DEVELOPMENT TIME OF *CULEX* MOSQUITOES IN STORMWATER MANAGEMENT STRUCTURES IN CALIFORNIA

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ABSTRACT. A widely recommended strategy to minimize mosquito production in structural stormwater Best Management Practices (BMPs) is to ensure they hold captured water for no more than 72 h. However, this standard may be overly conservative for many mosquito species found in urban environments and may impede or prevent the capacity of BMPs to fulfill more stringent water quality standards in environmentally sensitive areas. Egg-to-pupa development of *Culex tarsalis*, *Cx. pipiens*, and *Cx. quinquefasciatus* were examined during July, August, and September 2006 in stormwater management basins and in water collected from these basins in 3 climatically distinct regions of California: the Lake Tahoe Basin, Sacramento Valley, and Los Angeles Basin. The observed minimum times to pupal development were 6 days for *Cx. tarsalis* and *Cx. quinquefasciatus* and 8 days for *Cx. pipiens*. Multiple linear regression models were used to estimate minimum predicted development times under optimal conditions for each region. The results suggest that water residence times of up to 96 h will not significantly increase the potential for *Culex* mosquito production in stormwater BMPs in the 3 regions included in this study.

KEY WORDS Best Management Practices, *Culex tarsalis*, *Culex pipiens*, *Culex quinquefasciatus*, stormwater, urban runoff

INTRODUCTION

Structural Best Management Practices (BMPs) are being implemented throughout developed areas of the United States to mitigate the negative environmental impacts of stormwater and urban runoff. Unfortunately, many of these engineered structures unintentionally create mosquito larval habitat (Dorothy and Staker 1990, Santana et al. 1994, Metzger et al. 2002, Kwan et al. 2005, Gingrich et al. 2006, Wallace 2007, Metzger et al. 2008). A widely recommended strategy to minimize mosquito production in BMPs designed to drain completely is to hold captured water for no more than 72 h (FCCMC 1998, Metzger 2004, CDC 2005), a period that corresponds to the minimum time required for certain species to complete their life cycle under optimal conditions (Gunstream and Chew 1967). This umbrella policy provides effective mosquito prevention in these structures regardless of season, species, or the myriad of local and environmental factors that may influence production potential (Metzger 2004). However, this generic standard may be overly conservative for many species found in urban environments. Furthermore, it may significantly impede or prevent the capacity of BMPs in environmentally sensitive areas to fulfill more stringent water quality standards (Erlich, personal communication).

Lake Tahoe in northern California is a federal-state-designated Outstanding National Resource Water for its extraordinary clarity, purity, and deep blue color. This special provision is intended to ensure that water quality be maintained to the maximum extent by prohibiting further degradation attributable to human activities (CFR 2007). Since 1968, Lake Tahoe has lost nearly 9.14 m of transparency, due in part to increased water- and air-borne pollutant loading of fine sediments, nitrogen, and phosphorous. In an effort to slow this degradation, strict constituent limitations were created for stormwater and urban runoff that drain to the lake and its tributaries (CRWQCB 1995) and BMPs are being installed throughout the Tahoe Basin to meet these requirements. Relaxation of the 72-h drain-down policy would permit agencies responsible for installing and maintaining Tahoe-area BMPs greater flexibility in design and possibly result in enhanced ability to achieve discharge water quality standards.

In California, *Culex tarsalis* (Coq.), *Cx. pipiens* (L.), and *Cx. quinquefasciatus* (Say) have been documented in urban BMPs (Kwan et al. 2005, Metzger et al. 2008), thus presenting a public and veterinary health concern by potentially increasing the regional population of vector mosquitoes and the transmission pressure of pathogens such as West Nile virus, St. Louis encephalitis, and western equine encephalomyelitis (Reeves et al. 1962, Goddard et al. 2002). This study examined the egg-to-pupa development of these species in stormwater management basins and in water collected from these basins in 3 climatically

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distinct regions of California. The primary objective was to create a model to estimate the minimum development time of *Cx. tarsalis* in the Lake Tahoe Basin to determine if water residence times longer than 72 h could reasonably be implemented in stormwater BMPs region-wide. Similar models were created for *Cx. tarsalis*, *Cx. pipiens*, and *Cx. quinquefasciatus* in the Sacramento Valley and Los Angeles Basin for comparison.

**MATERIALS AND METHODS**

**Study regions**

Three geographical regions within California were selected: the Lake Tahoe (Tahoe) Basin, Sacramento Valley, and Los Angeles (LA) Basin. Study sites in the Tahoe Basin were located in the city of South Lake Tahoe, El Dorado County, the largest city bordering Lake Tahoe. The city is within the Sierra Nevada Mountain Range along the California–Nevada border at an elevation of approximately 1,900 m. South Lake Tahoe receives averages of 20.3 cm of rain and 546.1 cm of snow per year, for an annual total of 76.2 cm of precipitation, and the average high air temperature in July is 26.1°C. Study sites in the Sacramento Valley were in the city of Elk Grove, Sacramento County. Elk Grove has an elevation of 14 m, receives an average annual rainfall of 55.9 cm, and the average high air temperature in July is 33.3°C. Study sites in the LA Basin were in the city of Ontario, San Bernardino County. Ontario has an elevation of 282 m, receives an average annual rainfall of 37.6 cm, and the average high air temperature in July is 35°C.

**Study design**

Three existing stormwater management basins were selected in each geographical region based on the presence of permanent standing water with a depth of >8 cm and with sufficient surface area to deploy 3 floating larval rearing cages. Cages were constructed from small white plastic buckets (ca. 15 cm diameter by 15 cm deep). Two rectangular windows (ca. 10 cm²) were cut from opposing sides and replaced with fine mesh nylon fabric, and each cage was fitted with a foam ring for floatation. A 4th location, designated as an artificial site, was selected in each region to rear larvae outdoors in containers to simulate conditions associated with intermittent shallow pools common in many BMPs. At each artificial site, 3 clear plastic containers (57 × 42 × 33 cm deep, IRIS®. Iris USA Inc., Pleasant Prairie, WI) were prepared for each species at the beginning of every trial. Containers were filled with approximately 5 cm of stormwater collected from 1 of the local field sites and supplemented with ground alfalfa pellets to ensure that larval development was not food limited.

Three successive trials were conducted simultaneously in each region during July, August, and September 2006. Newly laid (<24 h old) *Cx. tarsalis* and *Cx. pipiens* egg rafts were obtained from stock colonies at the Sacramento–Yolo Mosquito and Vector Control District for the Tahoe Basin and Sacramento Valley sites; *Cx. tarsalis* egg rafts were obtained from the Aquatic Entomology Laboratory at the University of California, Riverside, and *Cx. quinquefasciatus* egg rafts were collected from alfalfa-infusion–baited egg traps located in Los Angeles, for the LA Basin sites. One egg raft was placed in each floating cage or artificial container at the beginning of each trial. *Culex tarsalis* egg rafts were placed in both field and artificial sites in all 3 regions, whereas *Cx. pipiens* and *Cx. quinquefasciatus* egg rafts were deployed only at the artificial sites in the Sacramento Valley and LA Basin, respectively. The latter 2 species have not been documented in the Tahoe Basin and were therefore not included in that region. Following deployment of the egg rafts, cages and containers were inspected once daily. Minimum development time was recorded for each cage or container, defined as the time from egg raft deployment (day 0) to the day on which the 1st pupa was observed. Water temperatures were recorded hourly at each site for the duration of each study phase using HOBO® electronic data loggers (MicroDAQ.com, Ltd., Contoocook, NH) submerged inside cages and containers.

**Statistical analysis**

Water temperature data were downloaded from the HOBO units into Microsoft® Excel 2003 and mean water temperatures were calculated for sites in each region during each trial. Statistical analyses were conducted with the use of standard software (Statistical Analysis System [SAS] 9.1; SAS Institute Inc., SAS Campus Drive, Cary, NC). Summary statistics for development data from each region were calculated and results expressed as mean time in days from egg to pupa with 95% confidence intervals (CI). As *Cx. pipiens* and *Cx. quinquefasciatus* egg rafts were deployed at artificial sites only in the Sacramento Valley and LA Basin, the mean development times for these species were compared to the mean development time of *Cx. tarsalis* in artificial containers in these 2 regions with the use of 2-sample *t*-tests. Multivariate linear regression analyses were used to assess the relationships between development time and average water temperature in each region. Study site was included in all regression models as a proxy for unmeasured factors that may impact larval development time, such as food abundance and
water quality. Homogeneity of the slopes of the regression equations was tested by examining the interaction term for region and water temperature in a model including data from all 3 regions. Statistical significance was defined at a level of $P < 0.05$. The fits of the final regression models were assessed with the use of residual plots and the adjusted $R$-square values. Equations generated from regression analyses were used to predict larval development time over a range of temperatures in each region. Finally, under the assumption that the data in this project are representative of the entire population, lower 1-sided 95% tolerance limits were calculated for development times of each species in each region. Tolerance limits were constructed with the use of an on-line tool (http://statpages.org/tolintvl.html).

RESULTS

Tahoe Basin

During the July trial, Cx. tarsalis pupae were observed in all 12 cages and containers at the 4 Tahoe Basin sites. The minimum development time from egg to pupa was 6 days, observed in 1 container at the artificial site. The maximum time to pupation was 15 days. The combined mean water temperature for all 4 sites was 21.6°C. In August, pupae were observed in only 3 cages at 2 field sites; there was either no observed egg hatch or the larvae died before pupating in the remaining cages and containers. Time to pupation was 16 days in each of the 3 cages and the mean water temperature was 16.7°C. During the 3rd trial in September, all eggs failed to hatch. The mean water temperature was 10.9°C. For the 3-month study period, the mean time from egg to pupa was 12 days (95% CI, 10–14 days) (Table 1).

Water temperature was significantly associated ($P = 0.01$) with development time in a multiple linear regression model controlling for study site. The regression coefficient for water temperature in this model was $-0.32$, indicating that larval development time decreased approximately 7 h for every 1°C increase in temperature (Fig. 1). The regression model fit the data well (adjusted $R$-square = 0.96). The regression equation was used to predict larval development times for mean temperatures of 12, 18, 24, and 30°C at the 4 study sites. As the study-site variable was significantly associated with development time, a range of values was obtained for each temperature. Predicted development times ranged from 10 to 18 days at 12°C, 8 to 16 days at 18°C, 7 to 14 days at 24°C, and 5 to 12 days at 30°C (Table 2). Based on lower 1-sided tolerance limit calculations, there was 95% confidence that 95% of the Cx. tarsalis population would have a development time greater than or equal to 2.8 days.

Sacramento Valley

In July, the minimum time to pupation for Cx. tarsalis was 7 days, observed in all 6 cages at 2 field sites. The maximum time to pupation was 8 days. Pupae were observed in all but 1 cage, which was found overturned on day 4. Culex pipiens in artificial containers pupated in 8 days. The mean water temperature at the 4 study sites was 28.1°C. In August, the minimum time to pupation for Cx. tarsalis was 9 days, observed in all 6 cages at 2 field sites. The maximum time to pupation was 14 days. One container at the artificial site failed to produce pupae. The 3 artificial containers with Cx. pipiens all contained pupae on day 12. Mean water temperature at the study sites was 24.6°C. In September, a windstorm overturned 2 Cx. tarsalis cages on day 10 at 1 field site. The pupal development times in the remaining 10 cages and containers ranged from a minimum of 10 days, observed in 1 cage at a field site, to a maximum of 20 days observed in the 3 containers at the artificial site. Culex pipiens larvae pupated in 14–15 days. The mean water temperature was 20.6°C. The overall mean development time from egg to pupa over the 3 months of the study was 11 days (95% CI, 9–12 days) for Cx. tarsalis and 12 days (95% CI, 9–14 days) for Cx. quinquefasciatus.

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<th>Region</th>
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<th>Mean development time</th>
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<tr>
<td>Lake Tahoe Basin</td>
<td>Cx. tarsalis</td>
<td>Field and artificial sites</td>
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<td>Sacramento Valley</td>
<td>Cx. tarsalis</td>
<td>Field and artificial sites</td>
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<td>Field and artificial sites</td>
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<td>Sacramento Valley</td>
<td>Cx. pipiens</td>
<td>Artificial site</td>
<td>12 days (9–14)</td>
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<td>Artificial site</td>
<td>7 days (6–8)</td>
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days) for *Cx. pipiens* (Table 1). There was 95% confidence that 95% of the *Cx. tarsalis* population would develop to pupae in 2.4 days or more and that 95% of the *Cx. p. pipiens* population would develop to pupae 2.9 days or more. There was no significant difference (P = 0.37) between development times of *Cx. tarsalis* and *Cx. p. pipiens* in the artificial sites.

The mean water temperature was significantly associated with *Cx. tarsalis* larval development time (*P < 0.01*) in a multivariate regression model adjusted for study site. The slope of the model was −0.81, indicating that larval development time decreased approximately 19 h for every 1°C increase in temperature (Fig. 1). The regression model fit the data well (adjusted *R* square = 0.71). With the use of the estimated regression equation, development times were predicted to be 20–24 days at 12°C, 15–19 days at 18°C, 11–14 days at 24°C, and 6–9 days at 30°C (Table 2). A separate regression analysis for *Cx. pipiens* suggested average water temperature was significantly associated with development time (*P < 0.01*). The slope for water temperature in this model was −0.58, indicating that larval development time decreased approximately 14 h for every 1°C increase in temperature. The regression model fit the data well (adjusted *R*-square = 0.82). Predicted mean development times for *Cx. pipiens* were 16 days at 12°C, 13 days at 18°C, 10 days at 24°C, and 6 days at 30°C.

### Los Angeles Basin

In the LA Basin study sites, pupae were observed in all *Cx. tarsalis* cages and containers throughout the 3 months of the study. In July, the minimum time to pupation for *Cx. tarsalis* was 7 days, observed in all containers at the artificial site and in all cages at 1 field site. The maximum time to pupation was 10 days. The mean water temperature at the 4 study sites was 28.4°C. *Culex quinquefasciatus* pupae were observed on day 6 in 1 container at the artificial site; however, larvae in the remaining containers failed to develop during the July phase of the study. In August, the minimum time to pupation for *Cx. tarsalis* was 10 days, observed in all containers at the artificial site. The maximum development time was 17 days. *Culex quinquefasciatus* pupated on day 8 in the 3 containers at the artificial site. The mean water temperature was 25.3°C. The minimum time to pupation for *Cx. tarsalis* in September was 10 days, observed in 4 cages at 2 field sites. The maximum time to pupation was 15 days. *Culex quinquefasciatus* pupated on Days 6 and 7 during the September trial. The mean water temperature was 22.6°C. The overall mean development times in the LA Basin for *Cx. tarsalis* and *C. quinquefasciatus* were 11 days (95% CI, 10–12 days) and 7 days (95% CI, 6–8 days), respectively (Table 1). There was 95% confidence that 95% of the *Cx. tarsalis* population would develop to pupae in 5.1 days or more, and that 95% of the *Cx. quinquefasciatus* population would develop to pupae in 3.7 days or more. The development time for *Cx. quinquefasciatus* was significantly shorter than the development time for *Cx. tarsalis* in artificial sites (*P < 0.01*).

In a multivariate regression model adjusted for site, mean water temperature was significantly associated with *Cx. tarsalis* larval development time (*P < 0.01*) and had a slope of −0.65, indicating that larval development time decreased approximately 14 h for every 1°C increase in temperature (Fig. 1). The model fit the data well (adjusted *R*-square = 0.7). Based on this model, predicted development times were 18–22 days at 12°C, 14–19 days at 18°C, 10–15 days at 24°C,
and 6–11 days at 30°C (Table 2). In a separate analysis for *Cx. quinquefasciatus*, predicted mean development times were 14 days at 12°C, 10 days at 18°C, 7 days at 24°C, and 3 days at 30°C.

**Estimated developmental times**

Based on linear regression models with a mean water temperature of 30°C, the minimum predicted mean development times from egg to pupa for *Cx. tarsalis* were 5 days in the Tahoe Basin, 6 days in the Sacramento Valley, and 6 days in the LA Basin. The mean observed water temperatures for all sites combined over the 3 months of the study were 16.4, 24.4, and 25.4°C for the Tahoe Basin, the Sacramento Valley, and the LA Basin, respectively (Table 1). Mean observed water temperatures at the artificial sites alone were 17.0, 20.4, and 24.7°C for the Tahoe Basin, the Sacramento Valley, and the LA Basin, respectively. The minimum predicted development time for *Cx. pipiens* in the Sacramento Valley was 6 days, whereas *Cx. quinquefasciatus* had a minimum development time of 3 days in the LA Basin. In all regions, observed and predicted development times were shortest as the average water temperature approached 30°C (Table 2). However, the study sites within each region were also significantly associated with larval development time, causing the predicted development time for *Cx. tarsalis* at 30°C to vary substantially by site. Predicted development times of *Cx. tarsalis* were consistently shortest at the artificial sites (Fig. 1), which had more space and food supplementation for developing immatures. The mean water temperature appeared to be less influential in predicting larval development time in the Tahoe Basin (Fig. 2); however, the slopes for water temperature were not significantly different between regions (P = 0.27).

**DISCUSSION**

Comparisons between observed and predicted egg-to-pupa development times for mosquitoes between the study regions differed slightly, usually by a day or two. Predictions of minimum development time were made based on multivariate linear regression models that assumed an average water temperature of 30°C and conditions optimal for immature development. This temperature represented the highest average observed during the study and beyond which larval mortality would be expected (Bailey and Geike 1968). Minimum development times observed for *Cx. tarsalis* were 6 days in the Tahoe Basin, 7 days in the Sacramento Valley, and 7 days in the LA Basin, whereas minimum predicted values for the same regions were 5, 6, and 6 days, respectively. The minimum development times observed for *Cx. pipiens* and *Cx. quinquefasciatus* were 8 days in the Sacramento Valley and 6 days in the LA Basin, respectively, with minimum predicted values of 6 and 3 days.

The lower 1-sided tolerance limit for *Cx. tarsalis* supports the notion that shorter development times may be possible for a small fraction of the population; greater than or equal to 2.4 days in the Sacramento Valley and 2.8 days in the Tahoe Basin. Tolerance limits do not take the mean water temperature, life history of the species, or other biotic and abiotic factors into account; rather these are conservative statistical estimates based only on the development time data. In addition, neither tolerance limits nor predicted egg-to-pupa development times take into account the time required for the pupal stage. The length of the pupal period varies and,
as in larvae, is negatively correlated with temperature; 1 study using Aedes albopictus pupae found that the time to emergence ranged from 1.76 to 8.15 days over a range of temperatures from 32°C to 12°C (Briegel and Timmermann 2001). At temperatures of 30°C, it would be reasonable to expect the emergence of adult Culex mosquitoes to occur within a minimum of 1–2 days following pupation (Subra 1981, Reisen and Reeves 1990).

As expected, mean water temperature was significantly associated with egg-to-pupa development times in the multivariate linear regression models constructed for each region. The effect of water temperature on larval development has been evaluated in many species of mosquitoes (Kramer and Garcia 1988, Rueda et al. 1990, Clements 1992, Focks et al. 1993, Eisenberg et al. 1995), in both controlled laboratory conditions and in the field. Temperatures ≤12°C or >32°C have been associated with high mortality of Cx. tarsalis larvae, but within this range higher temperatures correlate with an increased rate of development (Bailey and Geike 1968, Reisen et al. 1984). In the laboratory, Cx. tarsalis larvae pupated in 12, 9, and 5 days when reared in water maintained at 21, 27, and 30°C, respectively (Bailey and Geike 1968). The results of this study reflect these findings; shortest development times for Cx. tarsalis were observed during the month of July in all 3 regions when mean water temperatures were highest. Development times were similar for Cx. tarsalis and Cx. pipiens at artificial sites in the Sacramento Valley, but Cx. quinquefasciatus developed faster than Cx. tarsalis at artificial sites in the LA Basin.

In addition to temperature, the variability in observed egg-to-pupa development time between the study sites was likely due to variation in factors such as food abundance and water quality. The importance of food in determining larval development time has been well documented (Kramer and Garcia 1988, Clements 1992). At a water temperature of 21°C, most Cx. tarsalis larvae pupated 13 and 17 days posthatching in wild rice and white rice fields, respectively, with the difference attributed to greater nutrient abundance in wild rice fields (Kramer and Garcia 1987). In the current analysis, study site appeared to have a greater influence on development time than mean water temperature in the Tahoe Basin, but not in the Sacramento Valley or LA Basins. One factor contributing to this finding could be the lack of variability in the temperature data from the Tahoe Basin due to the small number of observed development times (n = 15). Most of the data were collected during the month of July, and few to no successful observations of development time were made during the months of August and September when water temperatures were lower. During a pilot study conducted in the Tahoe Basin during the summer of 2005, Cx. tarsalis larvae took up to 28 days to develop (California Department of Public Health, unpublished data). In the present study, there was high larval mortality below 15°C at the Tahoe Basin study sites. Although the high larval mortality is not reflected in the linear regression model, it has important implications for Cx. tarsalis production in stormwater structures in the Tahoe Basin; optimal conditions for immature development may be present in the region only during a very short summer time period.

Artificial sites were included in this study to mimic optimal conditions for mosquito development in stormwater BMPs that may occur during the summer months, or in warm microhabitats during other times of year. Furthermore, there is some evidence that immature mosquitoes subjected to fluctuating temperatures develop faster than those reared at constant water temperatures, even when the mean temperatures are equal (Clements 1963). Preliminary trials using HOBO electronic data loggers revealed greater temperature fluctuations in shallow water placed in plastic containers compared with the deeper water present in the stormwater management basins.

Structural stormwater BMPs have the potential to create temporary, shallow water habitats conducive to mosquito production. This study was conducted to evaluate the potential for relaxing the 72-h drain-down policy in California used to minimize mosquito production in BMPs designed without permanent water features. The impetus for the study was the need for improved BMPs in the environmentally sensitive Tahoe Basin where short water detention periods may deter achieving local water quality objectives (Erlich, personal communication). South Lake Tahoe, as the most populous city in this region, has the potential to create substantial runoff pollution into the lake and the city has thus required property owners to implement BMPs (TRPA 2004). This study suggests that increasing water retention periods up to 96 h would not significantly increase the potential for Cx. tarsalis production in BMPs installed in this region, although further research may be needed to determine whether a 24-h increase in water residence times will substantially enhance the quality of discharge water in this area. Similarly, a 96-h water residence time in the Sacramento Valley would not increase the risk of Cx. tarsalis and Cx. pipiens mosquito production; however, times exceeding 72 h in the LA Basin may occasionally lead to production of Cx. quinquefasciatus under optimal developmental conditions. Nevertheless, strict adherence to the intended design and maintenance schedules to ensure complete dewatering of BMPs remains essential.
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