Abundance of black flies (Simuliidae) and potential vector for *Onchocerca* sp. in San Gabriel Valley, California

Kimberly J. Nelson, J. Wakoli Wekesa, Marc Mitchell, Antonio Bishop, Angela Brisco, Melvin Cook and Kenn Fujioka

San Gabriel Valley Mosquito and Vector Control District, 1145 N. Azusa Canyon Road, West Covina, CA 91790
(626) 814-9466; wwekesa@sgvmosquito.org

ABSTRACT: Black flies (Simuliidae) are known throughout the San Gabriel Valley as nuisances because of their bites and are also intermediate hosts for *Onchocerca* spp., nematodes that may cause onchocercosis. Over the past 20 years, there have been nine cases of canine onchocercosis in southern California and one human infection in Arizona. The high incidence of onchocercosis and the history of black flies as a biting nuisance make it vital that the San Gabriel Valley Mosquito and Vector Control District evaluates its risk to public health. To monitor our black fly population, we sampled larvae and pupae by using one meter strips of yellow "caution tape" in areas with a good water flow and adults using carbon dioxide-baited traps. Black fly larvae were significantly more abundant than pupae and adults; however, larval and adult abundances were positively correlated. We will continue to monitor black fly abundance, black fly species diversity and the prevalence of *O. lupi* in 2015.

INTRODUCTION

The San Gabriel Valley Mosquito and Vector Control District (District) covers more than 54,000 hectares of Los Angeles County and is bordered to the north by the San Gabriel Mountains. This mountain range is drained by the San Gabriel River, and its many streams, creeks and flood channels provide ample breeding ground for black flies. These flies are a major biting nuisance to residents of the District. There are approximately 2,000 species of black flies worldwide; 254 occur in North America, and 24 are found in California (Crosskey and Howard 2004, Adler et al. 2004). Only two of the 24 black fly species in California bite; specifically, these are *Simulium vittatum sensu lato* and *Simulium tescorum*.

Black flies are known throughout the world as biting nuisances and as the intermediate host of parasitic nematodes in the genus *Onchocerca*. Infection by most *Onchocerca* spp. causes a variety of symptoms including visual impairment with eye lesions, inflammation of skin and/or lymph nodes. *Onchocerca volvulus* causes River Blindness, a disease with high morbidity and mortality in people of Africa, South America and Central America (Basáñez et al. 2006). *Onchocerca cervicalis* infects horses (Cummings and James 1985), *O. lienalis* and *O. guttturosa* infect cattle (Ferenc et al. 1986), and other *Onchocerca* spp. infect specific hosts throughout the world. Our district is concerned about *Onchocerca lupi* which recently was associated with canine ocular onchocercosis (Hassan et al. 2015), a disease of dogs, cats, and humans.

In the past 20 years, approximately 70 infections of *O. lupi* have been reported in canines worldwide (Eberhard et al. 2000, Zarfoss et al. 2005, Otranto et al. 2011, Hassan et al. 2015). Also, two infections were reported in cats (Labelle et al. 2011) and 11 infections in humans (Eberhard et al. 2013, Bergua et al. 2015, Hassan et al. 2015). In the United States there have been 15 canine infections [nine from Southern California (Hassan et al. 2015)], and in 2012 a 22-month old child from Arizona represented the first human case (Eberhard et al. 2013).

The increasing incidence of canine infections with *O. lupi* in southern California and the presence of black flies as a biting nuisance constitute a public health concern for the District. Here we discuss the abundance of black fly populations in the San Gabriel Valley and the role that black flies play in transmitting *O. lupi*.

METHODS

To determine the abundance of black fly populations, we performed monthly monitoring of black fly larvae at four locations in the District from April through November, 2014 (Figure 1). One of the four sampling sites was in a concrete-lined stream; the other three were in the San Gabriel River and its tributaries. Three one-meter long strips of yellow "caution tape" were placed in flowing water and checked weekly. We tallied the number of larvae and pupae on the "caution tape" and collapsed weekly data into monthly. We used an infrared laser thermometer (Cen-Tech 60725, Camarillo, USA) to determine water temperature during each sampling event.

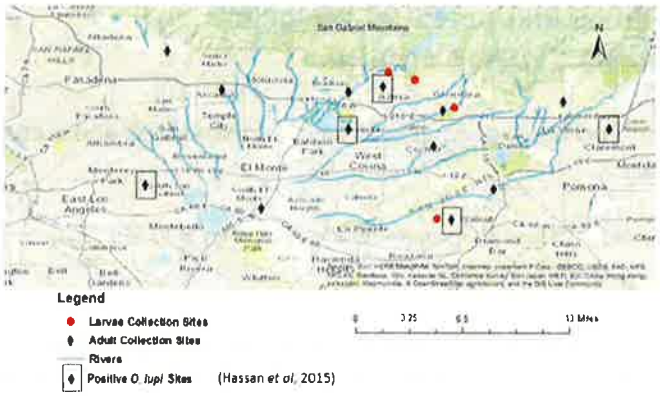

Figure 1. Larval and adult black fly collection sites in the San Gabriel Valley MVCD. Larvae (red dots) and adults (black diamonds) collection sites. Adult black fly sites that tested positive for *O. lupi* are black diamonds with boxes around them (Hassan *et al.* 2015).

To evaluate the impact of larviciding on adult black fly populations, we monitored 13 locations proximal to the San Gabriel River from April through November 2014 with carbon dioxide-baited (EVS) traps. Traps were set overnight and samples were processed in the laboratory. The black flies were anesthetized with Triethylamine then counted and recorded. We initiated larviciding with VectoBac® 12AS (*Bacillus thuringiensis* var. *israelensis*) whenever the larval counts were high and the District received increased complaints about biting nuisances.

We used one-way ANOVA to analyze the mean monthly larval and adult black fly abundance. To determine the impact of larviciding on larvae and adult populations, we also attempted to see whether the seasonal abundance of larval and adult black flies was correlated (JMP v 12.0, 2015, Cary, USA).

RESULTS AND CONCLUSIONS

We collected 22,969 black fly larvae at the four collection sites from April through November 2014. We observed seasonality during the study with more than 85 percent of the larvae collected between June and October of 2014 (Figure 2).

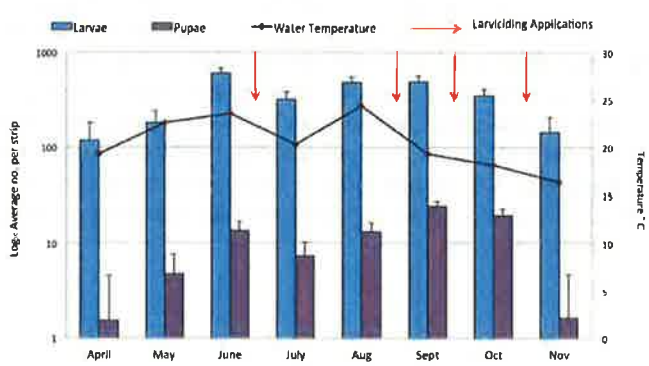

Figure 2. Average monthly black fly larvae and pupae collected from April – November 2014 in San Gabriel Valley, CA.

The peak abundance occurred in June followed by a slow steady decline through November. The decrease in larvae between June and July may have been caused by larviciding and lower than normal water temperature (Figure 2). The decrease in water temperature coincided with increased water flow of the San Gabriel River when water was released from Morris Dam (LA County Dept. of Public Works – Morris Dam Water Release Records). Our findings are consistent with research which found that warmer water temperatures causes faster development and increased abundance of larvae and lower water temperatures produce the opposite effect (Carlsson 1967, Becker 1973, Crosskey 1990, Bernotiene and Bartkeviciene 2013). The impact of subsequent larviciding was seen in September through October 2014. We did not larvicide consistently throughout the season. This is visible in August when larval counts were significantly higher (Figure 2).

We collected 4,152 black fly pupae at the four sites from April through November 2014. The pupae counts showed a seasonal pattern similar to that of the larvae with more than 90 percent occurring between June and October of 2014 (Figure 2). The inconsistent larviciding in August and September increased survival of larvae and subsequently increased the pupal count in September and October.

We collected 6,726 adult black flies at the 13 sites from April through November 2014. As with the larvae and pupae, more than 75 percent of adult black flies were collected from June through October 2014 (Figure 3).

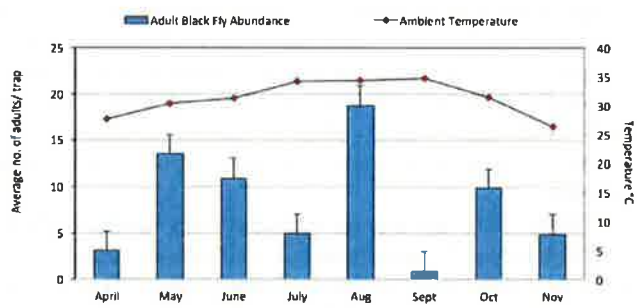

Figure 3. Average monthly adult black flies collected from April – November 2014 in San Gabriel Valley, CA.

The peak abundance of adult black flies was in August which coincided with the month when larviciding was inconsistent and water and ambient temperature increased (Figures 2 and 3). Carlsson (1967) found that higher ambient temperatures seem to increase adult activity of all species of black flies which is consistent with our data. Although larvae were present in much higher numbers than adult black flies, there was a consistent and significant positive correlation between the two measures throughout the season ($r = 0.58$, $p \leq 0.05$). This finding shows that as black fly larval abundance increases the adult population also increases, implying that larval black fly control can significantly reduce the biting nuisance.

Our study in 2014 showed that the abundance of black flies in the District is seasonal. Also, the study showed that our black flies can be managed with a consistent control program. In 2015 we will continue monitoring all larvae, pupae and adult black fly populations. Adult black flies will be identified to species to assess species diversity and richness over time.

We also evaluated *S. tribulatum*, a member of *S. vittatum* sensu lato, as a putative vector of *O. lupi* in San Gabriel Valley (Hassan et al. 2015). We collected 248 black flies from 13 locations in 2013 which were tested by PCR for infections with *O. lupi* by the University of South Florida. In the study, *O. lupi* was found in adult flies from 5 of the 13 collection sites (Figure 1). We will be looking further into the prevalence of *O. lupi* within the District. All *S. vittatum* s.l. will be tested for infections with *O. lupi,* to continue assessing the public health risks associated with biting black flies in the San Gabriel Valley.

ACKNOWLEDGEMENTS

Many thanks goes to the staff at San Gabriel Valley Mosquito & Vector Control District for helping with field work and processing of specimens in the laboratory; Richard Dubielzig and Melissa De Lombaert from the College of Veterinary Medicine, University of Wisconsin; Bruce Silverman from Complete Animal Eye Care; Jamie Schorling from the Eye Clinic for Animals in San Diego; Joseph Kubofcik and Thomas Nutman from National Institutes of Health (NIH); and Kelly Middleton currently at Greater Los Angeles Vector Control District for their many contributions on earlier work on black flies and incriminating *Simulium tribulatum* as a vector of *Onchocerca lupi*.

REFERENCES CITED

Adler, PH., Currie, DC., and Wood, DM. 2004. *The black flies (*Simuliidae) *of North America*. Cornell University Press, Ithaca, New York.

Basáñez, M., Pion., S., Churcher, T., Breitling, L., Little, M., and Boussinesq, M. 2006. River blindness: a success story under threat? *PLoS Med* 3: e371.

Becker, C. 1973. Development of Simulium (Psilozia) vittatum Zett. (Diptera: Simuliidae) from larvae to adults at thermal increments from 17.0 to 27.0 C. *American Midland Naturalist* 246-251.

Bergua, A., Hohberger, B., Held, J., Muntau, B., Tannich, E., and Tappe, D. 2015. Human case of *Onchocerca lupi* infection, Germany, August 2014. *Eurosurveillance* 20(16): 2-4.

Bernotiene, R. and Bartkeviciene, G. 2013. The relationship between water temperature and the development cycle beginning and duration in three black fly species. *Journal of Insect Science* 13(1):1-15.

Carlsson, G. 1967. Environmental factors influencing blackfly populations. Bulletin of the World Health Oragnization 37(1):139-150.

Crosskey, R. 1990. *The Natural History of Black Flies*. John Wiley & Sons Ltd.

Crosskey, R., and T, Howard. 2004. A revised taxonomic and geographical inventory of world blackflies (Diptera: Simuliidae). *The Natural History Museum of London* 8:78.

Cummings, E., and James, ER. 1985. Prevalence of equine onchocerciasis in southeastern and midwestern United States. *J Am Vet Med Assoc*. 186: 1202–3.

Eberhard, ML., Ortega, Y., Dial, S., Schiller, CA., Sears, AW., and Greiner, E. 2000. Ocular *Onchocerca* infections in two dogs in western United States. *Veterinary Parasitology,* 90(4), 333-338.

Eberhard, ML., Ostovar, GA., Chundu, K., Hobohm, D., Feiz-Erfan, I., Mathison, BA., ... and Cantey, P T. 2013. Zoonotic *Onchocerca lupi* infection in a 22-month-old child in Arizona: first report in the United States and a review of the literature. *The American Journal of Tropical Medicine and Hygiene, 88*(3), 601-605.

Ferenc SA, Copeman DB,Turk SR, Courtney CH. 1986. *Onchocerca gutturosa* and *Onchocerca lienalis* in cattle: effect of age, sex, and origin on prevalence of onchocerciasis in subtropical and temperate regions of Florida and Georgia. *Am J Vet Res*. 47:2266–8

Hassan HK, Bolcen S, Kubofcik J, Nutman TB, Eberhard ML, Middleton K, Wekesa, JW., Ruedas, G., Nelson, K., Dubielzig, R., De Lombaert, M., Silverman, B., Schorling, J., Adler, P., Unnasch, T., and Beeler, S. 2015. Isolation of *Onchocerca lupi* in dogs and black flies, California, USA. *Emerg Infect Dis* [Internet]. 2015 May 5. http://dx.doi.org/10.3201/eid2105.142011

JMP. 2015. *Statistics*. Retrieved May 4, 2015, from http://www.jmp.com

Labelle, AL., Daniels, JB., Dix, M., and Labelle P. 2011. *Onchocerca lupi* causing ocular disease in two cats. *Vet Ophthalmol*. 2011 Sep;14 Suppl 1:105-10.

Otranto, D., Sakru, N., Testini, G., Gürlü, V. P., Yakar, K., Lia, R. P., ... and Bain, O. (2011). First evidence of human zoonotic infection by *Onchocerca lupi* (Spirurida, Onchocercidae). *The American journal of tropical medicine and hygiene*, *84*(1), 55-58.

Zarfoss, MK., Dubielzig, R., Eberhard, ML., and Schmidt, KS. (2005). Canine ocular onchocerciasis in the United States: two new cases and a review of the literature. *Veterinary ophthalmology*, *8*(1), 51-57.