilm and algae suggested that there was a preferential enrichment of pyrethroids in these biological matrices. Algae and biofilms serve as the food source for a wide variety of organisms, including midges and other aquatic invertebrates that use such environments as their habitats (Parkyn et al., 2005). Although beyond the scope of this study, urban drainage systems, with stagnant water and rich organic matter, are breeding grounds for vectors such as mosquitoes (Kluh et al., 2001). Exposure of larval mosquitoes to trace levels of pyrethroids through water, algae, and biofilm may contribute to the evolution of insecticide resistance, presenting a challenge to the management of vectors and vector-borne diseases (Poupardin et al., 2012). The potential association of pyrethroid presence in USDS and insecticide resistance, however, in poorly understood and merits further research,

Partitioning within water samples was further evaluated (Tables 3.5 and 3.6). Overall, TSS ranged from 1.3 to 30.7 mg/L in the different water samples, and the apparent K_d values ranged from 1.9×10^3 to 2.09×10^6 L/kg. While no statistically significant differences were observed in K_d between sampling sites or between sampling time points, the median K_d for water from aboveground open channels (1.14×10^6 L/kg) was higher than those in the catch basins (1.89×10^5 L/kg) or drainage outfalls (9.5×10^4

L/kg). The mean % of pyrethroids associated with the suspended solids was 77% for open channels, 55% for catch basins, and 31% for outfalls. Elevated pyrethroid concentrations in suspended solids in open channels was the primary driver for the differences. The predominant association of pyrethroids with suspended solids in runoff drainage channels and catch basins highlights the importance of solid particles in the overall transport of pyrethroid residues form their application sites around residential homes to downstream urban surface streams.

Table 3.5 Total suspended solids, total pyrethroid concentrations, and pyrethroid partitioning in water samples. Values are expressed as mean ± standard deviation. TSS, pyrethroid concentrations in whole water (C_w), concentrations in aqueous phase (C_{dis}), and concentrations in suspended sediment (C_{ss}). B = catch basin; C = open channel; O = outfall.

Site	TSS (mg/L)	C_w (ng/L)	C_{dis} (ng/L)	C_{ss} (ng/g)
June 2021				
B1	10.0 ± 1.8	13.0 ± 0.9	10.6 ± 0.6	260 ± 42.6
B2	3.1 ± 0.5	15.4 ± 2.9	9.4 ± 2.5	1900.0 ± 373
B3	10.5 ± 3.0	129 ± 29.9	30.5 ± 4.8	9400.0 ± 760
C1	1.3 ± 0.1	2.1 ± 0.3	2.1 ± 0.3	<RL
C2	9.0 ± 2.4	52.1 ± 13.6	4.6 ± 1.2	5300.0 ± 555.0
C3	1.6 ± 0.2	31.2 ± 5.2	7.2 ± 0.5	15100.0 ± 2610.0
C4	11.3 ± 2.4	47.8 ± 12.9	2.8 ± 0.1	3980.0 ± 780.0
C5	6.2 ± 0.8	47.4 ± 13.7	<RL	7600.0 ± 1940.0
O1	10.2 ± 3.0	4.6 ± 0.3	4.6 ± 0.3	<RL
O2	8.0 ± 1.9	<RL	<RL	<RL
O3	5.5 ± 0.7	6.6 ± 1.0	2.8 ± 0.6	689.0 ± 110.0
O4	14.4 ± 1.9	320.0 ± 21.4	261.0 ± 18.9	4070.0 ± 425.0
O5	12.8 ± 1.3	473.0 ± 50.3	462.0 ± 50.2	870.0 ± 204.0
August 2021				
B1	13.8 ± 1.3	1890.0 ± 333.0	549.0 ± 46.8	97400.0 ± 22200.0
B2	6.8 ± 1.0	535.0 ± 149.0	48.7 ± 5.2	71300.0 ± 19000.0
B3	4.3 ± 0.6	6.9 ± 1.1	4.3 ± 0.8	610.0 ± 137.0
C1	4.9 ± 0.4	<RL	<RL	<RL
C2	30.7 ± 4.4	1710 ± 193	1040.0 ± 114.0	21900.0 ± 4000.0
C3	15.1 ± 7.6	78.2 ± 10.7	71.8 ± 10.2	424.0 ± 33.8
C5	7.3 ± 0.5	<RL	<RL	<RL
C6	5.3 ± 1.7	22.4 ± 6.4	3.2 ± 0.9	3660.0 ± 228.0
C7	20.8 ± 3.5	4.4 ± 0.7	3.2 ± 0.6	57.7 ± 2.9
O1	6.7 ± 4.8	60.7 ± 18	37.2 ± 3.4	3530.0 ± 701.0
O2	10.5 ± 6.6	3.1 ± 1.4	1.0 ± 0.3	198.0 ± 21.8
O3	4.5 ± 0.6	133 ± 8.8	102.0 ± 6.7	6920.0 ± 885.0
O4	8.5 ± 2.5	612 ± 87.1	473.0 ± 76.2	16400.0 ± 1280.0
O5	17.9 ± 2.3	16.3 ± 1.9	2.8 ± 0.3	750.0 ± 49.3
O6	2.7 ± 0.5	6.7 ± 1.2	2.9 ± 0.6	1380.0 ± 275.0

Table 3.6 Percentage of pyrethroid residues associated with aqueous phase, percentage of residues associated with suspended sediment, and partitioning coefficients (K_d). Values are expressed as mean ± standard deviation. B = catch basin; C = open channel; O = outfall.

Site	Dissolved Phase (%)	Suspended Sediment Phase (%)	K_d (x 10^3 L/kg)
		June 2021	
B1	82.1 ± 7.3	17.9 ± 7.3	24.4 ± 4.2
B2	61.5 ± 20.1	38.5 ± 20.1	201 ± 66.4
B3	23.6 ± 6.6	76.4 ± 6.6	308 ± 54.9
C1	-	-	-
C2	8.9 ± 3.2	91.1 ± 3.2	1140.0 ± 316.0
C3	23.1 ± 4.2	76.9 ± 4.2	2090.0 ± 391.0
C4	5.8 ± 1.6	94.2 ± 1.6	1430.0 ± 289.0
C5	-	-	-
O1	-	-	-
O2	-	-	-
O3	42.4 ± 11.7	57.6 ± 11.7	247.0 ± 69.5
O4	81.7 ± 8.0	18.3 ± 8	15.6 ± 2.0
O5	97.6 ± 14.8	2.4 ± 14.8	1.9 ± 0.5
		August 2021	
B1	29 ± 5.7	71 ± 5.7	178.0 ± 43.2
B2	9.1 ± 2.7	90.9 ± 2.7	1470.0 ± 421.0
B3	61.9 ± 15.3	38.1 ± 15.3	142.0 ± 42.0
C1	-	-	-
C2	60.7 ± 9.5	39.3 ± 9.5	21.1 ± 4.5
C3	91.8 ± 18.1	8.2 ± 18.1	5.9 ± 1.0
C5	-	-	-
C6	14.2 ± 5.6	85.8 ± 5.6	1150.0 ± 322.0
C7	73 ± 17.6	27 ± 17.6	17.9 ± 3.5
O1	61.3 ± 19	38.7 ± 19	95.0 ± 20.7
O2	33.1 ± 17.3	66.9 ± 17.3	193.0 ± 58.3
O3	76.8 ± 7.2	23.2 ± 7.2	67.7 ± 9.7
O4	77.2 ± 16.6	22.8 ± 16.6	34.8 ± 6.2
O5	17.4 ± 2.9	82.6 ± 2.9	266.0 ± 36.3
O6	44.1 ± 11.5	55.9 ± 11.5	468.0 ± 130.0

Spearman correlation analysis showed a strong association between DOC and TSS in water samples (Spearman's $\rho = 0.61$, $p < 0.01$), between C_w and C_{ss} (Spearman's $\rho = 0.79$, $p < 0.01$), between C_w and C_{dis} (Spearman's $\rho = 0.88$, $p < 0.01$), and between C_{dis} and C_{ss} (Spearman's $\rho = 0.57$, $p < 0.05$). The correlations between C_w, $C_{dis,}$ and C_{ss} supported the notion that elevated concentrations in one phase (e.g., suspended solids) of a sample would also translate to elevated levels in another phase (e.g., dissolved phase) due to equilibrium partition. Dissolved concentrations, as opposed to those in suspended solids, were likely a more reliable predictor of the relative distribution of pyrethroid residues in whole water samples, as TSS levels (median = 8.24 ± 6.37 mg/L) varied drastically among types of samples as well as sampling sites.

3.3.3 Ecological Risks

Using benchmark acute EC_{50} and LC_{50} values for *H. azteca*, TUs were calculated for bifenthrin, cyfluthrin, and cypermethrin based on the observed concentrations in water to estimate the potential risk to sensitive aquatic organisms (Weston and Jackson, 2009) (Tables 3.7 and 3.8). While TUs over 1 (i.e., OC-normalized concentrations greater than the toxicity benchmark) may not always result in a loss of ecosystem biodiversity, multiple ecotoxicological studies have associated this metric with invertebrate species reduction and community impairment (Iwasaki et al., 2020; Liess and Ohe, 2005; Rogers et al., 2016). Median TUs for bifenthrin were 1.3 (EC_{50}) and 0.5 (LC_{50}), and median total TUs were 1.4 (EC_{50}) and 0.6 (LC_{50}), indicating that most water samples posed potential risks for impairment, although not necessarily lethality. A total of 8 samples had

combined TUs over 10; all of these samples, except for water collected in August 2021 from outfall 1, consistently had high cyfluthrin concentrations.

Table 3.7 Toxic units calculated using dissolved pyrethroid concentrations in water from each site and EC50 values for H. azteca (Weston and Jackson, 2009). Values are expressed as mean ± standard deviation. BIF = bifenthrin; CYF = cyfluthrin; CYP = cypermethrin; B = catch basin; C = open channel; O = outfall.

	TU_{water} (EC_{50})			
Site	**BIF**	**CYF**	**CYP**	**$\Sigma_{TU,water}$**
		June 2021		
B1	3.2 ± 0.2	-	-	3.2 ± 0.2
B2	2.9 ± 0.8	-	-	2.9 ± 0.8
B3	9.2 ± 1.5	-	-	9.2 ± 1.5
C1	0.6 ± 0.1	-	-	0.6 ± 0.1
C2	1.4 ± 0.4	-	-	1.4 ± 0.4
C3	2.2 ± 0.2	-	-	2.2 ± 0.2
C4	0.8 ± 0.0	-	-	0.8 ± 0.0
C5	-	-	-	-
O1	1.4 ± 0.1	-	-	1.4 ± 0.1
O2	-	-	-	-
O3	0.8 ± 0.2	-	-	0.8 ± 0.2
O4	6.0 ± 0.4	127.0 ± 10.2	-	133 ± 10.6
O5	1.7 ± 0.2	240.0 ± 24.0	-	241.7 ± 24.2
		August 2021		
B1	72.4 ± 6.2	41.6 ± 4.1	-	114.0 ± 10.3
B2	7.7 ± 0.8	-	-	7.7 ± 0.8
B3	1.3 ± 0.2	-	-	1.3 ± 0.2
C1	-	-	-	-
C2	1.9 ± 0.2	544.0 ± 57.9	-	545.9 ± 58.1
C3	2.2 ± 0.3	33.9 ± 4.3	-	36.1 ± 4.6
C5	-	-	-	-
C6	1.0 ± 0.3	-	-	1.0 ± 0.3
C7	1.0 ± 0.2	-	-	1.0 ± 0.2
O1	1.2 ± 0.1	-	19.5 ± 3.0	20.7 ± 3.1
O2	0.3 ± 0.1	-	-	0.3 ± 0.1
O3	0.3 ± 0.0	53.3 ± 4.6	-	53.6 ± 4.6
O4	2.1 ± 0.3	245.0 ± 41.1	-	247.1 ± 41.4
O5	0.9 ± 0.1	-	-	0.9 ± 0.1
O6	0.9 ± 0.2	-	-	0.9 ± 0.2

Table 3.8 Toxic units calculated using dissolved pyrethroid concentrations in water from each site and LC50 values for H. azteca (Weston and Jackson, 2009). Values are expressed as mean ± standard deviation. BIF = bifenthrin; CYF = cyfluthrin; CYP = cypermethrin; B = catch basin; C = open channel; O = outfall.

	TU_{water} (LC_{50})			
Site	**BIF**	**CYF**	**CYP**	**$\Sigma_{TU,water}$**
		June 2021		
B1	1.4 ± 0.1	-	-	1.4 ± 0.1
B2	1.2 ± 0.3	-	-	1.2 ± 0.3
B3	4.0 ± 0.6	-	-	4.0 ± 0.6
C1	0.3 ± 0.0	-	-	0.3 ± 0.0
C2	0.6 ± 0.2	-	-	0.6 ± 0.2
C3	0.9 ± 0.1	-	-	0.9 ± 0.1
C4	0.4 ± 0.0	-	-	0.4 ± 0.0
C5	-	-	-	-
O1	0.6 ± 0.0	-	-	0.6 ± 0.0
O2	-	-	-	-
O3	0.4 ± 0.1	-	-	0.4 ± 0.1
O4	2.6 ± 0.2	105.0 ± 8.5	-	107.6 ± 8.6
O5	0.7 ± 0.1	199.0 ± 19.8	-	199.7 ± 19.9
		August 2021		
B1	31.0 ± 2.6	34.3 ± 3.4	-	65.3 ± 6.0
B2	3.3 ± 0.3	-	-	3.3 ± 0.3
B3	0.6 ± 0.1	-	-	0.6 ± 0.1
C1	-	-	-	-
C2	0.8 ± 0.1	449.0 ± 47.8	-	449.8 ± 47.9
C3	1.0 ± 0.1	28.0 ± 3.5	-	29.0 ± 3.7
C5	-	-	-	-
C6	0.4 ± 0.1	-	-	0.4 ± 0.1
C7	0.4 ± 0.1	-	-	0.4 ± 0.1
O1	0.5 ± 0.0	-	14.4 ± 2.2	14.9 ± 2.2
O2	0.1 ± 0.0	-	-	0.1 ± 0.0
O3	0.1 ± 0.0	44.0 ± 3.8	-	44.1 ± 3.8
O4	0.9 ± 0.1	203.0 ± 34.0	-	203.9 ± 34.1
O5	0.4 ± 0.0	-	-	0.4 ± 0.0
O6	0.4 ± 0.1	-	-	0.4 ± 0.1

For sediment samples, 10-day LC_{50} values for *H.* azteca were used to estimate TUs for pyrethroids in sediment (Amweg et al., 2005). Sediment TOC content, OC-normalized concentrations, and TUs are listed in Table 5. The range for $\Sigma_{TU,sediment}$ was 5.1-47.0; all 4 sediment samples posed significant toxicity to *H. azteca*. Amweg et al. (2005) compared total pyrethroid TUs in sediment with measured toxicity to *H. azteca* and found near total mortality when TUs were over 3. For the sediment samples considered in this study, bifenthrin was the dominant contributor of TUs. Earlier studies on sediments collected from urban regions in the United States also showed bifenthrin to be a major contributor to sediment toxicity, even when considering other classes of insecticides (Ding et al., 2010; Hintzen et al., 2009; Hladik and Kuivila, 2012; Huff Hartz et al., 2019; Mahler et al., 2020; Weston et al., 2011). In a study of 99 Australian urban wetlands, bifenthrin was detected in three quarters of the wetlands, and the associated TUs were the most predictive of toxicity. The bifenthrin associated toxicity was found to be a smaller contributor to sediment toxicity in other studies. For example, abamectin, fiproles, and cypermethrin were found to be the major sources of toxicity in an urban creek in Guangzhou, southern China (Li et al., 2013). Although the estimated toxicity from sediments in storm catch basins should not be equated to potential toxicity in urban streams receiving urban drainage, the high pyrethroid loads in underground catch basins may be expected to contribute greatly when rainfall-induced runoff remobilizes the trapped sediments downstream. In addition, periodical sediment-based transport of hydrophobic contaminants also occurs during the dry season, driven by irrigation intensity that is influenced by weather conditions (Gilbreath and McKee, 2015).

Furthermore, any potential toxicity from pyrethroids in water or sediment is likely to be compounded by the presence of other contaminants associated with urban runoff, such as other urban-use insecticides, metals, and PAHs (Hintzen et al., 2009; Hoffman et al., 1984; Walker et al., 1999).

Apart from effects on aquatic ecosystems, storm drains in regions with significant mosquito activity, including many tropical, sub-tropical and temperate regions, are significant habitats for the aquatic larvae of certain species (Arana-Guardia et al., 2014; Gao et al., 2018; Kluh et al., 2001; Seidahmed and Eltahir, 2016), including vectors of diseases, such as dengue fever and St. Louis encephalitis (Gubler, 1998; Marfin et al., 1993). Tolerance to pyrethroids, one of the main insecticides used for mosquito control, has been observed at varying levels in *Aedes* and *Culex* mosquitoes throughout the world (Scott et al., 2015; Smith et al., 2016). While associated fitness costs for resistance, which impose ecological disadvantages compared to susceptible phenotypes, limit resistance gene fixation (Kliot and Ghanim, 2012), environmental conditions like temperature and contaminants may encourage resistance development. Larval LC_{50}s for *Cx. quinquefasciatus* often range in the 1,000s-10,000s of ng/L, suggesting that there would be little selection pressure from pyrethroids in most storm drains (Shi et al., 2015; Zubair et al., 2022). However, pyrethroid concentrations may be elevated after periods of high flow, and larvae may also be exposed via contaminated food sources, such as algae and biofilms (Howland, 1930; Mahajan et al., 2011). A study selecting larval *Ae. aegypti* with 4,000-5,500 ng/L of permethrin over multiple generations showed that an additional pre-exposure to 400 ng/L permethrin resulted in a higher proportion of detoxification genes

being over-prescribed (Poupardin et al., 2012), implying that sublethal concentrations may have long-term effects on mosquito populations. The role of ubiquitous presence of pyrethroids in mosquito habitats, including urban catch basins, in insecticide resistance evolution needs to be better understood through experiments under controlled conditions and surveys of resistant mosquito strains in impacted urban areas.

3.4 Conclusion

Even though USDS is an important and prevalent feature of highly urbanized environments, occurrence and distribution of urban-use insecticides such as pyrethroids in USDS have been generally overlooked. In this study, water, sediment, algae, and biofilm samples were collected during dry season along urban drainage transects in Los Angeles, California. The vast majority of water samples contained various levels of pyrethroids, with bifenthrin and cyfluthrin detected most frequently. The simultaneous evaluation of water from catch basins, open channels, and outfalls demonstrated suspended solids as an important carrier of pyrethroid residues. Elevated levels of pyrethroids were found in sediment, algae, and biofilms along the urban drainage pathway, highlighting that sediments temporarily trapped in catch basins serve as a secondary source to supply pyrethroids to runoff water passing through the urban drainage systems, as well as a significant source for contaminant input during the initial phase of wet season. The finding of high levels of pyrethroids in algae and biofilms underscores ecotoxicological effects potentially beyond those derived from water or sediment-focused assays. Additionally, urban drainage systems are common habitats for

vectors such as mosquitos, and the ubiquitous occurrence of pyrethroids may exert selection pressure, potentially contributing to the evolution of insecticide resistance and posing challenges to public health.

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Chapter 4 Effects of larval Culex quinquefasciatus exposure to low concentrations of bifenthrin on adult pyrethroid resistance

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4.1 Introduction

Pyrethroids are widely used to control adult mosquito populations. For example, permethrin is one of the primary active ingredients for low volume sprays in urban settings. However, pyrethroid resistance has been observed with an increasing frequency, such as in a survey of sodium channel mutations in *Culex* mosquitoes in California (Yoshimizu et al., 2020). Pyrethroid resistance may render many pest control operations ineffective, as only a few other adulticides, i.e., organophosphates, are available and used sparingly by vector control professionals in the United States (McGregor and Connelly, 2021).

Previous research has shown that pyrethroids used on other insects frequently contaminate urban runoff water. After settling onto loose particles, pyrethroid residues may be transported with irrigation or rain-induced runoff into underground storm drains, with low levels of pyrethroids (up to 73 ng/L in water samples) being released into water bodies downstream of storm drain outfalls (Jiang and Gan, 2012; Weston et al., 2009). It has been well documented that contamination of creek and stream sediment may result in toxicity to aquatic organisms such as fish and benthic invertebrates (Carpenter et al.,

2016). In other settings, pyrethroid use has been associated with resistance in local populations of mosquitoes (Hien et al., 2017). Pyrethroids may be retained for an extended period in storm drains because of their strong affinity for solids and organic matter, and the general low light exposure and low ambient temperature further prolongs the persistence of pyrethroids. These conditions also favor mosquito breeding, and therefore urban underground catch basins are common mosquito breeding habitats and the target for larvicide applications. It may be hypothesized that exposure of mosquitoes to low levels of pyrethroid residues in these habitats provides selection pressure for pyrethroid resistance evolution in mosquitoes in urban areas.

While pyrethroid contamination of urban runoff has been extensively reported, there are few studies documenting concentrations in storm drain structures where mosquitoes may breed (Amweg et al., 2006; Bereswill et al., 2013). Our previous investigation of urban catch basins in California showed an especially high detection frequency of bifenthrin, a pyrethroid that is not generally used to control mosquitoes, with a median aqueous concentration of 20 ng/L and a maximum water concentration of 324 ng/L (Sy et al., 2022). In surface water samples, pyrethroids are most often detected in the 10s to 100s of ng/L. Some studies have found concentrations even in the 1,000s to 10,000s of ng/L (Tang et al., 2018).

Experimental studies have shown that populations of mosquito larvae show increased resistance after selection pressure, and the rate of resistance development varies with the intensity of selection (Machani et al., 2020; Shi et al., 2015). Exposure to permethrin concentrations as low as 4,000 ng/L has been shown to increase larval

permethrin resistance for a susceptible colony within 10 generations (Poupardin et al., 2012). Most studies of pyrethroid selection pressure have focused on resistance in the same life stage that mosquitoes are exposed (Saavedra-Rodriguez et al., 2012). Also, previously used methods of larval pesticide selection may not reflect environmental conditions. Compared to contaminated aquatic habitats where larvae may be constantly exposed, experiments often limit exposures to the latter stages of larval development to facilitate the measurement of larval mortality (Poupardin et al., 2012; Shi et al., 2015). Many exposure concentrations are continuously increased over the course of a study and thus are much higher than what has been measured in the environment.

This study attempted to simulate exposure conditions reflecting those observed in the urban environment to evaluate the effects of larval exposure to these residues and their implications for the control of adult mosquito populations. Strains of mosquitoes were collected from urban areas, and treatment strains' larvae were reared in water spiked with a low concentration of bifenthrin, one of the most common pyrethroids found in contaminated stormwater. Several bioassays were performed to assess the strains' resistance to permethrin and the role of the L1014F sodium channel mutation (*kdr*) and metabolism facilitated by cytochrome P450 enzymes (abbreviated as P450s or CYPs), which are the primary mechanisms of resistance in *Culex* mosquitoes (Scott et al., 2015). Based on previous experimental research on pyrethroid selection pressure in mosquitoes, it was expected that exposure to bifenthrin would result in maintained or increased resistance and that unexposed controls would exhibit a loss of resistance (Poupardin et al., 2012; Shi et al., 2015). Such a finding would contribute to establishing a connection

between the ubiquitous pyrethroid contamination in runoff and in the prevalence of pyrethroid resistance in mosquitoes.

4.2 Materials and Methods

4.2.1 Chemicals and Materials

Analytical standards of permethrin (94.2%) and piperonyl butoxide (PBO; 99.0%) were purchased from MilliporeSigma (St. Louis, MO). Insect cages used for housing adult mosquitoes were purchased from BugDorm (Taichung, Taiwan). Aspirators used for transferring adult mosquitoes between containers were purchased from John W. Hock Company (Gainesville, FL). Chicken blood in citrate used in mosquito blood feeding was purchased from HemoStat (Dixon, CA). DNeasy Blood and Tissue Kits used for extracting DNA were purchased from Qiagen (Hilden, Germany). Primers for PCR were purchased from Integrated Device Technology (San Jose, CA). Acetone, Invitrogen PCR mastermix, and ExoSAP-IT PCR product cleanup reagent were purchased from Thermo Fisher Scientific (Waltham, MA).

4.2.2 Mosquito populations

Two strains of *Cx. quinquefasciatus* were established from field collections of urban mosquitoes in southern California (Figure 4.1). The first strain, LA, was established in early October 2022 using egg rafts collected from 12 traps in a region of the Greater Los Angeles metropolitan area overseen by Greater Los Angeles County Vector Control District. The other strain, CV, was established in late October 2022 using

egg rafts collected from a trap in La Quinta, CA, in a region overseen by Coachella Valley Mosquito and Vector Control District. More information on mosquito collection, including trap locations, is detailed in Table 4.1. Egg rafts of a pyrethroid-susceptible reference strain (CQ1) were obtained from Coachella Valley Mosquito and Vector Control District. CQ1 was originally collected in Merced County in the 1950s and has been continuously maintained in multiple laboratories (Samra et al., 2012). All colonies used in the study were reared in a room in the Insectary & Quarantine Facility at the University of California, Riverside.

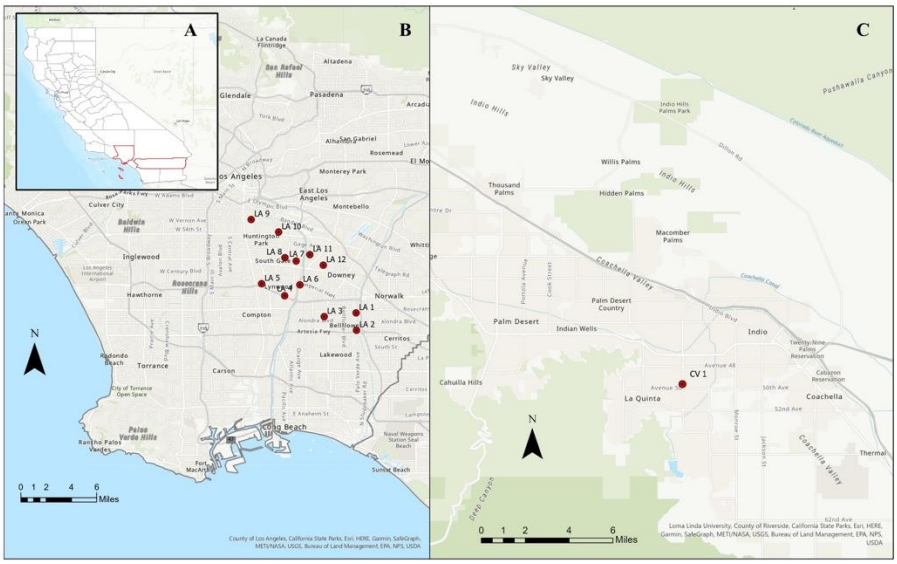

Figure 4.1 A) Location of Los Angeles and Riverside Counties within California. B) Location of mosquito traps in Los Angeles County from which egg rafts were used to establish LA strain. C) Location of mosquito trap in Riverside County from which egg rafts were used to establish CV strain.

Table 4.1 Locations of traps used to collect mosquitoes.

Site ID	City	Latitude	Longitude
LA 1	Bellflower, CA	33.89861	-118.11042
LA 2	Bellflower, CA	33.878738	-118.109978
LA 3	Paramount, CA	33.89438	-118.14691
LA 4	Lynwood, CA	33.91823	-118.19223
LA 5	Lynwood, CA	33.93206	-118.21865
LA 6	South Gate, CA	33.9307643	-118.174674
LA 7	Bell Gardens, CA	33.9580114	-118.1790586
LA 8	Bell Gardens, CA	33.9618498	-118.1919068
LA 9	Los Angeles, CA	34.0055086	-118.2305852
LA 10	Maywood, CA	33.9912109	-118.1991313
LA 11	Bell Gardens, CA	33.9653577	-118.1633931
LA 12	Downey, CA	33.9532622	-118.1479815
CV 1	La Quinta, CA	33.6882459	-116.2689837

Room conditions were maintained throughout the duration of the study as follows: temperature 27 °C, relative humidity 60%, and a light:dark cycle of 14:10 hr (with a 1 hr red-light overlap period). Mosquito larvae were raised in glass pans (43 cm × 28 cm × 6.4 cm) filled with 3 L tap water and fed with fish food ad libitum. Tap water was degassed for 24 hr prior to placement in pans to remove residual chlorine. On a daily basis, water was added to pans to maintain a consistent volume, and pans were gently stirred with stainless steel spoons to prevent biofilm formation. Pupae were collected into cups and placed in nylon net cages (32.5 cm × 32.5 cm × 32.5 cm; 650 µm aperture) for adults to emerge. Adult mosquitoes were reared in net cages and fed with 10% honey water (w/w). To obtain egg rafts for subsequent generations, mosquitoes were fed chicken blood (with sodium citrate added as an anticoagulant) via a Hemotek membrane

feeding system (Blackburn, United Kingdom). After blood-feeding, cups were placed in cages for oviposition, and egg rafts were transferred from cups to clean glass pans. To limit bottleneck effects, each generation of each population was seeded with 20 egg rafts.

4.2.3 Bifenthrin selection

Field-collected strains were each separated into treatment (LA treatment, CV treatment) and control (LA control, CV control) populations prior to exposures. LA was reared for 2 generations and CV was reared for 1 generation prior to the beginning of treatment. The last generation of each strain prior to the beginning of the treatment was designated as F_0. For each strain, 3 replicate lineages were reared. For treatment populations, bifenthrin dissolved in acetone was spiked into each pan at a concentration of 340 ng/L prior to egg rafts being introduced. For control lineages, an equivalent volume of pure acetone was added into each pan. Treatment populations were exposed for 3 consecutive generations (F_1, F_2, and F_3). All populations were reared in parallel.

4.2.4 Permethrin bottle bioassays

Modified Centers for Disease Control and Prevention (CDC) permethrin bottle bioassays were conducted for each generation of adult mosquitoes (CDC, 2022). For each population, 20-25 female adults (2-5 days old) were transferred with aspirators to 250 mL (8 oz) glass bottles. Each bottle was spiked with 1 mL of acetone containing a test concentration of permethrin and then rotated continuously until the solvent had completely evaporated. Prior to the treatment, a range finding test was performed to

determine a range of permethrin concentrations that resulted in approximately 10 – 90% 24 hr mortality for each strain. Bottle bioassay test concentrations (expressed as µg/mL permethrin in solution) ranged from 0.2 – 3.2 µg/mL for CQ1, 1 – 16 µg/mL for LA control and treatment, and 4 – 64 µg/mL for CV control and treatment (Table 4.2). Vehicle controls were spiked with 1 mL of acetone without permethrin. All vehicle controls and bioassay bottles were performed in triplicate for each population.

Table 4.2 Permethrin test concentrations in bottle bioassays. PBO = piperonyl butoxide

Strain	**Without PBO (µg/mL)**	**With PBO (µg/mL)**
CQ1	0.2, 0.4, 0.8, 1.6, 3.2	0.4, 1.6
LA control, LA treatment	1, 2, 4, 8, 16	2, 8
CV control, CV treatment	4, 8, 16, 32, 64	8, 32

After 1 hr of exposure, knockdown in each bottle was recorded. Mosquitoes were considered knocked down if they could not stand upright. Mosquitoes were then transferred to clean containers with no pesticide and provided cotton rounds soaked in 10% sugar water (w/w). After 24 hrs post-exposure, mortality in each clean container was recorded.

4.2.5 Piperonyl butoxide synergist bottle bioassays

Synergism tests with piperonyl butoxide (PBO), a cytochrome P450-inhibitor, were conducted to determine the effect of cytochrome P450 in a population's resistance. A separate set of glass bottles were spiked with 1 mL of acetone containing 400 µg of PBO and rotated until the acetone had evaporated. For each population, prior to

permethrin exposure, approximately 175 mosquitoes were introduced into a glass bottle containing PBO and kept for 1 hr before being transferred to a clean glass bottle. After 1 hr, mosquitoes were transferred to glass bottles with permethrin, and bottle bioassays were performed in a similar fashion to bioassays without pre-exposure to PBO, though a smaller range of test concentrations of permethrin was used for each strain (Table 4.2). Three replicates of each test concentration and one replicate of each vehicle control were included for each population.

4.2.6 DNA extraction and kdr allele detection

Adult females 2-5 days old from each strain were collected and frozen at −80 °C prior to DNA extraction. Genomic DNA was extracted from F_0 and F_3 mosquitoes using DNeasy Blood and Tissue Kits following the manufacturer's instructions. DNA quality and concentration were measured on a BioTek Epoch 2 microplate spectrophotometer (Agilent, Palo Alto, CA). Primers Cpp1 (forward) and Cpp2 (reverse) used for amplification of the *para*-sodium channel gene were obtained from a previous study of knockdown resistance molecular ecology in *Cx. pipiens pallens* in China (Chen et al., 2010).

PCR reactions were prepared in 25 μL total volumes with 12.5 μL of Invitrogen PCR mastermix, 1 μL each of primers Cpp1 and Cpp2, 1 μL of DNA extract (30-120 ng), and 9.5 μL of nuclease-free water. Thermocycler parameters were as follows: 94 °C for 5 min, 40 cycles of 94 °C for 40 s, 58 °C for 50 s, 72 °C for 40 s, and final extension at 72 °C for 8 min. The presence of PCR products was verified via electrophoresis on 2.0%

agarose gels. Confirmed PCR products were purified with ExoSAP-IT and submitted to the Genomics Core facility at the University of California, Riverside, for Sanger sequencing.

4.2.7 Data Analysis

PoloPlus software (LeOra Software Company, Parma, MO) was used to determine permethrin KC_{50} (the concentration producing 1 hr knockdown in 50% of mosquitoes) and LC_{50} (the concentration producing 24 hr mortality in 50% of mosquitoes) for bottle bioassays. Permethrin resistance ratios for KC_{50} (RR_{KC50}) and LC_{50} (RR_{LC50}) were calculated with the equation RR = (KC_{50} or LC_{50} of population) / (KC_{50} or LC_{50} of CQ1 in the same generation). DNA sequencing results were checked for proper amplification with BLAST searches in the NCBI GenBank database, and genotypes were determined from sequence chromatograms using Chromas software (Technelysium, Brisbane, Australia).

4.3 Results and Discussion

4.3.1 Permethrin bottle bioassays

Probit analysis of bottle bioassay data for each *Cx. quinquefasciatus* population provided KC_{50} and LC_{50} values. The KC_{50} and LC_{50} values of LA and CV populations were compared to those of CQ1 in their respective generations (Tables 4.3 and 4.4). For LA and CV strains, KC_{50}s, derived from 1 hr knockdown, ranged from 9.798 µg/mL to 20.391 µg/mL. These values were significantly higher than those for CQ1 (0.444 – 0.512

µg/mL), with RR_{KC50} ranging from 22.1 to 45.9. The CV treatment strain had the highest RR_{KC50} (29.3 – 45.9). However, no statistically significant differences in KC_{50} were observed between the two-field collected strains and across generations. LA strains and CV strains showed comparable KC_{50}s, and bifenthrin exposure in treatment strains did not result in a significant change in KC_{50} in subsequent generations.

The LC_{50} values, derived from 24 hr mortality, for each strain ranged from 11.419 µg/mL to 27.971 µg/mL. Notably, the CV treatment strain exhibited an increase in LC_{50} from F1 (13.19 µg/mL) to F2 (24.473 µg/mL; $p < 0.05$), and this increase was maintained in generation 3 (27.791 µg/mL). No other significant differences were observed in the LC_{50} values. Since all RR_{KC50} and RR_{LC50} values were over 10, all strains could be considered highly resistant according to previous established criteria of resistance magnitude (Mazzarri and Georghiou, 1995). However, it must be noted that these ratios and classifications of resistance depend on the KC50 and LC50 of the reference strain used. For example, a Colombian study of Cx. quinquefasciatus showed a permethrin KC50 of 3.152 µg/mL (up to 7 times higher than what was measured for CQ1 in the present study), which would result in much lower RRKC50 values (Maestre-Serrano et al., 2020). The LC_{50} for each strain was generally higher, although not statistically significantly, than its corresponding KC50, with a few exceptions.

Table 4.3The 50% 1 hr knockdown concentration (KC_{50}) and resistance ratios (RR_{KC50}) relative to the susceptible strain for permethrin in adult *Culex quinquefasciatus* populations. *N* = number of adult female mosquitoes tested; 95% CI = 95% fiducial confidence intervals; SE = standard error of slope; χ^2 = chi-square test statistic; df = degrees of freedom.

Strain	Generation	*N*	KC_{50} (95% CI; µg/mL)	Probit fit parameters			RR_{KC50}
				Slope (SE)	χ^2	df	
CQ1	F_1	326	0.512 (0.397-0.66)	2.108 (0.056)	0.77	3	
	F_2	307	0.444 (0.367-0.539)	3.263 (0.043)	0.927	3	
	F_3	304	0.507 (0.414-0.62)	2.98 (0.045)	0.089	3	
LA	F_1	992	13.39 (11.063-17.067)	1.434 (0.119)	2.567	3	26.2
Control	F_2	939	12.652 (11.081-14.834)	2.081 (0.154)	0.064	3	28.5
	F_3	973	13.53 (10.365-20.208)	1.975 (0.15)	4.4917	3	26.7
LA Treatment	F_1	1028	12.311 (8.874-21.534)	1.846 (0.132)	7.6429	3	24.0
	F_2	987	9.798 (6.691-18.756)	1.823 (0.134)	13.191	3	22.1
	F_3	1038	13.264 (11.49-15.774)	1.871 (0.135)	1.967	3	26.2
CV	F_1	1031	13.254 (11.047-15.864)	2.446 (0.131)	4.8448	3	25.9
Control	F_2	936	16.652 (12.53-22.933)	2.419 (0.14)	19.997	3	37.5
	F_3	992	16.333 (11.929-22.265)	2.217 (0.124)	11.119	3	32.2
CV Treatment	F_1	1169	15.012 (11.683-18.935)	2.827 (0.134)	20.287	3	29.3
	F_2	1050	20.391 (15.149-28.522)	2.154 (0.12)	20.259	3	45.9
	F_3	1079	20.275 (15.265-27.297)	2.083 (0.119)	16.834	3	40.0

Table 4.4 The 50% 24 hr lethal concentrations (LC_{50}) and resistance ratios (RR_{LC50}) relative to the susceptible strain for permethrin in adult *Culex quinquefasciatus* populations. *N* = number of adult female mosquitoes tested; 95% CI = 95% fiducial confidence intervals; SE = standard error of slope; χ^2 = chi-square test statistic; df = degrees of freedom

Strain	Generation	*N*	LC50 (95% CI; µg/mL)	Probit fit parameters			RRLC50
				Slope (SE)	χ^2	df	
CQ1	F_1	326	0.601 (0.465-0.771)	2.138 (0.055)	7.33	3	
	F_2	307	0.495 (0.237-0.777)	2.047 (0.224)	12.322	3	
	F_3	304	0.592 (0.434-0.788)	2.873 (0.28)	7.9037	3	
LA	F_1	992	11.419 (8.799-16.093)	1.001 (0.113)	2.27	3	19.0
Control	F_2	939	14.122 (12.516-16.356)	2.516 (0.196)	1.43	3	28.5
	F_3	973	17.895 (15.104-22.282)	2.021 (0.172)	2.023	3	30.2
LA	F_1	1028	14.372 (10.342-24.553)	1.711 (0.131)	5.2792	3	23.9
Treatment	F_2	987	11.555 (7.889-24.255)	1.949 (0.146)	13.732	3	23.3
	F_3	1038	16.422 (13.944-20.16)	1.865 (0.145)	2.327	3	27.7
CV	F_1	1031	14.457 (9.536-22.046)	1.728 (0.11)	24.019	3	24.1
Control	F_2	936	18.444 (13.47-26.574)	2.187 (0.13)	11.429	3	37.3
	F_3	992	19.319 (14.754-25.703)	2.094 (0.121)	8.1971	3	32.6
CV	F_1	1169	13.19 (11.947-14.279)	2.008 (0.11)	1.502	3	21.9
Treatment	F_2	1050	24.473 (16.157-42.858)	2.011 (0.118)	18.974	3	49.4
	F_3	1079	27.971 (19.513-45.933)	1.688 (0.111)	11.443	3	47.2

Resistance data has been similarly derived from modified bottle bioassays using permethrin in several other countries impacted by mosquito-borne diseases, including Mexico, Peru, Colombia, and Pakistan. Two strains of *Cx. quinquefasciatus* collected in the Caribbean region of Colombia showed similar levels of resistance to LA and CV strains: KC_{50}s were 15.712 µg/mL and 22.067 µg/mL, and LC_{50}s were 15.752 µg/mL and 30.705 µg/mL (Maestre-Serrano et al., 2020). A previous study of 9 *Ae. aegypti* strains in the same region showed KC_{50}s ranging from 0.901- 23.508 µg/mL and LC_{50}s ranging from 1.218 – 11.948 µg/mL (Maestre-Serrano et al., 2014). A strain of *Ae. aegypti* collected from Iquitos, Peru, showed no knockdown resistance (KC_{50} 0.3 µg/mL; RR_{KC50} < 1) but an elevated LC_{50} of 5.6 µg/mL. In the same study, five strains of *Ae. aegypti* from the Yucatán Peninsula of Mexico showed low to moderate KC_{50}s, with only 1 strain being highly knockdown resistant (KC_{50} 21.7 µg/mL); these strains had higher LC_{50}s ranging from 6.9 – 25.4 µg/mL (Saavedra-Rodriguez et al., 2012). Another study of 7 *Ae. aegypti* strains from Veracruz state, Mexico, had low to moderate KC_{50} (0.68 – 0.829 µg/mL) and LC_{50} (0.43 – 7.31 µg/mL) (Flores et al., 2013). *Ae. aegypti* collected in Puerto Rican cities had exceptionally high resistance, with 8 strains showing KC_{50}s 38 to 195 times higher and LC_{50}s 33 to 214 times higher than those of the susceptible reference (Ponce-García et al., 2016). Mosquitoes collected from urban areas of Punjab province in Pakistan exhibited low to high levels of resistance, with 12 strains of *Ae. aegypti* showing KC_{50}s of 3.31 – 21.68 µg/mL and similar LC_{50}s of 3.93 – 21.94 µg/mL (Khan and Akram, 2019). For 8 strains of *Ae. albopictus* from different areas of Punjab, LC_{50}s (8.71 – 37.14 µg/mL) were higher than KC_{50}s (6.85 – 15.47 µg/mL) (Khan, 2020).

In general, elevated pesticide KC_{50} (and RR_{KC50}) may indicate increased target-site resistance, whereas elevated LC_{50} (and RR_{LC50}) may indicate increased metabolic resistance. A higher frequency of *kdr* mutations is most likely to influence short-term response by limiting initial target-site binding and possibly preventing knockdown (Flores et al., 2013). Conversely, metabolism and other mechanisms of resistance may require a longer period to take effect. A higher LC_{50} compared to the respective KC_{50} implies that a proportion of mosquitoes knocked down when exposed to pesticide may eventually recover over time (Maestre-Serrano et al., 2014). Given that KC_{50} was not significantly altered in any of the strains in this study, *kdr*'s involvement in the strains' resistance likely remained similar as well. In the CV treatment strain, the increase in permethrin LC_{50} after F_1 may suggest that exposure to bifenthrin increased the strain's metabolic response.

Overall, the lack of broad changes in observed resistance suggests that the concentration used in the present study (340 ng/L bifenthrin) are unlikely to significantly affect permethrin resistance in populations that are already highly resistant to the insecticide. It was expected that later generations of unexposed strains would display a loss of resistance, but this was not observed in the measurements of knockdown or mortality. Noticeable losses in resistance for unexposed populations may require longer time periods, depending on the strain. For example, a strain of *Cx. pipiens pallens* removed from insecticide selection showed significantly reduced resistance to deltamethrin, another pyrethroid, after 22 generations (Shi et al., 2015). More pronounced

effects may be observed when susceptible populations are exposed, but significant increases in resistance may also take many generations.

It is possible that the treatment populations' resistance in the bottle bioassays may have been reduced by constant exposure to bifenthrin as larvae. In this case, a subsequent generation removed from treatment may show increased tolerance. A study of *Aedes aegypti* larvae selected with permethrin (4,000 ng/L – 5,500 ng/L) for 24 hr showed that an additional 72 hr pre-exposure to a sub-lethal concentration of permethrin (400 ng/L) resulted in slightly decreased LC_{50} compared to the population without pre-exposure (Poupardin et al., 2012). However, additional investigation is needed to confirm this assumption.

4.3.2 Synergist bottle bioassays with PBO

Knockdown and mortality from bottle bioassays with PBO as a synergist were compared to those from bioassays without PBO to determine whether cytochrome P450 enzymes were involved in the strains' resistance (Table 4.5). While tests without PBO consistently showed lower mortality than knockdown, all tests with PBO resulted in higher mortality relative to knockdown. In all field-collected strains, knockdown after exposure to the lower concentration (2 µg/mL for LA strains, 8 µg/mL for CV strains) was increased (2.3-fold on average) when mosquitoes were pre-exposed to PBO. For nearly all LA strains, tests with PBO resulted in an increased knockdown from the higher permethrin concentration (8 µg/mL); the only exception was the F_2 generation of LA treatment mosquitoes. Conversely, except for F_3 CV treatment mosquitoes, exposure to

PBO and the higher permethrin concentration (32 µg/mL) decreased knockdown. For all test concentrations in all strains, the addition of PBO consistently increased mortality. An average 3.6-fold increase in mortality was observed at the lower concentration, and an average 1.6-fold increase was observed at the higher concentration. The decrease in knockdown observed when a low concentration of permethrin was paired with PBO was also reported for *Ae. aegypti*, though the same study showed the same treatment to increase knockdown in *Anopheles gambiae* (Gross et al., 2017). Another study found that PBO decreased permethrin penetration into adult *Ae. aegypti* (Kasai et al., 2014). In this study, PBO may have reduced absorption such that CV treatment mosquitoes were more tolerant to the lower concentration.

Table 4.5 The 1 hr knockdown and 24 hr mortality observed in each strain in bottle bioassays without and with PBO.

Strain	Generation	Permethrin concentration	1 hr knockdown (%)		24 hr mortality (%)	
		(ug/mL)	Without PBO	With PBO	Without PBO	With PBO
LA	F_1	2	13.8	28.5	28.2	44.0
Control		8	33.7	55.0	47.2	73.8
	F_2	2	4.5	24.5	1.5	41.8
		8	34.3	53.7	24.7	71.1
	F_3	2	5.1	20.6	2.0	39.0
		8	37.6	49.2	27.2	78.1
LA	F_1	2	7.0	31.9	7.0	49.3
Treatment		8	42.0	42.3	37.5	69.2
	F_2	2	4.3	24.6	2.1	41.2
		8	47.4	34.7	41.5	61.4
	F_3	2	7.4	20.5	5.9	45.9
		8	36.1	44.3	26.3	72.7
CV	F_1	8	32.2	50.0	27.8	74.0
Control		32	78.5	60.3	65.5	88.7
	F_2	8	31.7	49.3	22.9	71.4
		32	73.5	60.9	72.7	82.1
	F_3	8	23.8	51.3	20.9	79.3
		32	71.6	60.1	63.7	95.2
CV	F_1	8	13.6	39.5	32.2	58.0
Treatment		32	86.4	55.4	77.7	75.1
	F_2	8	24.9	43.0	18.1	61.8
		32	72.3	55.9	66.5	74.9
	F_3	8	17.3	50.5	20.3	71.6
		32	60.5	64.8	47.3	87.6

For the CV treatment strain, PBO had a greater effect on increasing mortality in subsequent generations. For F_1, F_2, and F_3, mortality at 8 µg/mL increased 1.8-fold, 3.4-fold, and 3.5-fold, respectively. At 32 µg/mL, mortality was roughly equivalent at F_1, increased slightly (1.1-fold) at F_2, and nearly doubled (1.9-fold) at F_3. This finding was in agreement with the CV treatment LC_{50} increasing after F_1 and implies that increased resistance in the strain was at least partially driven by increased P450 activity.

The cytochrome P450 family of enzymes is widely represented in insects and are involved in many species' resistance to various insecticides (Feyereisen, 2005). A variety of P450s may be involved in pyrethroid-resistant mosquitoes, depending on the species and strain. Analysis of the JPAL strain of *Cx. quinquefasciatus* revealed the overexpression of the genes *CYP4H34, CYP6Z10,* and *CYP9M10* (Komagata et al., 2010). For certain P450 isoforms, other contaminants may select for or induce cross-resistance in mosquitoes. For example, CYP6M2 has been shown to metabolize pyrethroids along with DDT and carbamates, two other types of insecticides, in *Anopheles gambiae* (Edi et al., 2014). *CYP6M11* has been shown to be induced by both permethrin and fluoranthene, a polycyclic aromatic hydrocarbon (Poupardin et al., 2012). Predicting the effects of larval pesticide exposure and selection on metabolic resistance is complicated by the fact that many P450 genes, such as *CYP9M10*, may only be significantly expressed in the larval or adult stage, though some, such as *CYP9M6*, may be continuously expressed throughout the lifespan (Kasai et al., 2014; Komagata et al., 2010).

Besides cytochrome P450, other enzymes may be involved in pyrethroid interactions, but their roles in directly mediating resistance have not been confirmed. Esterases and glutathione-*S*-transferases have been shown to metabolize other insecticides, including organophosphates, and xenobiotics in mosquitoes, but they have not been directly associated with pyrethroid metabolism in *Culex* species (Enayati et al., 2005; Hemingway and Karunaratne, 1998). Other enzymes may not directly degrade pyrethroids but may be involved in detoxification pathways. A study of *Ae. aegypti* showed that alanine aminotransferases, epoxide hydrolases, and epoxide dehydrogenases were over-transcribed in permethrin-selected strains (Poupardin et al., 2012).

4.3.3 *kdr* allele frequencies

The distribution of the *kdr* (L1014F) mutation in mosquitoes of F_0 and F_3 populations is detailed in Table 4.6. All F_0 and F_3 CQ1 mosquitoes contained the susceptible (L1014) genotype. At F_0, LA mosquitoes were 80% homozygous resistant, 15% were heterozygous, and 5% s susceptible, while all CV mosquitoes were homozygous resistant. After 3 generations of exposure, LA control mosquitoes were 95% homozygous resistant and 5% heterozygous, while LA treatment mosquitoes were 100% homozygous resistant. F_3 CV control and treatment mosquitoes remained 100% homozygous resistant. Though the resistant mutation L1014S (serine residue) has been reported for *Cx. quinquefasciatus*, it was not detected in mosquitoes in the present study.

Table 4.6 Distribution of kdr target-site mutations in Culex quinquefasciatus strains.

Population	**Generation**	***N***	**R/R[a]**	**R/S[b]**	**S/S[c]**
CQ1 (susceptible)	F_0	20	0	0	20
	F_3	19	0	0	19
LA	F_0	20	16	3	1
LA Control	F_3	20	19	1	0
LA Treatment	F_3	20	20	0	0
CV	F_0	20	20	0	0
CV Control	F_3	20	20	0	0
CV Treatment	F_3	20	20	0	0

[a] Number of mosquitoes with homozygous resistant (TTT/TTT) genotype.
[b] Number of mosquitoes with heterozygous (TTT/TTA) genotype.
[c] Number of mosquitoes with homozygous susceptible (TTA/TTA) genotype.

The ubiquity of the homozygous resistant genotype in F_0 CV mosquitoes suggests that *kdr* was already at or near fixation before collection. F_0 LA mosquitoes were somewhat less resistant, and it is more likely that a reversal from fixation could occur for this population. After 3 generations, both LA control and resistant mosquitoes had a similarly increased prevalence of *kdr*. In *Culex* mosquitoes collected from Riverside County, where La Quinta is located, in 2014, approximately 40% were homozygous resistant, 50% were heterozygous, and 10% were homozygous susceptible (Yoshimizu et al., 2020). Though the same study did not measure *kdr* frequency in Los Angeles County, mosquitoes in nearby Orange County in 2014 were approximately 20% homozygous resistant, 50% heterozygous, and 30% resistant, and were overall less frequently resistant than those in Riverside County (Yoshimizu et al., 2020).

While selection pressure may increase the prevalence of *kdr* in a mosquito population, the high frequency of the resistant mutation in these strains is unlikely to be increased by the low concentration of bifenthrin (340 ng/L) used. This may explain the

lack of changes in strains' KC_{50} across generations, where target site insensitivity would prevent knockdown depending on the concentration of permethrin. Differences in KC_{50} can also be explained by the frequency of mosquitoes homozygous or heterozygous for *kdr*. A study comparing *Cx. quinquefasciatus* strains found that *kdr* heterozygotes had up to 48-fold increased permethrin resistance, whereas homozygotes had 70-fold increased resistance (Hardstone et al., 2009).

In *Culex* mosquitoes, other target-site mutations conferring resistance to permethrin and other pyrethroids have been documented, though they are much less prevalent than 1014F and even 1014S. An alternative mutation, 1014C (cysteine; TGT) was detected in *Cx. pipiens* complex mosquitoes collected in China (Wang et al., 2012). The JPal-per strain, from which JPAL was derived, contained the V1016G mutation, which is more commonly found in other mosquitoes such as *Ae. aegypti* and *Ae. Albopictus* (Komagata et al., 2008; Nachaiwieng et al., 2021; Pichler et al., 2022).

4.3 Conclusion

Strains of wild *Cx. quinquefasciatus* collected from urban areas in southern California were exposed as larvae to bifenthrin at a concentration reflecting those observed in environmental monitoring studies of stormwater runoff. All strains exhibited high levels of resistance compared to the susceptible reference strain, and nearly every mosquito from these strains was homozygous or heterozygous for the *kdr* mutation conferring target site resistance. In one strain exposed to bifenthrin, results from bottle bioassays, including synergist bioassays with the synergist PBO, implicated increased

involvement of cytochrome P450-mediated metabolism in permethrin resistance. The results suggest that the exposure concentration used in the present study is unlikely to consistently influence permethrin resistance in populations that are already moderately or highly resistant. Any risk in contaminated urban sites is likely to come from exposure to susceptible populations, longer time scales comprising many generations, and/or additive or synergistic effects from multiple contaminants. These findings highlight the importance of considering strain-specific (in addition to species-specific) effects when evaluating environmental factors influencing pesticide resistance. Further research should focus on longer time scales or more specific aspects of resistance dynamics, primarily target site mutations and metabolic activity, to improve understanding of the impacts of contaminated habitats on insecticide resistance.

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Chapter 5 Summary and Future Work

5.1 Summary of Findings

This dissertation research evaluated the occurrence and distribution of pyrethroid residues in urban underground storm drain systems, as well as their potential influence on pyrethroid resistance in larval mosquitoes that may use storm drains as habitats. Environmental and experimental data were collected and analyzed to achieve the outlined objectives. The primary findings and conclusions are summarized below.

5.1.1 Pyrethroid insecticides in urban catch basins: A potential secondary contamination source for urban aquatic systems

A total of 205 water samples were collected from 79 different urban catch basins during the summer, when pyrethroid use is often highest and runoff is almost completely driven by irrigation. Residues were detected in 98% of samples, and bifenthrin was detected in 97% of samples. Detection frequencies for the other 8 pyrethroids targeted ranged from 0% to 30%. The prevalence of bifenthrin in this study is in agreement with previous research on urban runoff in the United States, much of which has found bifenthrin to be one of the most frequently detected pesticides in general.

Aqueous pyrethroid concentrations ranged from 3 to 726 ng/L, whereas concentrations in suspended solids ranged from 42 to 93,600 ng/g. Bifenthrin was detected at up to 75,400 ng/g in suspended solids, which is a higher maximum than has been previously observed in urban samples. The proportion of residues varied widely depending on the location, and sometimes other pyrethroids such as lambda-cyhalothrin

and permethrin made up the majority of residues in a region's samples. Values for the partition coefficient K_d indicated enrichment of residues on suspended solids, though much of the residues were associated with the aqueous phase of samples.

Toxic units were to measure the potential risk to sensitive invertebrates such as the amphipod *Hyalella azteca*. The majority of samples contained bifenthrin at concentrations above the reference values for toxicity. Though conditions in catch basins is not representative of discharge into urban waterways, their water and sediment may be a source of aquatic toxicity, especially during periods of high water flow.

5.1.2 Distribution of pyrethroid insecticides in urban storm drain structures: Catch basins, open channels, and outfalls

In this study, a variety of media were collected during the dry season from multiple types of storm drain structures to obtain information on the distribution of pyrethroids in storm drains. A total of 28 water samples, 4 sediment samples, 8 algae samples ,and 4 biofilm samples were collected from 3 catch basins, 7 open channels, and 7 outfalls. Overall, 89% of water samples contained pyrethroid concentrations above the reporting limit. Bifenthrin was again the most frequently detected pyrethroid, being found in 85% of water samples and 100% of all other samples, while detection of other pyrethroids ranged from 0% to 43% of water and 0% to 50% of other samples. Pyrethroids were detected at up to 1,890 ng/L in water, 271 ng/g in sediment, 2,290 ng/g in algae, and 102,000 ng/g in biofilms.

The sites sampled did not exhibit strong hydrological connectivity, and such a connection between sampling sites may be generally difficult to establish during the dry

season. Bifenthrin concentrations were elevated in water from catch basins, suggesting that highly contaminated runoff may be diluted when transported across channels or to outfalls. Water samples with high total suspended solids were more likely to have an increased proportion of residues associated with their suspended sediment. The high concentrations detected in certain biofilm and algae samples underline the significance of these media, especially since certain aquatic organisms may feed on them.

Calculated toxic units for bifenthrin, cyfluthrin, and cypermethrin showed that most water samples posed risks for impairment of *H. Azteca*, though potentially lethal concentrations were not as prevalent. Cyfluthrin was in most water samples with high estimated toxicity. Potential toxicity in sediment samples was primarily due to high bifenthrin concentrations.

5.1.3 Effects of larval Culex quinquefasciatus exposure to low concentrations of bifenthrin on adult pyrethroid resistance

Wild *Cx. quinquefasciatus* were collected from two urban areas of southern California and used to establish strains in the laboratory. Some of these strains were exposed to a low concentration of bifenthrin as larvae for multiple generations, and the pyrethroid resistance status of each strain was evaluated. All strains showed high levels of resistance to permethrin compared to the susceptible reference strain. The primary change observed was a decrease in mortality for one of the treatment strains' subsequent generations, suggesting that, even for highly resistant populations, pyrethroid exposure in aquatic habitats such as storm drains may have strain-specific influences.

Synergist bottle bioassays with piperonyl butoxide revealed mixed results in knockdown and increased mortality for the resistant strains. Past studies indicate that piperonyl butoxide may reduce the potency of low concentrations of permethrin by limiting its penetration into mosquitoes. The synergist also had a more pronounced effect on subsequent generations of the same strain that showed decreased mortality, implying that the change in resistance was driven by cytochrome P450 enzyme activity.

DNA sequencing revealed a nearly 100% frequency of *kdr*, a mutation conferring target site resistance to pyrethroids, in resistant strains. This suggests that *kdr* may be at or near fixation in wild *Cx. quinquefasciatus* populations from the collection areas, which would reduce the efficacy of local adult mosquito control. Minimal changes were observed in the mutation frequency, which may explain why knockdown did not significantly change for any strain.

5.1.4 Overall Conclusions

The research presented in this dissertation provides further documentation of the near-ubiquitous presence of pyrethroids in urban runoff along with evidence that the concentrations observed in environmental samples may be sufficient to affect the resistance status of urban mosquitoes. The pyrethroids were detected in the vast majority of water collected during low flow conditions, often at concentrations which may potentially cause toxicity in susceptible fish and invertebrates. Bifenthrin was the most prevalent substance by a wide margin, but other pyrethroids, such as lambda-cyhalothrin, permethrin, and cyfluthrin, sometimes contributed a high proportion of residues. Comparison between storm drain structures found that catch basins had higher bifenthrin

concentrations relative to other structures, suggesting that toxic risk is attenuated by the storm drain system while there is low flow. When larvae of pyrethroid resistant *Cx. quinquefasciatus* were treated with bifenthrin, a strain-specific increase in adult *Cx. quinquefasciatus* survival after permethrin exposure was observed. This indicates that residues of these insecticides may influence populations that use storm drains as breeding habitats, posing a risk for vector control operations.

5.1 Future Research

Future research can provide insights into the environmental fate and transport of pyrethroids in urban runoff or larval exposure's effects on mosquito populations. With regards to the environmental chemistry of pyrethroids and similar contaminants, the low flow conditions of the dry season posed challenges in finding sites with hydrological connectivity. More insightful results may be obtained by in-depth sampling and analysis of a smaller number of sites that are more closely related to each other.

While bifenthrin exposure did not produce broad changes in the resistance status of highly resistant *Cx. quinquefasciatus* strains, further research could be conducted to confirm whether environmental concentrations may support the maintenance of resistance over the long term. Also, other regions have wild populations that are susceptible to permethrin, and the risks of larval exposure to low concentrations of pyrethroids are still poorly understood. Comparisons could be made between various methodologies for pesticide selection, such as exposing larvae for the full life stage versus limiting exposure to the 4^{th} instar. Lastly, many other contaminants have been detected in urban runoff. These include other pesticides, such as neonicotinoids, metals,

pharmaceuticals and personal care products, and product additives, such as polymer plasticizers and stabilizers. Many of these contaminants have been shown to have adverse effects on aquatic organisms, and mosquito populations may also be affected. Determining whether certain contaminants have additive or synergistic roles in insecticide resistance development may better inform strategies for managing runoff and vectors.