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Long term impacts of combined sewer overflow remediation on water quality and population dynamics of *Culex quinquefasciatus*, the main urban West Nile virus vector in Atlanta, GA

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ABSTRACT

Background: Combined sewers are a significant source of urban water pollution due to periodic discharges into natural streams. Such events (called combined sewer overflows, or CSOs) contribute to the impairment of natural waterways and are associated with increased mosquito productivity and elevated risk of West Nile virus transmission.
Objectives: We investigated the impact of CSOs on water quality and immature mosquito productivity in the city of Atlanta, Georgia, one year before and four years after CSO facility remediation.
Methods: Water quality (ammonia, phosphate, nitrate and dissolved oxygen concentrations), immature mosquitoes (larvae and pupae), water temperature and rainfall were quantified biweekly between June–October at two urban creeks during 2008–2012. A before–after control–intervention design tested the impact of remediation on mosquito productivity and water quality, whereas generalized linear mixed-effect models quantified the factors explaining the long term impacts of remediation on mosquito productivity.
Results: Ammonia and phosphate concentrations and late immature (fourth-instar and pupae) mosquito populations were significantly higher in CSO than in non-CSO creeks, while dissolved oxygen concentrations were lower. Remediation significantly improved water quality estimates (particularly ammonia and dissolved oxygen) and reduced the number of overflows, mosquito productivity and the overall contribution of CSO-affected streams as sources of vectors of West Nile virus.
Conclusions: The quality of water in CSOs provided a suitable habitat for immature mosquitoes. Remediation of the CSO facility through the construction of a deep storage tunnel improved water quality indices and reduced the productivity of mosquito species that can serve as vectors of West Nile virus.

1. Introduction

Since its new world emergence in New York City in 1999, West Nile virus has spread over much of North America and the Caribbean, and become a threat to human and veterinary health (Kramer et al., 2008). During the last decade, more than 36,000 human cases of West Nile were reported in the US, including more than 1500 fatalities (Centers for Disease Control, 2013). Heterogeneities in the occurrence and competence of vector and reservoir hosts throughout the US (Reisen et al., 2005; Turell et al., 2005), together with local variations in vector host-feeding patterns (Hamer et al., 2009; Kilpatrick et al., 2006), bird herd immunity and infectiousness (Loss et al., 2009; Reisen et al., 2008), viral genetic variability (Bertolotti et al., 2008; Kilpatrick et al., 2008), human demographic and socio-economic factors (Harrigan et al., 2010; Rochlin et al., 2009, 2011; Ruiz et al., 2004, 2007), climate and other environmental variables (Kilpatrick et al., 2008; Soverow et al., 2009) strongly modulate West Nile transmission dynamics in urban environments. As a consequence, vector abundance and virus amplification tend to be highly localized in space and time (Chung et al., 2013; Ruiz

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et al., 2004, 2007; Vazquez-Prokopec et al., 2010) due to prevailing environmental conditions favoring immature mosquito development and bird–mosquito contacts (Kilpatrick, 2011).

In the US, urban water pollution due to effluents emanating from combined sewer facilities is considered a major source of water impairment, and a significant human health concern (Tibbetts, 2005; US Environmental Protection Agency, 2009). Combined sewer systems collect and convey storm and waste water through a single pipe network. Under dry conditions, the mix of precipitation and sewage is channeled to a treatment plant before being discharged into water bodies. During heavy precipitation, storm and waste water exceeding a treatment plants′ processing capacity are discharged into local surface waters, a process known as a combined sewer overflow (CSO) (Tibbetts, 2005). In Atlanta, GA, *Culex quinquefasciatus* is the main urban vector of West Nile virus (Vazquez-Prokopec et al., 2010). CSO-affected streams provide optimal habitat for *Cx. quinquefasciatus* by enhancing female oviposition rates (Calhoun et al., 2007; Chaves et al., 2011, 2009; Nguyen et al., 2012), accelerating immature development and increasing female body size (Chaves et al., 2011). Furthermore, proximity to CSO-impaired streams was the most important risk factor of West Nile infection in *Cx. quinquefasciatus*, birds (mainly corvids) and humans (Vazquez-Prokopec et al., 2010). Understanding the long-term contribution of CSO-impaired streams to the dynamics of West Nile virus ecology is of significant interest for the design of disease surveillance and control strategies, particularly since approximately 772 cities in 32 US states rely on combined sewers for wastewater management (Tibbetts, 2005).

In 1999, the City of Atlanta was issued a consent decree to improve its combined sewer system due to the constant sewer discharges exceeding environmental standards and the violation of the federal Clean Water Act and the Georgia Water Quality Control Act (Hunter and Sukenik, 2007; US Environmental Protection Agency, 1999). A $1 billion capital investment was required to make the necessary improvements, which included the separation of combined sewers into distinct sewer and storm water lines and the construction of off-line storage facilities (see Supplementary material for details on CSO remediation policies and technologies). As remediation through deep storage tunnels can still have the possibility of generating sporadic discharges, there is a need to assess the environmental and public health impacts of such remediation strategy on stream health and mosquito productivity. This study describes and quantifies the long-term (5-year) changes in water quality and mosquito abundance in Tanyard Creek, the urban creek in Atlanta most heavily affected by sewer discharges, one year before and four years after the remediation of the CSO facility by the construction of a deep storage tunnel.

2. Methods

2.1. Study sites

This study was performed from June 2008 through October 2012 in two urban creeks in the city of Atlanta, Georgia. Tanyard Creek (from now on, CSO creek), flows for 3.4 km through private residential areas and public green spaces before emptying into Peachtree Creek, a major tributary of the Chattahoochee River (Supplementary Fig. S1A). The creek is approximately 10–15 m wide and 0.1–2.0 m deep. A 1000 m long concrete channel connects the CSO facility to the stream′s headwaters. The Tanyard Creek CSO facility was improved in 2008 by the construction of a deep-storage tunnel that diverts overflows to the local water reclamation center for treatment (see Supplementary material for a description of the remediation operations). This improvement was expected to significantly reduce (but not eliminate) discharges into the CSO creek (Carlile, 2010). Not associated with a CSO facility, Peavine Creek (from now on, non-CSO creek), located near the Emory University campus is similar to Tanyard Creek in its flow characteristics and served as a control stream (Supplementary Fig. S1B). While both creeks are affected by non-point source pollution (i.e. stormwater runoff), wastewater at the non-CSO creek is managed by a separated sewer system.

2.2. Sampling design

For this study, six sampling locations (three along each creek, see Supplementary Fig. S1) were chosen according to physical characteristics considered suitable for mosquito larvae (Calhoun et al., 2007). A drought in 2011 forced us to select three new pools (similar in characteristics) in the CSO creek (see Supplementary Fig. S1). Biweekly water and mosquito samples from both creeks were obtained between June and October over five consecutive mosquito breeding seasons (2008–2012). Samples collected from the CSO creek were divided into pre- (2008) and post- (2009–2012) facility remediation.

Immature mosquitoes were collected using standard 350 ml dippers (Bioquip, Rancho Dominguez, CA). Five dips were performed at each pool (constant collecting effort of 15 dips per creek) and the mosquito larvae and/or pupae collected in dips were taken back to the lab where the number of larvae (by instars, from first to fourth) and pupae per dip were registered. Fourth instar larvae and adults that emerged from pupae were identified to species using morphological keys (Slaff and Apperson, 1989).

Chemical and physical properties of water in each pool were measured on each sampling date. Dissolved oxygen, pH and water temperature were recorded using hand-held meters (YSI-55 and YSI EcoSense pH10, YSI Inc., Yellow Springs, OH). Concentrations of ammonia, nitrate, and phosphate were assessed by testing 300–500 mL samples of creek water with a digital photometer (CHEMetrics V-2000, CHEMetrics Inc., Midland, VA). The concentration ranges of each chemical were: 0.20–1.50 ppm for nitrate (Cadmium reduction method), 0.20–30.0 ppm for ammonia (Salicylate method) and 0.30–8.00 ppm for phosphate (Stannous chloride method). Macroinvertebrate collections were performed post-remediation (2009–2012) to further elucidate water quality and identify presence of mosquito predators. Aquatic sampling D-nets (Wilco D-Frame Multifilment, Wildco, Yulee, FL) were used over an hour sampling period in and around each sampling location. Specimens were stored in 70–90% ethanol for further identification using proprietary keys (Merritt and Cummins, 1984). The principal limitation of these data is the inconsistency of our sampling effort; each creek was visited just once in each 2009 and 2010, resulting in a small number of collected specimens in these years compared to 2011 and 2012. Macroinvetebrate samples were not collected pre-remediation.

2.3. Data management

Discharge monitoring reports for Tanyard Creek containing information on the monthly number of sewer discharges, the volume and duration of discharge events for the years 2008, 2009, 2011 and 2012 were provided by the City of Atlanta′s Department of Watershed Management. Daily weather data from the nearest weather station to each creek were used to calculate average and total monthly rainfall. Productivity (i.e., the potential to produce adult mosquitoes) at each pool was quantified by summing the number of fourth instar larvae and pupae collected across the five dips. As trapping effort was the same on each sampling pool and creek, such measure of productivity was both comparable across creeks and appropriate for statistical analysis. To account for temporal dependence in data points collected every week, the monthly sum of fourth instar/pupae and average of all water quality measures (water temperature, pH and concentrations of ammonia, nitrate, phosphate and dissolved oxygen) were calculated. Water quality data from 2009 were excluded from all analyses because of an improperly calibrated photometer.

2.4. Statistical analysis

Statistical analyses were performed to address two research questions: (a) did CSO facility remediation have an impact on water quality and mosquito productivity indicators?; and (b) what physical and chemical factors determine changes in mosquito productivity within and between creeks? Both questions aimed at determining the impact and consequences of remediation on stream health and mosquito population dynamics. Within a creek, sampling pools cannot be considered truly independent because similar factors (i.e. sewer overflows) could similarly affect their water quality and mosquito productivity measures. All analyses accounted for this spatial and temporal pseudo-replication (Chaves, 2010) issue.

For research question (a) we used a before–after control–intervention design, commonly used in impact assessment (Wiens and Parker, 1995), in which a treatment and a control creek are compared before and after an environmental impact (i.e., CSO facility remediation). A mixed effects ANOVA was performed on all variables that met the normality assumption (monthly average of water quality indicators and proportion of *Cx. quinquefasciatus* at each pool). The test included creek (CSO vs. non-CSO) and period (before vs. after remediation) together with their interaction as the main effects, and sampling site as a random effect. The interaction of creek and period (i.e., the before–after control–intervention effect) was used to quantify the impact of facility remediation on each variable. Adding a random effect accounted for spatial dependence between sites within a creek. As mosquito productivity was highly skewed and non-normal, a before–after approach (Wiens and Parker, 1995) was used to compare the effect of CSO facility remediation. Briefly, monthly values for the CSO creek were compared before and after facility remediation using the Kruskal–Wallis test. Monthly aggregates of mosquito and water quality before facility remediation were compared between creeks by using the non-parametric Mann–Whitney matched pairs signed rank test.

For research question (b) we implemented generalized linear mixed-effects models (GLMM) (Zuur et al., 2009) to assess the relationship between mosquito productivity, water quality and the impact of CSO facility remediation. Given the large number of variables (15) and limited number of observations (144) we only fit models using variables with $P<0.2$ in the univariate tests performed in (a). Such threshold value allowed including variables potentially important in explaining the data (yet not statistically significant). A Poisson distribution was chosen to model the monthly mosquito counts using a log link function. The models accounted for the spatial dependence in the data (sampling sites within a creek) by a random intercept associated with each sampling site (Zuur et al., 2009). The structure of the full model was

$$\begin{aligned} f(\text{No. mosquitoes}) = {} & \beta_0+\beta_1(\text{CRK})+\beta_2(\text{TIME})+\beta_3(\text{AMM})+\beta_4(\text{PHOS})+\beta_5(\text{DO}) \\ & +\beta_6(\text{TEMP})+\beta_7(\text{DO}\times\text{TEMP})+\beta_8(\text{AMM}{*}\text{TIME}) \\ & +\beta_9(\text{PHOS}{*}\text{TIME})+\beta_{10}(\text{PERIOD}) \end{aligned}$$

where CRK is the creek (CSO vs. non-CSO), AMM and PHOS the mean monthly concentrations of ammonia and phosphate, DO × TEMP accounts for depletion of dissolved oxygen at increasing water temperatures, PERIOD distinguishes observations before or after facility remediation and terms with a * represent interaction factors. The variables CRK and PERIOD were included in all tested models as structural variables reflecting the study design.

Model inference was based on a multi-model selection approach (Burnham and Anderson, 2002). Under this analytic framework we contrasted a set of candidate models (representing different hypotheses) with each other and identified the best model (or the best set of models) based on model fit (Burnham and Anderson, 2002), where the best model was the one with the lowest Akaike Information Criterion (AIC) value. When the lowest AIC value differed from the next best model by <2 units, we identified a best set of models rather than a single best model (Burnham and Anderson, 2002). We estimated the Akaike weight (ω_i) for each model as a measure of the probability that a particular model fitted the data better than the alternative set of candidate models (Burnham and Anderson 2002). For each independent variable (j) evaluated we estimated the sum of Akaike weights ($\sum\omega_i$) as the sum of the ω_i from each model in which j was a present (Burnham and Anderson, 2002). This metric (bounded between 0 and 1) evaluates the relative importance of each independent variable for predicting the dependent variable (Burnham and Anderson, 2002). We contrasted nine models for *Cx. quinquefasciatus* abundance (see Supplementary Table S2 for details on each model). ANOVA and GLMM were performed using the packages *nlme* (Pinheiro et al., 2012) and *lme4* (Bates et al., 2013), respectively, from the R statistical software (version 2.15) (R Core Team, 2012), and non-parametric tests were performed using SAS 9.3 (Cary, NC).

3. Results

3.1. Impact of remediation

The deep storage tunnel of the remediated CSO facility became operational in November 2008. Prior to the remediation, the facility averaged 8.4 overflows a month into the CSO creek with a mean (SD) overflow size of 9.82 (10.7) million gallons and an average (SD) duration of 3.28 (2.07) hours (Supplementary Fig. S2). Following the completion of the tunnel, the number of discharges into the CSO creek declined dramatically, even during heavy rain events (Supplementary Fig. S2). A total of 42 sewer discharges were recorded in 2008, none in 2009, one in 2011 and two in 2012 (Supplementary Fig. S2). The total annual volume discharged from the CSO facility was reduced from 154.15 million gallons in 2008 to 0 in 2009, 6.17 million gallons in 2011 and 6.24 million gallons in 2012 (Supplementary Fig. S2).

A total of 11,580 immature mosquitoes were collected at both creeks over five years. Most mosquitoes were collected from the CSO creek (93.4%) and prior to the facility remediation (89% in 2008) (Fig. 1). The difference between creeks in the monthly sum of mosquito pupae and fourth-instar larvae was significantly different pre- and post-facility remediation (Kruskal–Wallis test,

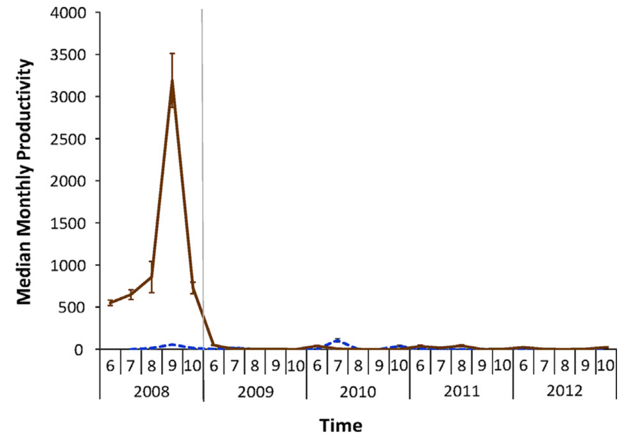

Fig. 1. Temporal distribution of the median monthly productivity, expressed as number of late immature (IV-instar and pupae) mosquitoes at the CSO (solid brown) and non-CSO (dashed blue) creeks pre- (2008) and post- (2009–2012) CSO facility remediation. Vertical gray line indicates the time when the remediated CSO-facility became operational and error bars indicate the interquartile range (Q1–Q3). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

$\chi^2=9.6$; df=1; $P=0.002$). Prior to remediation, median [Q1–Q3] mosquito productivity was 7.5 [0 – 99] at the CSO creek and 0 [0–0] at the non-CSO creek (Fig. 1). Following remediation median productivity dropped to 0 [0–0] both in the CSO and non-CSO creek (Fig. 1). In the years that followed the remediation (2009–2012), mosquito productivity did not differ significantly between CSO and non-CSO creeks (Mann Whitney test, $W=1.79$; $P>0.05$; Fig. 1).

The composition of immature mosquito species collected from the CSO creek also changed over time (Fig. 2). While *Cx. quinquefasciatus* comprised a substantial proportion of immature species collected throughout the study period, its dominance in the CSO creek became diminished post-remediation (Fig. 2). The monthly proportion of *Cx. quinquefasciatus* was significantly lower after CSO facility remediation in comparison to the non-CSO creek (Mixed Effects ANOVA creek:period interaction term; $t=2.02$; df=58; $P=0.04$; Supplementary Table S1). Ninety percent of immature mosquitoes collected from the CSO creek in 2008 were *Cx. quinquefasciatus*, compared to less than 20% in 2009 and 2010, 60% in 2011 and 13% in 2012 (Fig. 2). Although the proportion of *Cx. quinquefasciatus* jumped in 2011, relative abundance post-remediation never reached pre-remediation levels. During the post-remediation period, *Cx. restuans* became the predominant species in the CSO creek, and other species, namely *Cx. territans* and *Aedes vexans* comprised larger proportions (yet a small number) of the larval mosquito community in the CSO creek.

Prior to remediation, Calhoun et al. (2007) reported aquatic midges (family:Chironomidae) as the most frequently collected macroinvertebrate taxon at the CSO creek and vertebrates were only found downstream of the sampling sites, near the confluence of Tanyard and Peachtree Creeks. Our post-remediation data show increases in macroinvertebrate diversity, with species composition in the CSO creek at levels comparable to the non-CSO creek (Fig. 3). Post-remediation, we found that the most prevalent macroinvertebrates were from the Order Trichoptera (caddisflies), making up 76% of collected samples at the non-CSO creek and 25% of collections at the CSO creek (Fig. 3). Odonata (dragonflies and damselflies), major predators of larval mosquitoes (Shaalan and Canyon, 2009), were prevalent in 15% of the collections at the CSO-creek and 6% of the collections at the non-CSO creek. Other vertebrates and invertebrates not observed by Calhoun et al. (2007) prior to remediation (tadpoles, fish, turtles, crayfish

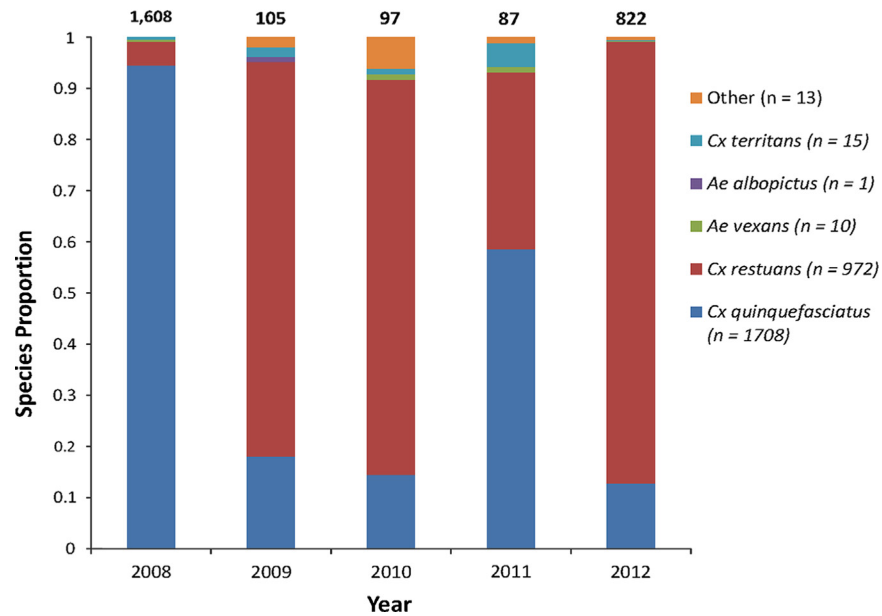

Fig. 2. Larval mosquito species composition, expressed as proportion of each monthly collection at the CSO creek during 2008–2012. Total number of specimens collected shown above each bar. The category "Other" includes *Culex erraticus*, *Culex salinarius*, *Anopheles crucians* and *Anopheles punctipennis*.

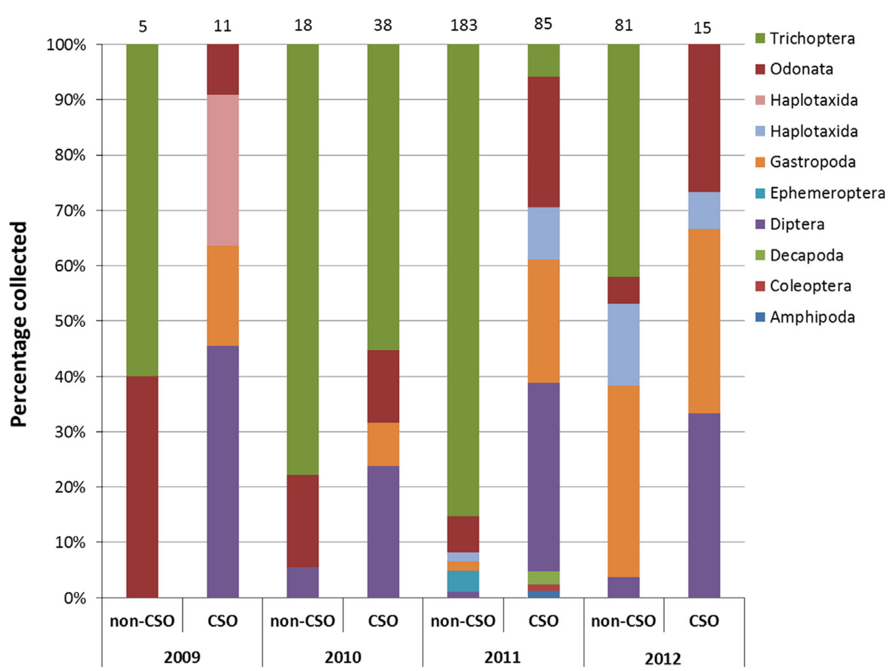

Fig. 3. Macroinvertebrate community composition, expressed as percentage of individuals collected from each family in the CSO and non-CSO creeks post-facility remediation (2009–2012). Total number of specimens collected shown above each bar.

and salamanders) were observed (but not quantified) post-remediation.

Remediation had significant impacts in water quality indicators, particularly ammonia (Mixed Effects ANOVA creek:period interaction term; $t=2.09$; df$=85$; $P=0.037$) and phosphate (Mixed Effects ANOVA creek:period interaction term; $t=3.69$; df$=100$; $P=0.0004$) (Fig. 4 and Supplementary Table S1). The remaining water quality indices showed no statistically significant change before and after facility remediation (Fig. 4). Ammonia concentrations were reduced from a pre-remediation mean [SD] of 1.03 [0.8] ppm to a four-year post-remediation median of 0.45 [0.4] ppm and phosphate concentrations from 1.03 [0.6] ppm to a four-year post-remediation value of 0.39 [0.4] ppm. Dissolved oxygen concentrations increased from 1.42 [0.6] mg/L in 2008 to 2.41 [1.5] mg/L following facility remediation, reaching a level comparable to the non-CSO creek by 2011. Conversely, water quality measurements remained relatively constant at the non-CSO creek with no dramatic changes over time (Fig. 4).

3.2. Determinants of mosquito productivity

The best-fitting GLMM model (among nine tested, see Supplementary Table S2) evaluating the association between mosquito productivity and water quality pre- and post-facility remediation is shown in Table 1. The standard error of the random intercept of the best fitting model was 1.617. The full model included terms for ammonia (together with its time interaction), DO $\times$ water temperature, a term for creek and a term for pre–post-CSO

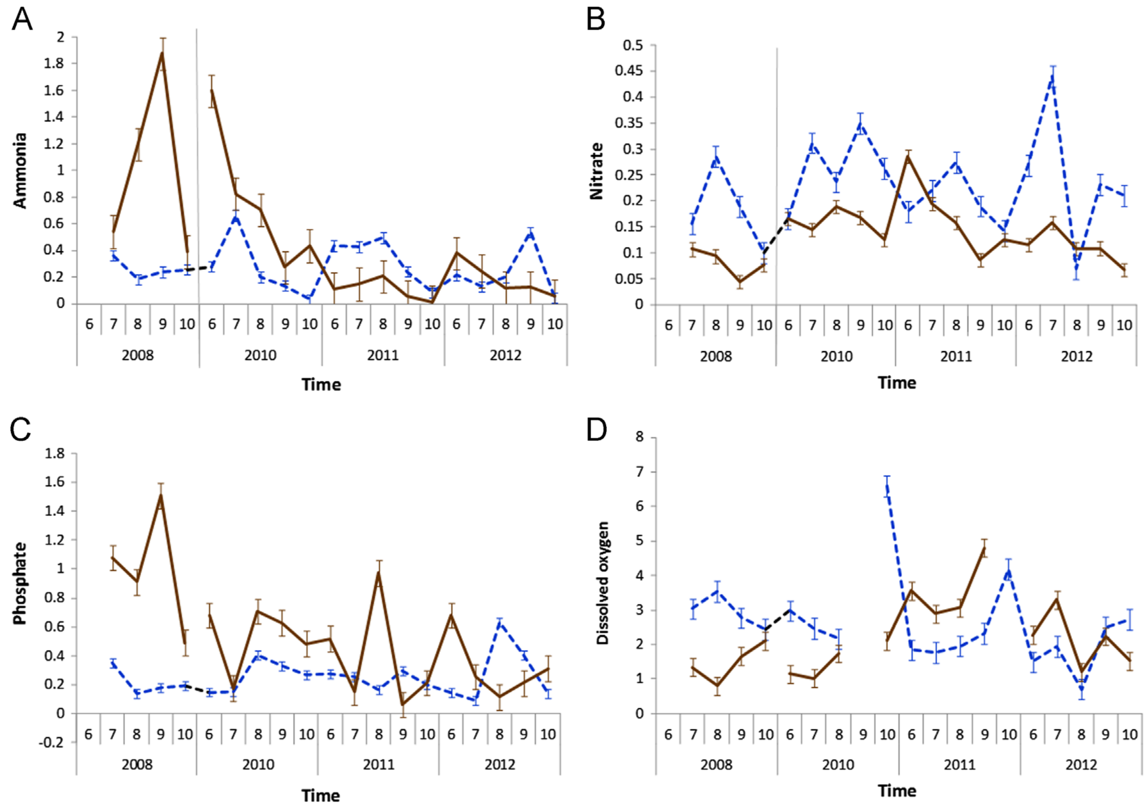

Fig. 4. Median water quality indicator concentrations ((A) ammonia; (B) nitrate; (C) phosphate; and (D) dissolved oxygen) in CSO (solid brown) and non-CSO (dashed blue) creeks before (2008) and after (2010–2011) CSO facility remediation. Vertical gray line indicates the time when the remediated CSO-facility became operational and error bars the interquartile range.

Table 1
Rate, standard errors and 95% confidence intervals from best-fitting GLMM model predicting mosquito productivity within a CSO- and a non-CSO affected stream pre- 2008 and post-CSO facility remediation (2010–2012).

Parameter	Rate	SE	95% CI
Intercept	0.0142	0.894	0.002, 0.113**
Creek	45.563	1.038	4.610, 450.344*
Ammonia	2.814	0.031	2.686, 2.949**
Time since remediation	0.929	0.008	0.915, 0.943**
Dissolved oxygen	13.939	0.107	9.419, 20.626**
Water temperature	1.156	0.012	1.114, 1.199**
Remediation period	4.463	0.103	3.735, 5.334**
Ammonia × Time	0.987	0.005	0.977, 0.997*
DO × Water temperature	0.901	0.005	0.886, 0.917**

** $P < 0.001$.
* $P < 0.01$.

remediation was best supported by the data (ΔAIC = 410; Supplementary Table S2). Ammonia (positively) and dissolved oxygen × water temperature (negatively) were significantly associated with high mean mosquito productivity (Table 1). The sum of Akaike weights show that ammonia, although statistically significant in the best model, was the best predictor of mosquito productivity only when included as an interaction term with the time since facility remediation (Table S2).

4. Discussion

Urban runoff, improperly located, designed and maintained wastewater treatment systems (such as combined sewer systems), pet wastes, industrial point discharges, household chemicals and vehicle emissions all contribute to urban water pollution (Alberti, 2008; Paul and Meyer, 2001; US Environmental Protection Agency, 2009). The introduction of nitrogen or phosphorus into the environment can result in explosive production of phytomass (Camargo and Alonso, 2006; Smil, 2000), leading to oxygen depletion, harmful algal blooms, and overall reductions in aquatic biodiversity (Paul and Meyer, 2001; Smil, 2000; Townsend et al., 2003). In particular, changes to the nutrient cycle by sewer overflows have the capacity to dramatically alter the ecology of receiving waters. Prior to CSO facility remediation in Atlanta, periodic sewer overflows increased the concentration of ammonia in receiving streams, enhancing mosquito development by increasing food sources for *Cx. quinquefasciatus* larvae. Changes in ammonia concentrations at the CSO creek in the four years following facility remediation were significantly associated with reductions in immature mosquito abundance, and were complemented by the re-appearance of mosquito predators absent from pre-remediation samples (Calhoun et al., 2007).

Changes in aquatic community composition in the CSO creek also reveal water quality improvements. In 2008, *Cx. quinquefasciatus*, a species whose larvae are commonly associated with eutrophic and polluted waters (Savage and Miller, 1995), dominated mosquito collections at the CSO creek. The significant reduction of *Cx. quinquefasciatus*' dominance in the post-remediation years signifies changing conditions in the CSO creek as a larval mosquito habitat. Reductions in frequency and size of sewage overflows made the CSO stream habitat less attractive for *Cx quinquefasciatus* oviposition (Chaves et al., 2009; Chung et al., 2013; Nguyen et al., 2012) and more suitable for pollution-sensitive species (Crans, 2010). The appearance of *Cx. territans*, a species whose larvae do not survive in polluted water (Crans, 2010), at the CSO creek in 2009, 2010 and 2011 indicate improving water quality conditions, making the habitat suitable for a wider range of species. Similarly, the post-remediation habitat at the CSO creek was suitable for a large number of vertebrate and invertebrate taxa, some of them predators of mosquito larvae. Since remediation, it has become increasingly common to find fish, tadpoles, crayfish, snakes, turtles and water fowl at the CSO creek. In addition to our measurements of water quality, these observations point to an overall improvement of stream health in the CSO creek.

Frequent pre-remediation overflows originating from the CSO facility not only introduced high concentrations of organic nutrients, but also caused physical damage to the stream through increased water flow, erosion and the movement of boulders, trees and other debris. Such frequent hazards may have rendered the creek uninhabitable for resident species such as fish, frogs and water fowl. Conversely, the pools of stagnant water isolated from the main creek – generated by overflow events – represented key breeding habitats for *Cx. quinquefasciatus* mosquitoes (Calhoun et al., 2007). In addition to changes in water quality indicators, physical changes associated with the reduction of overflows may have contributed to changes in the aquatic community composition and the effective reduction of mosquito productivity.

The best fitting GLMM models indicate greater mosquito productivity at the CSO creek, which decreased over time following remediation as a consequence of reductions in ammonia and increases in dissolved oxygen. These results, coupled with the univariate analyses comparing water quality indicators between creeks, indicate that reductions in water quality indicators due to sewer discharges can dramatically modify the chemistry and ecology of local streams, turning them into suitable *Cx. quinquefasciatus* habitats. These results suggest that habitats created by CSOs are similar to other urban mosquito habitats such as catch basins, where ammonia concentrations were found to be positively associated with larval mosquito abundance (Gardner et al., 2013). While significant reductions in phosphate concentrations were associated with remediation at the CSO creek, model results indicate that phosphate was not an important predictor of mosquito abundance, suggesting the influence of non-point sources of pollution (i.e. fertilizer runoff from residential and recreational areas) in the creek environment. A previous study in the CSO creek found that immature mosquito abundance was often regulated by the stream's flow patterns (Calhoun et al., 2007), with increased flow associated with diminished immature mosquito abundance. Large rain events resulted in larger flow volume through the creeks and were hypothesized to be responsible for flushing mosquitoes downstream, significantly reducing the number at each study site.

Despite local and federal pressure to improve CSO facilities throughout the US Environmental Protection Agency (2001), little documentation is available regarding which of the 772 communities affected with combined sewer systems have completed remediation projects. The deep storage tunnel constructed in Atlanta, GA, appears to have been an effective means of controlling CSOs and, consequently, mosquito larval development. Increased storage capacity of the CSO facility resulted in the ability to divert heavy flows and reduce discharges into receiving streams. Consequently, water quality improved significantly compared to pre-remediation measurements, reaching levels comparable to a non-CSO creek. While the CSO creek remains on EPA's list of impaired water bodies (due to fecal coliform contamination, most likely originating from sewer discharges) (US Environmental Protection Agency, 2010), the stream has become habitable again for many pollution sensitive taxa. Observed changes in water quality and community composition indicate a measurable positive impact of the City's CSO remediation effort.

Spatial clustering of West Nile virus infection in urban environments occurs in areas where favorable conditions for larvae, competent reservoir hosts and opportunities for human exposure overlap (Hamer et al., 2009; Nielsen et al., 2008; Reisen, 2010; Ruiz et al., 2004). In Atlanta, GA, hot-spots of high West Nile infection in mosquitoes, humans and birds were identified in close proximity to CSO-impacted streams (Vazquez-Prokopec et al., 2010). Our study shows that the overall contribution of CSO streams as sources of *Cx. quinquefasciatus* mosquitoes was dramatically reduced after CSO facility remediation (evidenced by the dramatic reduction in the absolute number of fourth instar larvae and pupae). It remains to be studied whether this overall reduction in vector species and mosquito productivity in general has the potential to reduce the risk of West Nile virus transmission near CSO streams.

5. Conclusions

This study demonstrated that the concentration of wastewater and runoff originating from CSOs not only had significant impacts on aquatic environments (as evidenced by excess nitrogen and phosphorus, reduced dissolved oxygen and biodiversity) but also had an important role in modifying ecological conditions to favor the development and population growth of certain pollution-tolerant mosquito species. We provide evidence of the association between urban stream pollution due to CSOs and *Cx. quinquefasciatus* immature development and demonstrate that CSO facility remediation through the construction of a deep storage tunnel improved water quality indices and reduced mosquito productivity, potentially diminishing the role of CSO-impacted creeks as sources of West Nile virus vectors.

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Appendix A. Supplementary material

Supplementary material associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.envres.2013.12.008.

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