Journal of Environmental Science and Health, Part A
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/lesa20

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Available online: 19 Dec 2009

To cite this article: P. A.M. Bachand, S. M. Bachand, S. E. Lopus, A. Heyvaert & I. Werner (2010): Treatment with chemical coagulants at different dosing levels changes ecotoxicity of stormwater from the Tahoe basin, California, USA, Journal of Environmental Science and Health, Part A, 45:2, 137-154

To link to this article: http://dx.doi.org/10.1080/10934520903425459

Journal of Environmental Science and Health Part A (2010) **45**, 137–154

ISSN: 1093-4529 (Print); 1532-4117 (Online)
DOI: 10.1080/10934520903425459

Treatment with chemical coagulants at different dosing levels changes ecotoxicity of stormwater from the Tahoe basin, California, USA

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In recent decades, the transport of stormwater-associated fine particles and phosphorus into Lake Tahoe has led to decreased water clarity and related ecological changes. Polyaluminum chloride coagulants (PACs) have shown great promise in removing these constituents from stormwater before it enters the lake. However, the potential risks of coagulant treatment to aquatic organisms are not well understood. This study investigated stormwater and coagulant toxicity under non-dosed, optimally-dosed, and over-dosed conditions using the US EPA 3-species test through growth of green algae (*Selenastrum capricornutum*), zooplankton (waterflea, *Ceriodaphnia dubia*) mortality and reproduction, and larval fish (fathead minnow, *Pimephales promelas*) mortality and biomass. Stormwater samples were collected during a 2005 spring snowmelt runoff event from three sites representing various forms of developed regions around Lake Tahoe. Samples were dosed with two different coagulants (a chitosan and a PAC) at levels optimized with a streaming current detector (SCD). Non-treated highway runoff was toxic to zooplankton and fish. Optimal coagulant dosing increased algal growth and reduced zooplankton toxicity. Overdosing at two and three times the optimal level of a PAC decreased zooplankton reproduction and increased fish mortality. PAC-related toxicity was correlated with increasing total unfiltered aluminum and decreasing alkalinity, pH, and DOC. Because of toxicity risks, we recommend keeping PAC coagulant dosing at or below optimal levels in practice.

Keywords: Coagulants, ecotoxicity, stormwater, runoff, overdosing.

Introduction

Progressive reduction of clarity in Lake Tahoe, a large and deep subalpine lake located in the Sierra Nevada mountains of the western United States, has been attributed to accumulations of fine particles and phosphorus.[1,2] Urban runoff within the Tahoe basin has been identified as a major contributor of small particles and nutrients to this oligotrophic lake,[3] and many structural best management practices (BMPs) designed to capture and treat surface runoff and increase infiltration have been recently implemented.[4,5]

Small particles in urban runoff routinely contain high concentrations of constituents of environmental concern, such as heavy metals and nutrients.[6–8] Because settling of fine particles occurs slowly, natural sedimentation is an ineffective method for their removal.[9–11] This is frequently the case in the Tahoe region, where hydraulic residence times of stormwater treatment BMPs are too short to effectively settle fine particles and associated phosphorus and other pollutants.[5,12–13] Chemical coagulation is one possible treatment alternative to achieve highly efficient removal of small particles. Treatment programs utilizing conventional metal salts have demonstrated low removal efficiencies under many conditions,[9,14] but polymerized coagulants have shown promise in successfully removing fine particles from runoff even at low dosages.[9,15] Low-intensity chemical dosing with polyaluminum chloride (PAC) coagulants at dosing levels optimized to efficiently remove constituents of concern has promise as a viable approach to achieving environmental standards (turbidity <20 NTU and total phosphorus $<100\ \mu g/L$) for stormwater entering Lake Tahoe.[15] PAX-XL9® was found by Trejo-Gayton[15] to be a particularly effective PAC

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Received June 22, 2009.

coagulant for reducing dissolved phosphorus and fine solids under a range of temperatures, mixing regimes, and stormwater qualities.

For coagulation treatment of stormwater to move forward as a viable means to decrease fine particle and phosphorus pollution in Lake Tahoe, concerns of toxicity associated with coagulant treatment need to be addressed. It is necessary to investigate the potential toxic effects of coagulants at different dosing levels when added to stormwater. Biomonitoring has become many researchers' preferred method of investigating ecotoxicity of complex contaminant mixtures because chemical analysis may not address total effluent effects.[16–18] In a recent study, Lopus et al.[19] showed toxicity of stormwater in the Tahoe basin dosed with coagulants was generally the same or higher than of non-dosed stormwater. In that study, optimal coagulant doses were determined subjectively through jar testing and visual inspection of flocculant formation.

The study presented here used streaming current detectors (SCDs) measuring charge neutralization to provide precise estimates of optimal dosing levels. SCDs are used to minimize overdosing or underdosing under varying water flow and quality conditions.[15,20–21] Because SCDs are reliable and can be automated, they are becoming a standard tool applied in the water treatment industry. We assessed the effects of raw (non-dosed) and dosed stormwater on algae, zooplankton and fish toxicity using the standard US EPA 3-species test.[22] Toxicity associated with optimal dosing of Liquid Floc, a natural chitosan product, and PAX-XL9 was investigated. Additionally, we assessed toxicity of stormwater treated at up to 3 times optimal dosing levels of PAX-XL9. The mechanisms of ecotoxicity were investigated by analyzing the water for a suite of metals and basic water quality parameters such as pH, TSS, and dissolved oxygen (DO).

Materials and methods

Sample Collection

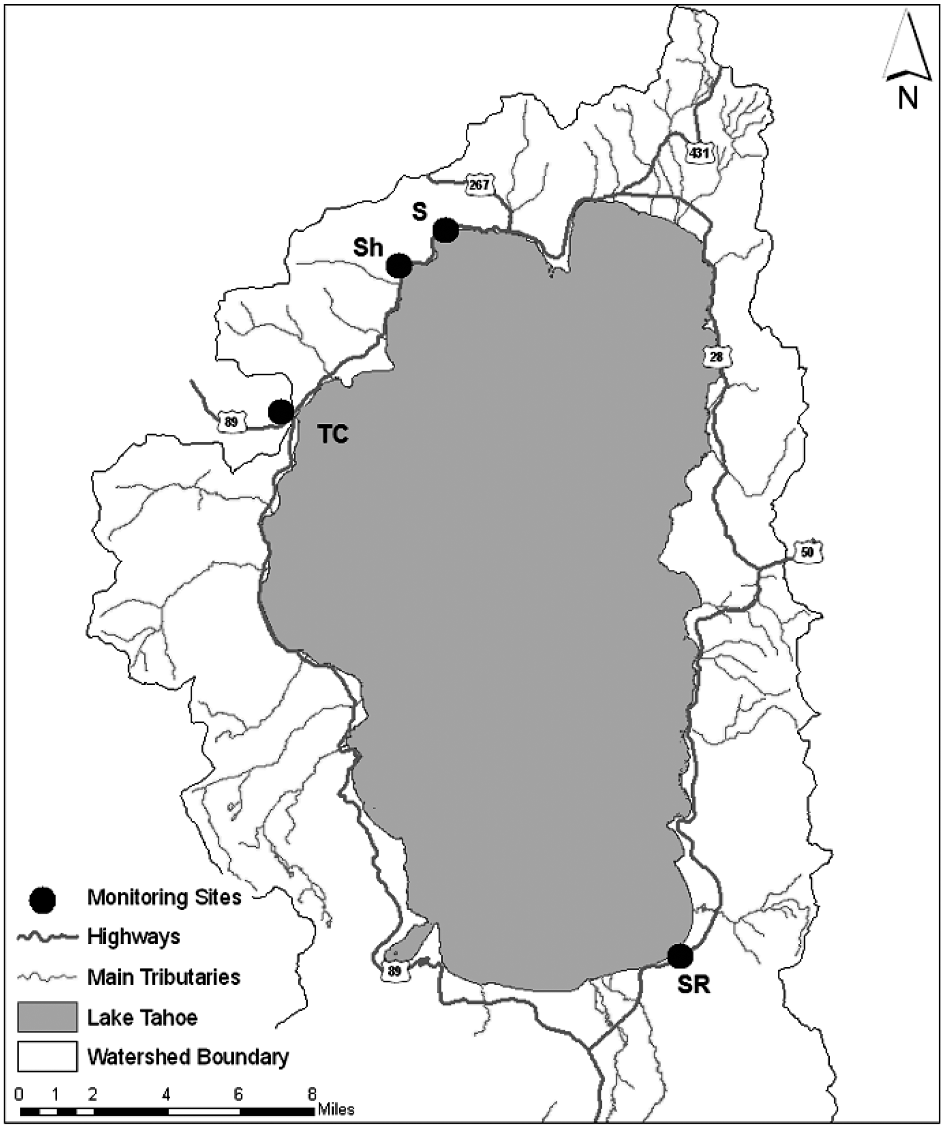
Stormwater samples were collected into high-density polyethylene (HDPE) buckets from four Tahoe basin locations (Fig. 1) representing various combinations of residential, commercial, and highway land use areas (Table 1). Stormwater from the urban sites (Tahoe City, Ski Run, and Stag) was considered representative of what would likely be targeted for treatment with coagulants in stormwater basins. Shivagiri was a non-urban site. Sampling occurred on March 21, 2005 during a spring snowmelt runoff event.

Two coagulants were used in this study: Liquid Floc, a chitosan product derived from shells of sea crustations, and PAX-XL9, a cationic PAC. Optimal coagulant dosing levels were determined with a Streaming Current Detector (SCD), which measures net ionic and colloidal surface charge in the stormwater and converts that measurement to a reading defined as the streaming current voltage (SCV). In theory, the optimal precipitation of suspended sediments occurs when particle charge is neutralized, and the SCV is near zero. For the purpose of this study, optimum dose was

Table 1. Composition of drainage areas for sampled stormwaters.

			Shivagiri	*Tahoe City*	*Ski Run*	*Stag*
% Coverage	Residential	Single Family: Pervious	0	17	25	47
		Single Family: Impervious	0	8	6	14
		Multifamily: Pervious	0	7	8	0
		Multifamily: Impervious	0	8	6	0
	Com/Ind*	Pervious	0	3	8	1
		Impervious	0	16	6	0
	Roads	Primary	0	6	0	0
		Secondary	0	15	26	19
		Unpaved	0	0	0	0
	Other	Ski Run	0	0	0	0
		Unimpacted Area	100	19	16	19
		Vegetation: Recreational	0	0	0	0
		Vegetation: Burned	0	0	0	0
		Vegetation: Harvested	0	0	0	0
		Turf	0	2	0	0
% Impervious Area			0	53	44	33
% Erosion Hazard		Slight	80	87	100	57
		Moderate	20	13	0	43
		High	0	0	0	0
Drainage Area (m^2)			994, 591	273, 043	101, 031	87, 846

* Com/Ind categorization includes commercial, industrial, communications, and utilities.



S=Stag, Sh=Shivagiri, SR=Ski Run, TC=Tahoe City.

Fig. 1. Map of stormwater sampling locations.

defined as a zero mV reading on the SCD. The stormwater samples were treated across a gradient of PAC dosing levels from 50% to 300% of the optimum dosing level by mass. We studied stormwater dosed at the optimum dose (1x, 100%) of Chitosan and at 0.5x (50%), 1x (100%), 2x (200%), and 3x (300%) the optimum dose of PAX-XL9 (Table 2).

Non-treated and coagulant-treated stormwaters were tested for toxicity to green algae (*S. capricornutum*, growth), zooplankton (*C. dubia*, reproduction and mortality), and fish (*P. promelas*, biomass and mortality) at the Aquatic Toxicology Laboratory, University of California at Davis (UCD ATL) using standard US EPA methods.[22] Prior to exposing test organisms, water was shaken rigorously

Table 2. Coagulant specifications and optimal dosing levels (1x) for each stormwater.

			1x dosing level (mg-Al/L)			*1x dosing level (mg-coag/L)*		
Coagulant	*Designation*	*% Al*	*Ski Run*	*Stag*	*TC*	*Ski Run*	*Stag*	*TC*
PAX-XL9® (Kemiron)	Polyaluminum chloride	5.6	6.85	2.55	6.82	120.17	44.80	119.66
Chitosan	Amino-polysaccharide	0	0.00	0.00	0.00	109.96	248.77	294.81

in its original sampling container to homogenize the sample. Water for *C. dubia* and *P. promelas* tests was poured through a 53-μm screen, warmed to 25°C, and briefly aerated at a rate of 100 bubbles/minute until the DO was near saturation. Before *S. capricornutum* cells were introduced to the sample, water was filtered through a type A/E glass fiber filter (nominal pore size 1.0 μm) and warmed to 25°C.

Green algae (*S. capricornutum*)*:* *S. capricornutum* were obtained from the University of Texas Starr collection (#1648), and cultures were grown in US EPA algae nutrient media with EDTA for 4 to 7 days prior to test initiation to ensure the cells were in the exponential growth phase. Test treatments consisted of four replicate flasks, each containing 100 mL of sample. Each flask was inoculated with nutrients according to protocol, but without EDTA, and 10,000 cells/mL of *S. capricornutum*. Test flasks were randomly placed on shaker tables and continuously agitated at 100 rpm. Tests were performed at 25 ± 1°C under a continuous light source (400 ± 40 ft-candles) for 96 h. Flasks were moved to random positions twice daily. Upon test termination, cell concentration was measured using a Coulter Counter® model Z1. Glass-distilled water was used as the control.

Water flea (*C. dubia*)*:* *C. dubia* were obtained from in-house cultures. At test initiation, a single <24-h-old *C. dubia* was placed into each of ten replicate glass vials per test treatment. Each vial contained 15 mL of sample and food (a mixture of *S. capricornutum*, yeast, CEROPHYLL® and trout chow). Animals were transferred daily into new vials containing 15 mL of fresh sample water and food. The test was performed in a temperature-controlled room maintained at 25 ± 1°C with a 16:8 h light:dark photoperiod. Mortality and number of neonates (reproductive success) were recorded daily and upon test termination after the third release of neonates in controls (6–8 days). Commercial bottled water amended to a hardness of 80 to 100 mg/L as $CaCO_3$was used as the control.

Fathead minnow (P. *promelas*): Fish larvae were obtained from Aquatox, Hot Springs, Arkansas. Each test treatment consisted of four replicate 600 mL beakers, each containing 250 mL of sample and 10 <48-h-old *P. promelas* (at test initiation). *P. promelas* were fed three times daily with brine shrimp (*Artemia spec.)* nauplii. Approximately 80% of the test solution was renewed daily, and dead fish, food and debris were removed. Water temperature was maintained at 25 ± 1°C. The test was performed with a 16:8 h light:dark photoperiod for 7 d. Mortality was recorded daily and at test termination. Surviving fish were euthanized with MS-222, dried to constant weight at 103–105°C (approximately 16 h), and weighed with a Mettler AE-163 balance to determine relative growth. Final weight per fish was calculated by dividing total dry weight by the number of surviving fish. Laboratory control water was deionized water amended to a hardness of 80 to 100 mg/L as $CaCO_3$.

Quality assurance/quality control

Positive control reference-toxicant tests were conducted monthly for each species during the study period. These tests included a laboratory control and a dilution series of NaCl or $ZnCl_2$ in laboratory control water. Resulting data points were plotted in a control chart to assess changes in organism sensitivity to a known toxicant.

Test acceptability criteria

Test acceptability for all *C. dubia* and larval *P. promelas* 7-d tests require ≥80% mean survival in controls. In addition, 60% of the surviving *C. dubia* adult females in the control must have their third brood within 7 ± 1 days, and the average number of surviving young must be ≥15 per surviving female. For *S. capricornutum* 96-h tests, the mean cell number of the control must equal or exceed 200,000 cells/mL, and control variability must be ≤20%. When control performance does not meet test acceptability criteria, the test data are rejected.

Water quality parameters

Initial DO, pH, electrical conductivity (EC), hardness and alkalinity of each sample were measured at UCD-ATL prior to the toxicity tests. DO and pH were also measured at each water renewal, and temperature was monitored continuously. At the conclusion of the toxicity tests, water samples were collected for more extensive testing. The UC Davis Department of Agriculture and Natural Resources (DANR) laboratory determined total, filtered total and soluble concentrations of metals; filtered and unfiltered pH (SM 4500-H+) and hardness (SM 314A – by calculation); unfiltered alkalinity (SM 2320); total dissolved solids (TDS, SM 2540-C); total suspended solids (TSS, SM 2540-D); and turbidity (nephelometric method; SM 2130-B).[23–28] Total and filtered total metals were determined by AAS and ICP-AES.[29] Filtered soluble metals were determined by AAS, AES and ICP-AES using US EPA Methods 200.7.[30] All filtered analyses represent analytes that pass through a 0.45 micron filter. UC Davis Soils Laboratory determined dissolved organic carbon (DOC) concentrations using persulfate digestion according to SM 5310-C.[31]

Statistical analysis

For each endpoint, toxicity was defined as a statistically significant difference ($P < 0.05$) to the laboratory control (control). Data obtained from the US EPA 3-species tests were analyzed using ANOVA and Tukey–Kramer HSD. Data was analyzed using Statistica 8.0.[32] To investigate mechanisms of toxicity, results were analyzed using correlation analysis with mean values from each of the five US EPA 3-species toxicity metrics, dosing level, and 29 water quality factors. Significant correlations with absolute values of $R \geq 0.70$ were considered to be highly correlated.

A post-hoc analysis focused on changes to toxicity and water quality caused by PAX-XL9 overdosing; the data set included data for water dosed with PAX-XL9 (0.5-3x) and excluded cases in which significant decreases in toxicity were observed between the raw stormwater and optimally dosed stormwater. In this way, we eliminated the confounding effects of toxicants initially in the stormwater. Using Tukey–Kramer HSD analysis on this data set, we grouped treatment regimes (site x dose) by "high" or "low" toxicity level for each metric; we then investigated differences in water quality parameters between toxicity groups using ANOVA.

Results and discussion

Water quality of non-dosed stormwater

Stormwater samples collected for this study all had near neutral pH (7.45–7.72) but varied widely with respect to other water quality constituents (Table 3). Stormwater from Stag generally contained the highest concentrations of solids and metals, and Shivigiri stormwater contained the lowest concentrations of these constituents. Turbidity and TSS of Stag stormwater were about 6 times higher than at the other sites. Dissolved and particulate aluminum, iron, and other metal concentrations were also highest in the Stag runoff. DOC levels were highest in stormwater from Tahoe City. Samples from Ski Run and Tahoe City had similar hardness, alkalinity, and related constituents, such as calcium and magnesium, but Ski Run had much higher EC and TDS.

Ecotoxicity of non-dosed stormwater

Ecotoxicity tests were performed with non-treated stormwater to investigate the toxicity of runoff (Table 4). Stormwater from Stag was toxic to zooplankton (reproduction) and fish (biomass). It reduced zooplankton brood sizes by about 77% of the control and fish biomass by about 22% of the control. Stormwater from Tahoe City significantly enhanced zooplankton reproduction to 25% greater than the control. Stormwater did not affect algae cell count, zooplankton mortality, or fish mortality.

Toxicity of dosed stormwater at optimal coagulant doses

Stormwater samples from Tahoe City, Stag, and Ski Run were tested with optimal doses of PAX-XL9 or Chitosan. Stormwater source, coagulant treatment, and their interaction significantly affected algal growth and zooplankton reproduction (Table 5). Algal growth significantly increased with optimal dosing of PAX-XL9 in Ski Run water and with optimal dosing of Chitosan in Tahoe City water (Fig. 2). For Stag runoff, zooplankton brood size significantly increased with optimal dosing (Fig. 2); this increase was particularly pronounced for Chitosan. Fish biomass was only significantly affected by stormwater source and not by coagulants (Table 5). Stormwater source, coagulants, and their interactive effects did not affect zooplankton or fish mortality (Table 5).

Toxicity of overdosed stormwaters

Mean toxicity levels of water dosed with PAX-XL9 at 0.5x and 1x (with "x" representing optimal dose) were not significantly different from each other (Table 6). However, overdosing at 2x and 3x significantly increased toxicity to zooplankton and fish compared with 0.5-1x levels. Mean zooplankton brood size decreased from 19.0 at optimal dosing to 3.2 at 3x dosing; decreases in brood size were significant for each stormwater (Fig. 3). Mean zooplankton mortality increased from 2% at optimal dosing to 67% at 3x dosing. The increase in zooplankton mortality was significant for two of three stormwaters, Ski Run and Tahoe City (Fig. 3). Acute fish toxicity increased significantly with dosing when averaged across all sites but was not significant for individual sites. Average fish mortality increased from about 5% at optimum to 13–14% at 2x and 3x levels. Averaged across all sites, algal growth and fish biomass were not significantly affected by overdosing (Table 6).

Water quality as it relates to dosing and toxicity

Of 54 water quality parameters, 29 were detected in some or all dosed stormwater samples (Table 7). Concentrations of soluble Ag, As, Cd, Cu, Cr, Pb, Zn and Ni were below reporting limits. Over dosing with PAX-XL9 decreased alkalinity, pH, and DOC and increased filtered zinc, total unfiltered aluminum, and TSS (Fig. 4, Table 8). These water quality parameters were highly correlated ($R \geq |0.7|$) to toxicity metrics in PAX-XL9 dosed waters (Table 8).

Zooplankton reproduction decreased as unfiltered pH (UpH), unfiltered alkalinity (Ualk), and DOC decreased and as unfiltered total aluminum (UAl[T]) and total suspended solids (TSS) increased (Fig. 5, Table 8). Brood size decreased in a roughly linear relationship with decreasing alkalinity, pH, and DOC. Brood size dropped precipitously when TSS and UAl(T) increased above about 10 mg/L and 1mg/L, respectively.

Zooplankton mortality increased with rising UAl(T) (Fig. 6, Table 8). Zooplankton mortality increased markedly and consistently when UAl(T) increased above 12–16 mg/L. Zooplankton mortality tended to be higher when alkalinity, pH and DOC dropped below 0.4 meq/L, 6.7, and 1.6 mg/L, respectively, and when TSS increased above 60 mg/L. These trends were not consistent. For instance, both 100% and 0% mortalities were seen at TSS

Table 3. Initial water quality conditions for stormwaters.

		Stormwater			
	Water Quality Parameters	*Shivagiri*	*Ski Run*	*Stag*	*Tahoe City Wetland*
Oxygen and EC					
	Initial DO (mg/L)	*8.2*	8.4	8.4	***8.6***
	Final DO	***7.4***	*7.2*	7.3	7.3
	Initial EC (us/cm)	*66.5*	***326.3***	*247.4*	203
pH					
	Initial pH	***7.75***	**7.72**	7.63	*7.45*
	Final pH	***7.93***	7.84	*7.65*	**7.9**
	Filtered pH	7.4	7.4	*7.3*	7.4
	Unfiltered pH	***7.7***	*7.3*	*7.3*	7.6
Hardness					
	Initial hardness, grains/gal	28	***64***	*20*	**56**
	Unfiltered hardness, grains/gal	25.3	***59.4***	*20.4*	**55.5**
	Filtered Hardness, grains/gal	26.3	***59.8***	*19.8*	**56.8**
	Filtered, Soluble Ca, meq/L	*0.3*	***0.8***	*0.3*	**0.7**
	Filtered, Soluble Mg, meq/L	0.2	***0.4***	*0.05*	***0.4***
	Filtered, Total Ca, mg/L	*5.3*	***15.7***	6.1	**13.4**
	Filtered, Total Mg, mg/L	2.9	**4.5**	*1*	***5.6***
	Unfiltered, Total Ca, mg/L	*5.2*	***16.4***	11.1	**13.9**
	Unfiltered, Total Mg, mg/L	*2.9*	**5.2**	*2.9*	***5.9***
Alkalinity					
	Initial Alkalinity, meq/l	32	***50***	*28*	***50***
	Unfiltered alkalinity, meq/L	0.6	***0.9***	*0.5*	***0.9***
Solids					
	TDS, mg/L	*30*	***220***	*170*	130
	TSS, mg/L	*2*	34	***185***	*37*
	Turbidity, NTU	*0.7*	**66.6**	***458.9***	62.2
Carbon					
	DOC, mg/l	*1.2*	**4.4**	2.9	***5.3***
Miscellaneous Metals					
Filtered, Soluble	Al, mg/L	*0.23*	0.36	***0.97***	**0.65**
	Fe, mg/L	*0.05*	0.1	***0.8***	**0.3**
	Zn, mg/L	*0.01*	***0.04***	***0.04***	0.03
Filtered, Total	Al, mg/L	*0.25*	*0.25*	***0.9***	*0.25*
	As, ug/L	*0.5*	*0.5*	*0.5*	***1***
	Fe, mg/L	*0.05*	0.2	***1***	**0.3**
Unfiltered, Total	Al, mg/L	*0.25*	2.2	***10.1***	2.3
	As, ug/L	*0.5*	*0.5*	***2***	***2***
	Cr, mg/L	*0.005*	*0.005*	***0.01***	*0.005*
	Fe, mg/L	*0.05*	**2.2**	***8.7***	1.6
	Mn, mg/L	*0.05*	*0.05*	***0.1***	*0.05*

Bold italicized print identifies highest concentration or level. **Bold print** identifies second-highest concentration or level. Plain print identifies third-highest concentration or level. *Italicized print* identifies lowest concentration or level.

concentrations above 60 mg/L. Fish mortality increased in a roughly linear relationship with decreasing alkalinity, pH, and DOC (Fig. 7, Table 8). Fish mortality tended to be higher at TSS levels above 10 mg/L and at UAl(T) above 1 mg/L.

A posthoc analysis was performed to clarify causes of toxicity associated with PAX-XL dosing but not with consitituents initially found in stormwater. Zooplankton mortality was significantly related to unfiltered pH ($P = 0.002$) and total unfiltered aluminum ($P < 0.001$, Fig. 8); it was

Table 4. Stormwater toxicity as indicated by five metrics, means and standard deviations (SDs) shown.

			Control	*Shivagiri*	*Ski Run*	*Stag*	*Tahoe City*	*P-value*
Algae	Cell Count	Mean (#)	1.4E+06	1.6E+06	1.3E+06	1.5E+06	1.5E+06	
		SD	2.7E+05	8.2E+04	8.4E+04	1.3E+05	1.7E+04	
		Sig ($P < 0.05$)	a	a	a	a	a	0.300
Zooplankton	Reproduction	Mean (#)	22	16	18	**5**	28	
		SD	4	6	6	**2**	3	
		Sig ($P < 0.05$)	cd	bc	bcd	**a**	e	0.000
	Mortality	Mean (%)	2.5%	0.0%	0.0%	0.0%	0.0%	
		SD	15.8%	0.0%	0.0%	0.0%	0.0%	
		Sig ($P < 0.05$)	a	a	a	a	a	0.915
Fish	Survivor Biomass	Mean (mg)	0.27	0.26	0.27	**0.21**	0.26	
		SD	0.03	0.02	0.01	**0.03**	0.03	
		Sig ($P < 0.05$)	b	ab	ab	**a**	ab	0.020
	Mortality	Mean (%)	1.7%	2.5%	2.5%	5.0%	2.5%	
		SD	3.9%	5.0%	5.0%	5.8%	5.0%	
		Sig ($P < 0.05$)	a	a	a	a	a	0.814

Different letters indicate statistical differences ($P < 0.05$) within rows between control, Shivagiri (non-urban site), and stormwaters. Designation of letters begins with "a" assigned to the lowest value. Significance calculated within rows using Tukey-Kramer HSD; ANOVA *P*-values displayed in right column. **Bolded values** represent significantly higher ($P < 0.05$) toxicity than control. Zooplankton reproduction and fish survivor biomass were negatively affected by Stag stormwater. *P*-value from ANOVA analysis.

Table 5. Factorial ANOVA *P*-values comparing toxicity metrics with stormwater source, coagulant treatment regime (no dose, 1x PAX-XL9, or 1x chitosan), and interactive effects between stormwater source and treatment regime.

	Algae Cell Count	*Zooplankton Reproduction*	*Zooplankton Mortality*	*Fish Biomass*	*Fish Mortality*
Stormwater (Stag, Ski Run, Tahoe City)	**0.029**	**0.000**	0.678	**0.005**	0.171
Coagulant (No Dose, Optimal PAX-XL9, Optimal Chitosan)	**0.000**	**0.000**	0.616	0.053	0.740
Stormwater x Coagulant	**0.043**	**0.001**	0.745	0.085	0.700

Bolded values are statistically significant ($P < 0.05$).

Table 6. Coagulant dosing effects on stormwater according to five toxicity metrics.

		Control	*Shivagiri*	*0x*	*0.5x*	*1x*	*2x*	*3x*	*P-value*
Algae cell count (n = 88)	Mean	**1.42E+06**	**1.62E+06**	**1.45E+06**	**1.49E+06**	1.65E+06	**1.59E+06**	**1.48E+06**	0.008
	Sig (P < 0.05)	**a**	**ab**	**ab**	**ab**	b	**ab**	**ab**	
Zooplankton reproduction (n = 230)	Mean (#)	22.0	15.6	17.0	21.3	19.0	**3.7**	**3.2**	<0.001
	Sig (P < 0.05)	*c*	*bc*	b	*bc*	*bc*	**a**	**a**	
Zooplankton mortality (n = 230)	Mean (%)	2.5%	0.0%	0.0%	0.0%	1.7%	13.3%	**66.7%**	<0.001
	Sig (P < 0.05)	a	a	a	a	a	a	**b**	
Fish survivor biomass (n = 100)	Mean (mg/survivor)	0.27	0.26	0.25	0.26	0.26	0.25	0.24	0.113
	Sig (P < 0.05)	a	a	a	a	a	a	a	
Fish mortality (n = 100)	Mean (%)	1.7%	2.5%	3.3%	5.0%	4.6%	**14.2%**	**13.4%**	<0.001
	Sig (P < 0.05)	a	ab	a	a	a	**c**	**bc**	

Different letters indicate statistical differences ($P < 0.05$) within rows between control, Shivagiri (non-urban site), stormwaters, and stormwaters dosed with PAX-XL9. Designation of letters begins with "a" assigned to the lowest value. Significance calculated within rows using Tukey-Kramer HSD; ANOVA *P*-values displayed in right column. **Bolded values** represent highest toxicity amongst statistically significant ($P < 0.05$) differences. *Italicized values* represent lowest toxicity, when three discrete levels emerged with Tukey–Kramer analysis.

Table 7. Analytes above reporting limits measured for stormwater toxicity tests, with means and ranges shown for each dosing level.

		0x (n = 3)		*0.5x (n = 3)*		*1x (n = 6)*		*2x (n = 3)*		*3x (n = 3)*	
		mean	*range*	*mean*	*range*	*mean*	*range*	*mean*	*range*	*mean*	*range*
Oxygen and EC											
	Initial DO	8.4	(8.4–8.6)	8.2	(8.0–8.4)	8.3	(8.1–8.4)	8.3	(8.2–8.4)	8.5	(8.4–8.5)
	Initial EC	258.9	(203.0–326.3)	275.8	(222.0–346.3)	287.5	(233.8–369.9)	310.3	(270.6–385.4)	327.7	(281.3–405.6)
pH											
	Initial pH	7.6	(7.5–7.7)	7.5	(7.4–7.7)	7.4	(7.1–7.8)	7.3	(7.1–7.5)	7.1	(6.9–7.0)
	Filtered pH	7.4	(7.3–7.4)	7.4	(7.3–7.5)	7.2	(7.1–7.3)	7.1	(6.9–7.3)	7.0	(6.9–7.1)
	Unfiltered pH	7.4	(7.3–7.6)	7.4	(7.2–7.6)	7.2	(7.0–7.5)	7.0	(6.7–7.4)	6.6	(6.2–6.8)
Hardness											
	Initial hardness, grains/gal	47	(20–64)	45	(20–60)	43	(16–60)	44	(20–60)	43	(20–56)
	Filtered hardness, grains/gal	45.5	(19.8–59.8)	45.1	(18.6–60.4)	45.3	(19.4–60.4)	45.5	(19.6–59.6)	46.5	(20.0–61.6)
	Unfiltered hardness, grains/gal	45.1	(20.4–59.4)	43.9	(17.8–58.4)	43.8	(18.1–57.7)	44.4	(18.5–58.2)	44.5	(19.2–58.9)
	Filtered, Soluble Ca, meq/L	0.6	(0.3–0.8)	0.6	(0.3–0.8)	0.6	(0.3–0.9)	0.6	(0.3–0.8)	0.6	(0.3–0.9)
	Filtered, Soluble Mg, meq/L	0.28	(0.05–0.40)	0.28	(0.05–0.40)	0.27	(0.05–0.40)	0.28	(0.05–0.40)	0.28	(0.05–0.40)
	Filtered, Total Ca, mg/L	11.7	(6.1–15.7)	11.5	(5.9–15.5)	11.5	(6.1–15.3)	11.6	(6.2–15.5)	11.9	(6.4–15.9)
	Filtered, Total Mg, mg/L	3.7	(1.0–5.6)	3.5	(0.7–5.4)	3.5	(0.7–5.4)	3.5	(0.7. 5.3)	3.6	(0.8–5.2)
	Unfiltered, Total Ca, mg/L	13.8	(11.1–16.4)	11.7	(6.0–15.8)	11.6	(6.1–15.6)	12.2	(7.1–15.8)	12.3	(7.4–16.0)
	Unfiltered, Total Mg, mg/L	4.7	(2.9–5.9)	3.6	(0.7–5.5)	3.5	(0.7–5.3)	3.7	(1.3–5.3)	3.8	(1.4–5.4)
Alkalinity											
	Initial alkalinity, meq/L	0.9	(0.6–1.0)	0.7	(0.5–0.9)	0.6	(0.4–0.7)	0.4	(0.4–0.5)	0.3	(0.2–0.5)
	Unfiltered alkalinity, meq/L	0.8	(0.5–0.9)	0.7	(0.4–0.8)	0.6	(0.4–0.7)	0.4	(0.3–0.4)	0.2	(0.2–0.3)
Solids											
	TDS, mg/L	173	(130–220)	180	(160–220)	175	(140–230)	190	(160–250)	200	(170–260)
	TSS, mg/L	85	(34–185)	2	(2–2)	3	(2–8)	27	(4–58)	76	(70–79)
	Turbidity, NTU	195.9	(62.2–458.9)	5.4	(2.0–12.1)	3.2	(1.3–5.5)	25.3	(1.5–70.3)	33.7	(10.7–69.3)
Carbon											
	DOC, mg/L	4.2	(2.9–5.3)	2.6	(2.2–3.1)	2.1	(1.9–2.4)	1.8	(1.7–1.9)	1.4	(1.3–1.5)
Miscellaneous Metals											
Filtered, Soluble	Al, mg/L	0.66	(0.36–0.97)	0.07	(0.06–0.07)	0.05	(0.025–0.060)	0.05	(0.025–0.060)	0.06	(0.025–0.080)
	Fe, mg/L	0.40	(0.1–0.8)	0.05	(0.05–0.05)	0.05	(0.05–0.05)	0.05	(0.05–0.05)	0.05	(0.05–0.05)
	Zn, mg/L	0.04	(0.03–0.04)	0.02	(0.01–0.03)	0.03	(0.01–0.06)	0.03	(0.01–0.04)	0.05	(0.02–0.07)
Filtered, Total	Al, mg/L	0.47	(0.25–0.90)	0.25	(0.25–0.25)	0.25	(0.25–0.25)	0.25	(0.25–0.25)	1.67	(0.25–4.50)
	As, ug/L	0.7	(0.5–1.0)	0.5	(0.5–0.5)	0.5	(0.5–0.5)	0.5	(0.5–0.5)	0.5	(0.5–0.5)
	Fe, mg/L	0.50	(0.20–1.00)	0.05	(0.05–0.05)	0.09	(0.05–0.30)	0.05	(0.05–0.05)	0.10	(0.05–0.20)
Unfiltered, Total	Al, mg/L	4.87	(2.2–10.1)	0.33	(0.25–0.50)	0.38	(0.25–0.50)	3.90	(1.2–6.3)	14.77	(9.3–17.7)
	As, ug/L	1.5	(0.5–2.0)	0.7	(0.5–1.0)	0.5	(0.5–0.5)	0.5	(0.5–0.5)	0.7	(0.5–1.0)
	Cr, mg/L	0.007	(0.005–0.010)	0.005	(0.005–0.005)	0.005	(0.005–0.005)	0.005	(0.005–0.005)	0.005	(0.005–0.005)
	Fe, mg/L	4.17	(1.6–8.7)	0.20	(0.05–0.50)	0.14	(0.05–0.30)	0.98	(0.05–2.70)	1.40	(0.5–3.0)

When analytes were not detected, values of 50% the reporting limit were assigned.

not significantly related to unfiltered alkalinity, TSS, or DOC (data not shown; "high toxicity" level = SR3, TC 3; "low toxicity" level = all other treatments). Zooplankton reproductive toxicity was significantly related to unfiltered pH, unfiltered alkalinity, UAl(T), and DOC ($P = 0.017$, $P < 0.001$, $P = 0.010$, and $P = 0.047$, respectively) but not to TSS (data not shown; Stag data excluded; "high toxicity" = SR2, SR3, TC2, TC3; "low toxicity" = all other treatments). None of the water quality parameters measured were correlated to fish mortality (data not shown; "high toxicity" = SR3, TC3; "low toxicity" = all other treatments), possibly because of its weak response to dosing; fish mortality did not show significant changes with dosing for individual water samples.

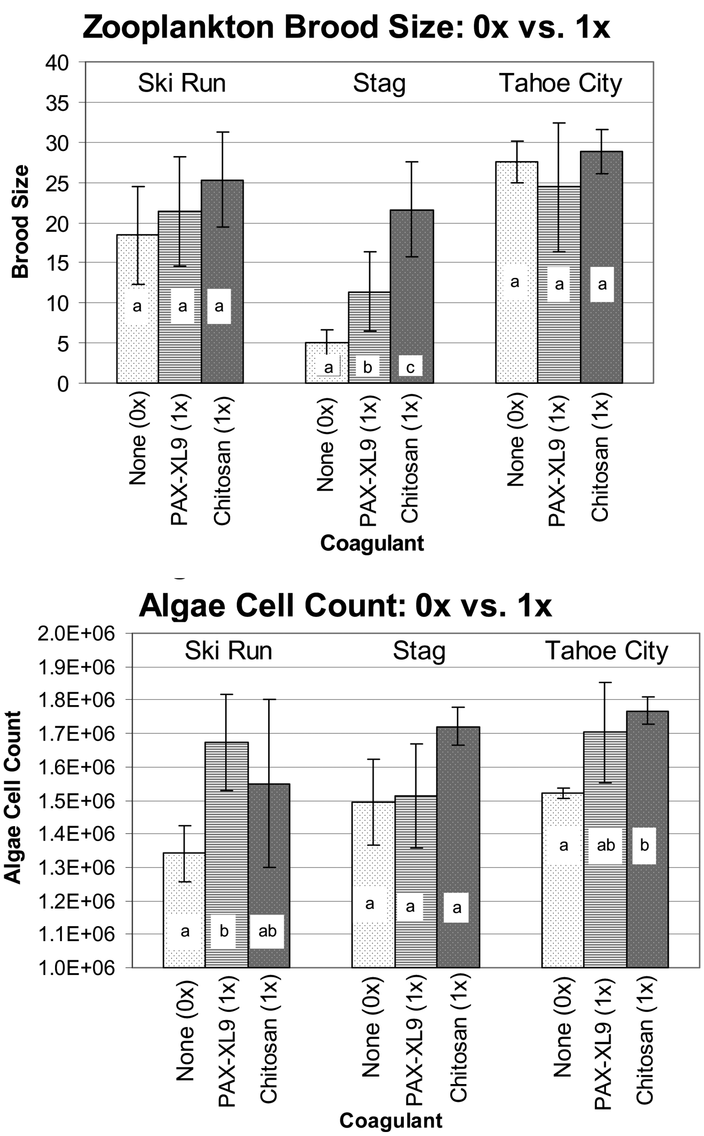


Fig. 2. Algae cell reproduction and zooplankton brood size for non-dosed and optimally dosed stormwaters (PAX-XL9 and Chitosan). Different letters indicate statistical differences (Tukey Kramer HSD, $P < 0.05$) within sites between non-dosed and optimally dosed stormwaters.

Toxicity of stormwater

To understand the effects of dosing on stormwater toxicity, we considered the initial stormwater toxicity and the toxicity of optimally dosed and overdosed stormwaters.

Each sampling site represented unique landscape features (Table 1) and was located in an area with a different population density, so different chemical signatures were expected. We found that stormwater from urban areas in the Tahoe Basin can be either toxic or beneficial to organisms, depending on the organism and the stormwater quality. In this study, zooplankton reproduction was the most sensitive toxicity metric to stormwater. The only toxic site, Stag, does not show remarkable differences in landscape classification from other sites (Table 2), but the water sampling point was from a pipe flowing directly off a heavily-sanded highway. Stag water had the highest concentrations of metals and TSS and significantly reduced both zooplankton reproduction and fish survivor biomass. Samples from another urban stormwater (Tahoe City) were taken downstream of the junction of several buried pipes draining various types of landscapes, including a golf course. These samples contained the highest concentrations of DOC and the lowest pH, and they enhanced zooplankton reproduction above both the laboratory control and runoff from the non-urban site (Shivigiri).

Depending on the nature of contaminants present in drainage areas, stormwater may affect bioassay species differently. For example, commonly-used insecticides are generally more toxic to zooplankton than to fish or algae, whereas some heavy metals are more toxic to algae than to zooplankton.[33] Roofs and building sidings can be major sources of Cd, Cu, Pb, and Zn to urban stormwater; vehicle brake emissions can contribute Cu; tire wear can contribute Zn; atmospheric deposition can contribute Cd, Cu, and Pb; and car washes can be major contributors of Cd, Cr, Pb, and Zn.[34–35] Since stormwater generally contains multiple contaminants, the combined effects of contaminant mixtures are poorly understood and therefore not predictable.

Effects of dosing on stormwater toxicity

Optimal dosing improved algae cell counts and zooplankton brood sizes compared with non-dosed stormwaters (Fig. 2). Since there was no evidence of toxicity of untreated water to algae, it is likely that increases in algae cell counts were due to improved water clarity rather than to reduced toxicity. Significant increases in zooplankton brood size were observed for Stag stormwater, the only water that caused toxicity when untreated. The coagulants apparently removed constituents that were responsible for the initial toxicity.

Although optimal coagulant dosing reduced the toxicity of stormwater to zooplankton, over-dosed water samples were highly toxic to zooplankton (Table 6). The increase in zooplankton toxicity at high doses was more severe for stormwater from Ski Run and Tahoe City than from Stag (Fig. 3). Since Ski Run and Tahoe City waters were not toxic when untreated, it appears that coagulant-related water quality changes caused toxicity. Similarly, the comparatively low toxicity of overdosed Stag water may be related to the low dosing regime for Stag stormwater (Table 2).

An increase in zooplankton toxicity after coagulant treatment was also observed by Lopus et al.[19] In that study, stormwater was treated with optimal dosing levels of PAX-XL9 and other PACs. However, the optimal doses were estimated using jar tests, which are more subjective and approximated than the SCD methods used in this study. Our results suggest that toxicity increases seen by Lopus et al.[19]

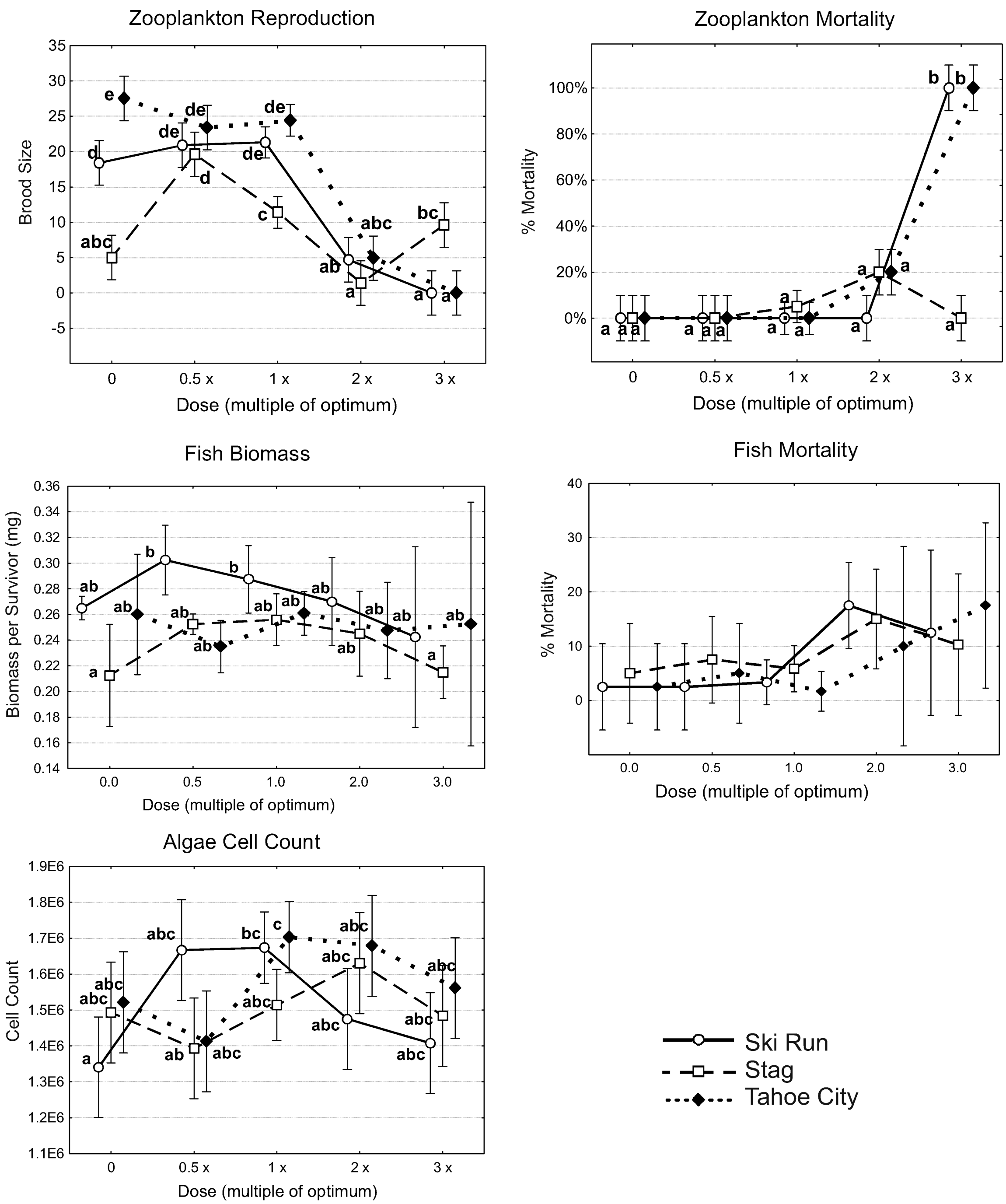


Fig. 3. Effects of optimal- and over-dosing of PAX-XL9 on zooplankton brood size, zooplankton mortality, fish biomass, fish mortality, and algae cell count. Dosing level of PAX-XL9 relative to optimal dose. Different letters indicate statistical differences (Tukey Kramer HSD, $P < 0.05$) between treatment group (dose × stormwater); no significant differences, between treatment groups for fish mortality. Vertical bars represent 95% confidence intervals.

were likely due to accidental over-dosing of the stormwater samples.

Likely mechanism of toxicity associated with overdosing

Dosing with a cationic coagulant (i.e., PAX-XL9) consumes alkalinity linearly according to a stoichimometric relationship[36]. Free hydrogen ions are released, resulting in a decrease in pH. DOC and other negatively charged dissolved constituents are removed by coagulation, as suspended solids and particulates form. At optimal dosing, solids tend to aggregate and settle out of solution. However, when overdosed, coagulants provide nearly complete coverage of the particles,[37] resulting in positively-charged

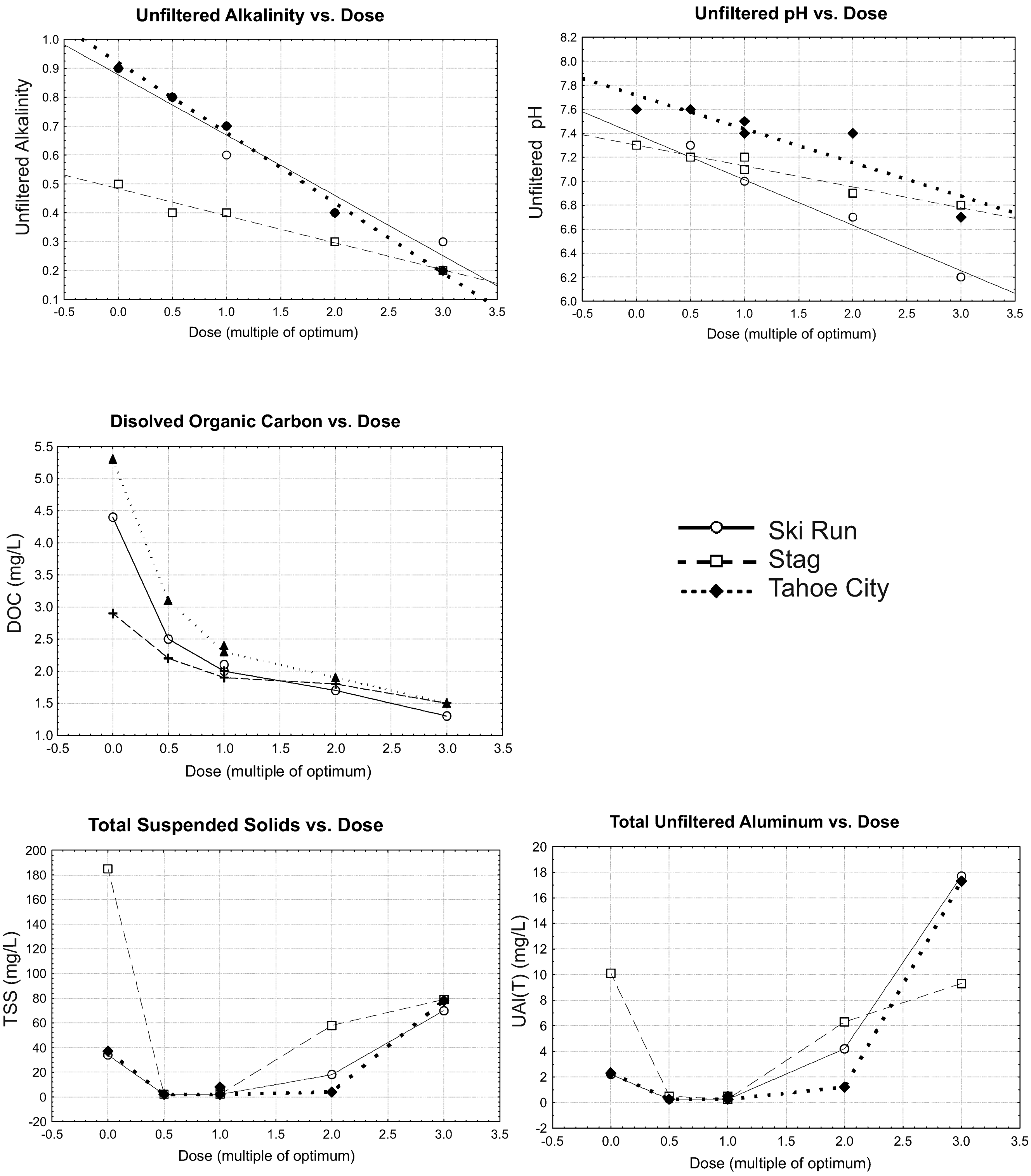


Fig. 4. Effects of optimal- and over-dosing on unfiltered alkalinity, unfiltered pH, dissolved organic carbon, total suspended solids, and total unfiltered aluminum. Dosing level of PAX-XL9 relative to optimal dose. Fit lines are provided for unfiltered alkalinity and unfiltered pH, by site, with equations and fits displayed.

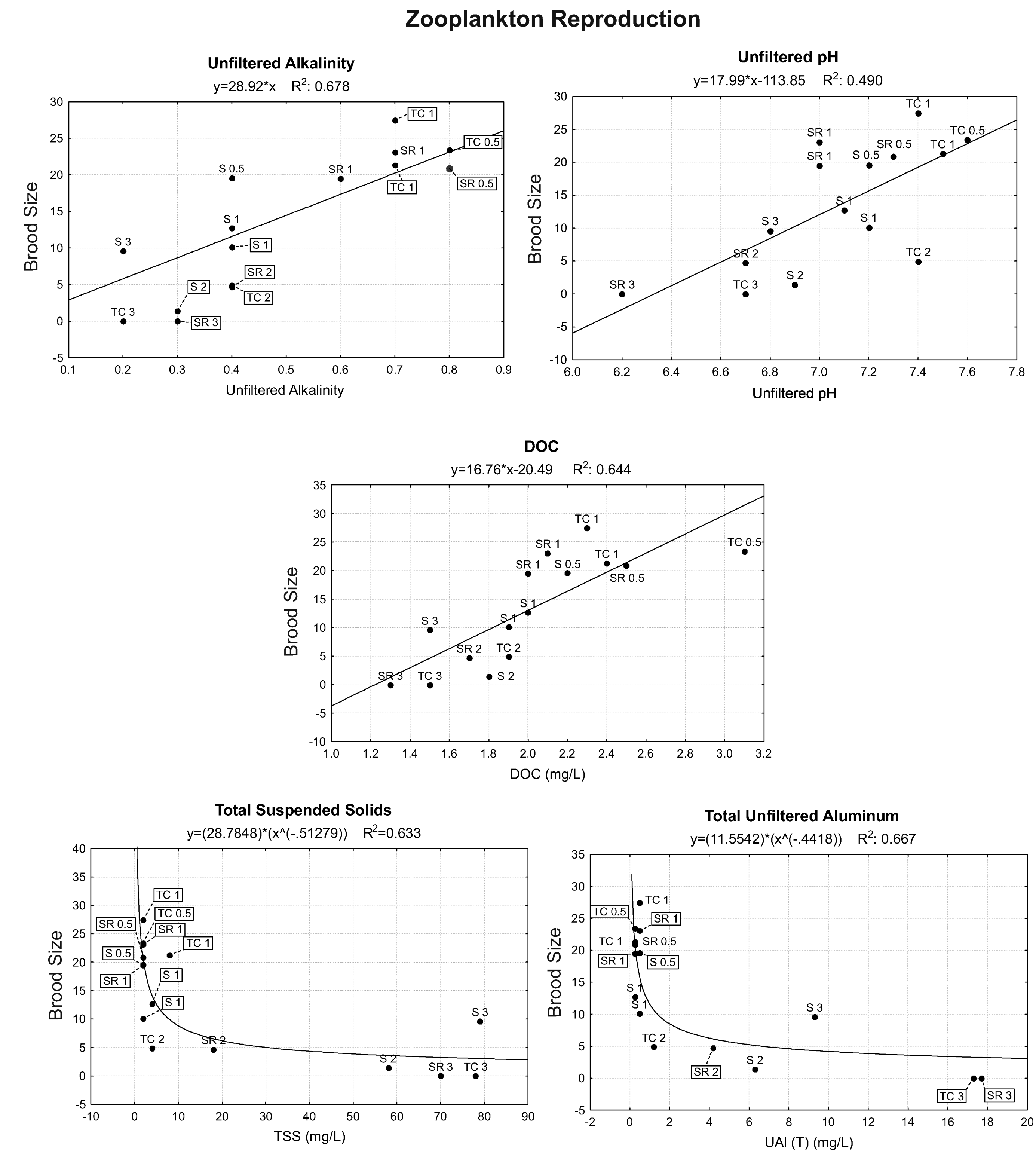


Fig. 5. Zooplankton reproduction with unfiltered alkalinity, unfiltered pH, dissolved organic carbon, total suspended solids, and total unfiltered aluminum. Values are plotted for all stormwaters (SR = Ski Run, S = Stag, TC = Tahoe City Wetland) for dosing levels 0.5x-3x (numbers following site descriptions in point labels). Equations and fits displayed for fit lines.

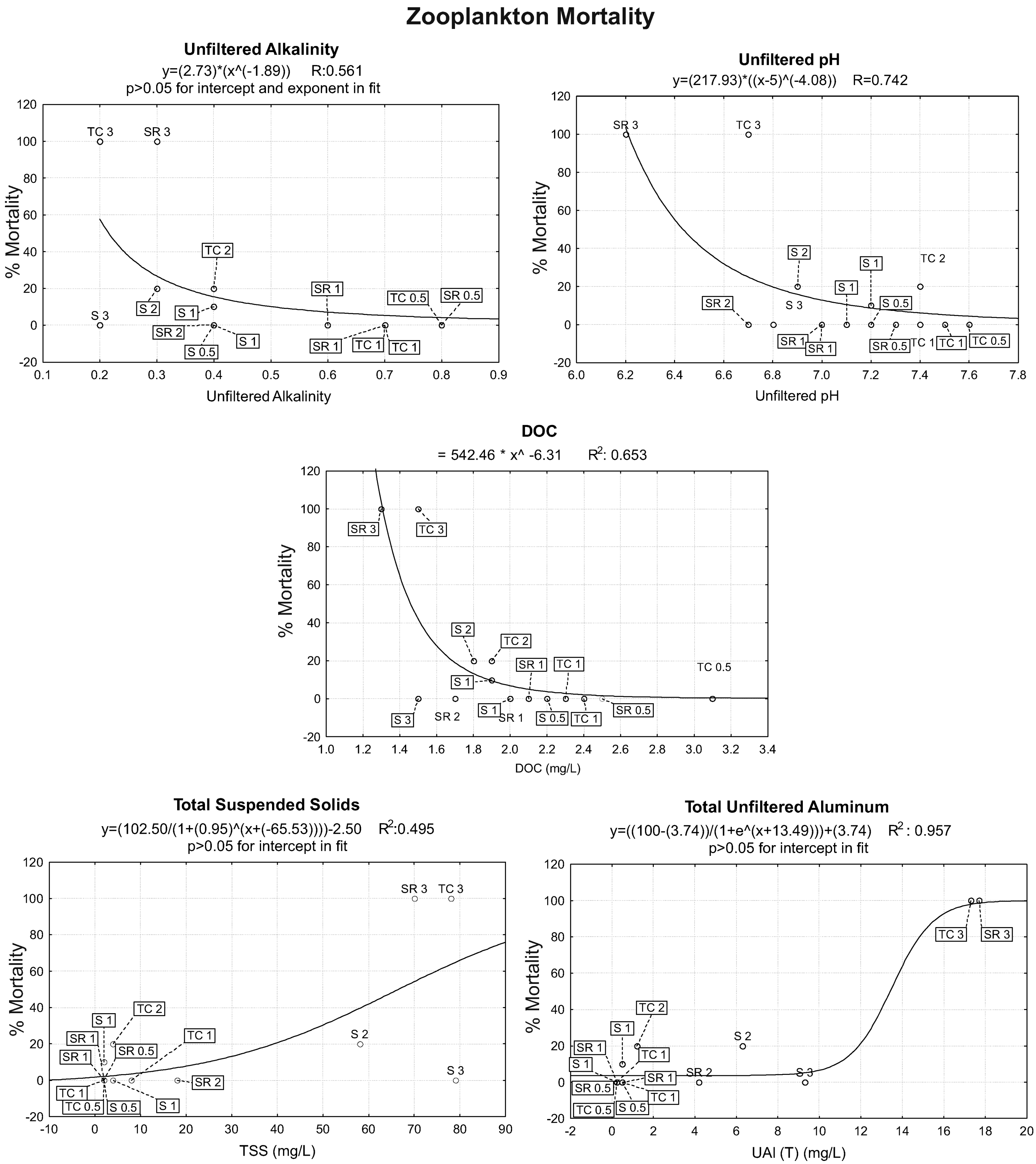


Fig. 6. Zooplankton mortality with unfiltered alkalinity, unfiltered pH, dissolved organic carbon (DOC), total suspended solids (TSS), and total unfiltered aluminum. Values are plotted for all stormwaters (SR = Ski Run, S = Stag, TC = Tahoe City Wetland) for dosing levels 0.5x-3x (numbers following site descriptions in point labels). Equations and fits displayed for fit lines.

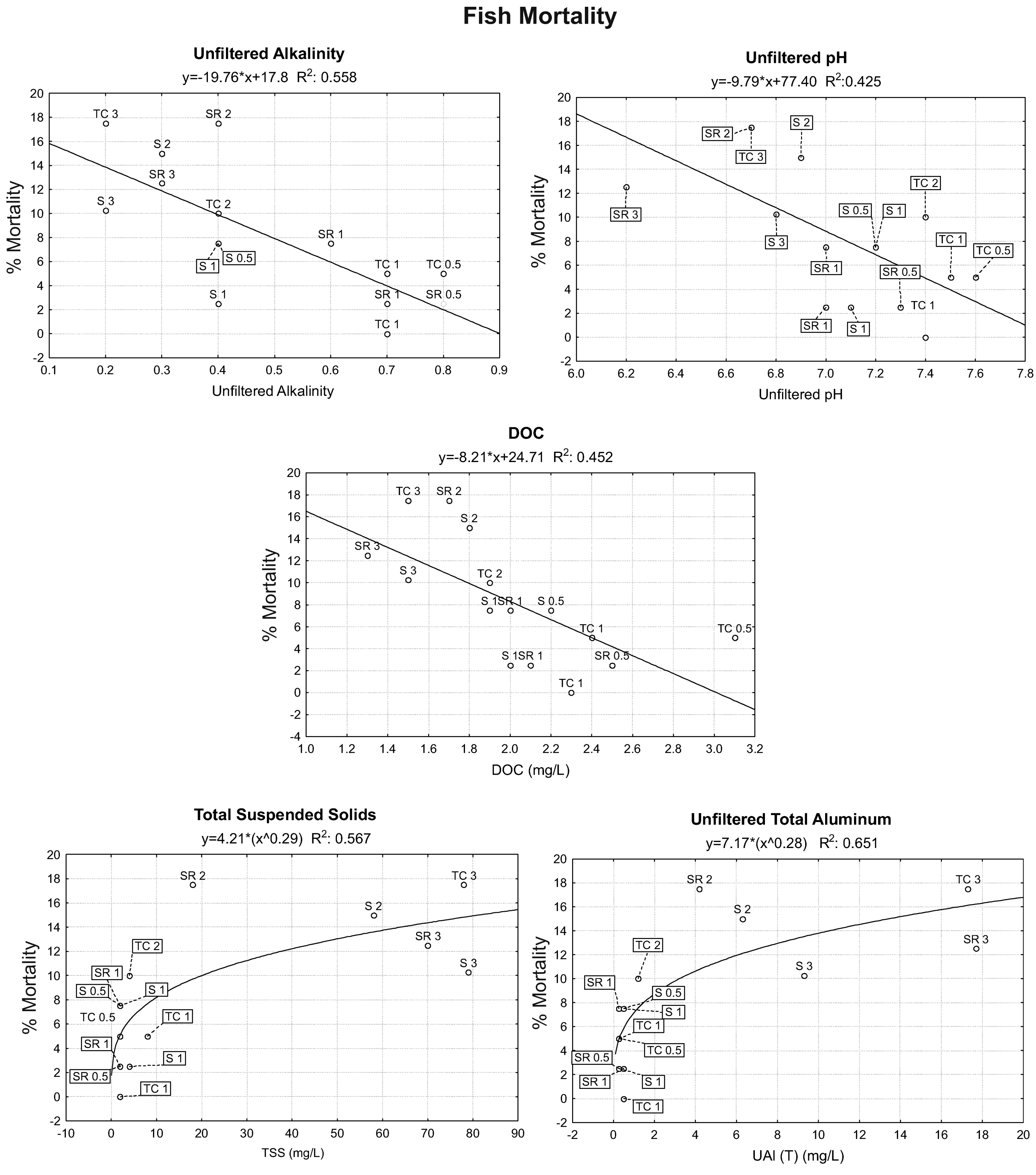


Fig. 7. Fish mortality with unfiltered alkalinity, unfiltered pH, dissolved organic carbon, total suspended solids, and total unfiltered aluminum. Values are plotted for all stormwaters (SR = Ski Run, S = Stag, TC = Tahoe City Wetland) for dosing levels 0.5x-3x (numbers following site descriptions in point labels). Equations and fits displayed for fit lines.

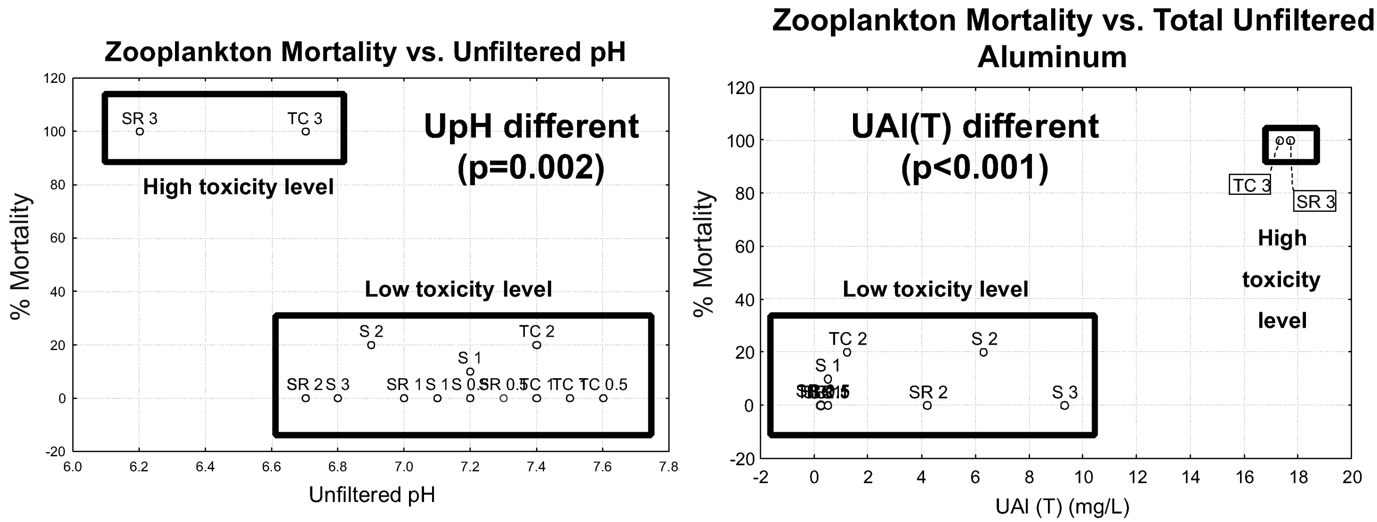


Fig. 8. Toxicity levels ("high" and "low") for zooplankton mortality, displaying differences between groups' UpH and total unfiltered aluminum levels. Toxicity grouping determined using Tukey-Kramer HSD analysis.

particles that do not aggregate. For example, we observed that the aluminum added with PAX-XL9 stayed in the water column when overdosed, leading to increased concentrations of UAl(T). We observed that the fraction UAl(T)/TSS increased with overdosing, indicating that UAl(T) was becoming more concentrated in the suspended flocculates (results not shown).

Post-hoc analysis to discern constituents associated with coagulant-related toxicity indicated that coagulant-related toxicity was associated with increased UAl(T) and decreased UAlk, pH, and DOC. These water quality components interact with each other[38,39] and are affected by dosing, so it is difficult to discern a principle actor causing toxicity.

Only one treatment (3x dosing for Ski Run, pH = 6.2) lowered the pH to outside the US EPA standard range of 6.5 to 9.0. Belanger and Cherry reported that survival and reproduction of *C. dubia* were reduced at pH $\leq$ 4.6 and $\leq$5.3, respectively,[40] and Locke reported a threshold for C. dubia reproduction at pH 5.0–5.5.[41] Acidification has been shown to negatively affect fathead minnow survival when pH levels drop to 6.0 or below.[42] Although pH alone is probably not causing toxicity, H+ ions and aluminum can interact synergistically or antagonistically in their effects on toxicity.[43]

Total aluminum exceeded the US EPA chronic water quality criteria (0.087 mg/L)[44] at all dosing levels, and zooplankton toxicity increased when total aluminum was above approximately 1 mg/L (Fig. 5). Gostomski observed chronic *C. dubia* toxicity at a similar concentration, 1.9 mg/L, under conditions similar to those in this study (pH of 7.15 and hardness of 50 mg/L).[45] Havas reported toxicity to another zooplankton species, *Daphnia magna*, at pH 6.5 when total aluminum concentrations exceeded about 0.3 mg/L, with greater toxicity reported at low calcium concentrations (2.5 mg/L–12.5 mg/L); aluminum was found to accumulate in legs, carapace, and hind gut and seemed to be associated with chloride cells.[38] This association with chloride cells suggests that aluminum interferes with ion regulation.[46] Winter et al. studyied aluminum toxicity to fish over a pH range of 5–10 and found that the greatest aluminum accumulation occurred in gills at pH 6.2–8.[47] At this pH range, aluminum polymers or amorphous aluminum are present, resulting in Al precipitation and polymerization. Poleo found that aluminum deposition on fish led to toxicity through a combination of hypoxia and compromised ion regulation.[48] Based on the results of these studies, it is likely that aluminum is a principle cause of the toxicity observed in this study, and aluminum toxicity is related to the tendency of positively charged aluminum-rich flocculates to precipitate or polymerize onto negatively-charged surfaces of the organisms, causing hypoxia and/or compromised ion regulation.

The toxicities of overdosed stormwaters may vary due to the presence of ameliorating factors. Alkalinity and DOC were found to negatively correspond to toxicity in this study. Alkalinity buffers pH changes, and some components of alkalinity, such as carbonate and bicarbonate, complex with heavy metals and reduce their toxicity.[49] Natural organic matter has been shown to have a protective effect against Al toxicity in near-neutral water.[39–50] Organic matter complexes with aluminum and reduces aluminum toxicity by rendering it unavailable to bind to gills.[47,50] Calcium was not strongly correlated to dosing-related toxicity in this study, although it has been shown to ameliorate aluminum toxicity at near-neutral pH conditions.[38] The calcium concentrations present in the water may have been sufficient to reduce gill membrane permeability and protect against aluminum toxicity in all waters. Such threshold Ca levels have been reported for some aquatic species.[39]

Table 8. Correlations between dosing, zooplankton, and fish toxicity metrics and all variables with significant correlations to any metric.

	Means	*Std.Dev.*	*Dose (mult of opt)*	*Dose mg-Me/L*	*Algae Count*	*Zooplankton Reproduction*	*Zooplankton Mortality*	*Fish Biomass per Survivor*	*Fish Mortality*
Dose (mult of opt)	2	0.9	1.000**	***0.808***	−0.188*	***−0.821***	**0.703**	−0.476*	***0.752***
Dose mg-Me/L	8	6.2	***0.808***	1.000**	−0.066*	−0.656**	***0.825***	−0.099*	0.651**
Algae Count	1.57E+06	1.20E+05	−0.188*	−0.066*	1.000**	0.257*	−0.222*	0.574**	−0.264*
Zooplankton Reproduction	13	9.5	***−0.821***	−0.656**	0.257*	1.000**	−0.671**	0.422*	***−0.868***
Zooplankton Mortality	17	34.6	**0.703**	***0.825***	−0.222*	−0.671**	1.000**	−0.210*	0.569**
Fish Biomass per Survivor	0	0.0	−0.476*	−0.099*	0.574**	0.422*	−0.210*	1.000**	−0.279*
Fish Mortality	8	5.6	***0.752***	0.651**	−0.264*	***−0.868***	0.569**	−0.279*	1.000**
Filtered pH	7	0.2	**−0.727**	***−0.756***	0.023*	0.538**	−0.688**	0.404*	−0.423*
Unfiltered pH	7	0.4	***−0.762***	−0.660**	0.376*	**0.700**	−0.663**	0.191*	−0.652**
Initial hardness	43	18.6	−0.025*	0.464*	0.342*	0.237*	0.171*	0.587**	−0.049*
Filtered hardness	46	19.2	0.027*	0.527**	0.350*	0.167*	0.249*	0.556**	−0.026*
Unfiltered hardness	44	18.8	0.015*	0.515**	0.351*	0.180*	0.230*	0.549**	−0.035*
Filtered Soluble Ca	1	0.2	0.032*	0.520**	0.295*	0.146*	0.257*	0.587**	−0.012*
Filtered Soluble Mg	0	0.2	0.023*	0.523**	0.324*	0.157*	0.250*	0.517**	−0.006*
Filtered Total Ca	12	4.1	0.037*	0.521**	0.312*	0.142*	0.240*	0.596**	−0.007*
Unfiltered Total Ca	12	4.0	0.071*	0.528**	0.313*	0.116*	0.233*	0.586**	0.023*
Unfiltered Total Mg	4	2.0	0.068*	0.530**	0.357*	0.164*	0.256*	0.414*	−0.010*
Initial alkalinity	28	9.6	***−0.750***	−0.363*	0.353*	***0.778***	−0.389*	0.651**	−0.672**
Unfiltered alkalinity	0	0.2	***−0.790***	−0.466*	0.405*	***0.856***	−0.528**	0.612**	**−0.747**
TSS	22	31.2	***0.901***	0.614**	−0.267*	**−0.712**	0.696**	−0.497*	0.686**
Turbidity	14	23.2	0.544**	0.005*	−0.130*	−0.441*	0.100*	−0.525**	0.442
DOC	2	0.5	***−0.848***	−0.650**	0.158*	***0.802***	−0.592**	0.294*	−0.672**
Filtered Soluble Zn	0	0.0	0.493*	***0.795***	0.124*	−0.292*	0.650**	0.338*	0.377*
Filtered Total Al	1	1.1	0.448*	0.555**	−0.365*	−0.386*	0.667**	−0.171*	0.215*
Unfiltered Total Al	4	6.1	***0.884***	***0.807***	−0.336*	**−0.732**	***0.900***	−0.370*	0.691**
Unfiltered Total Fe	1	0.9	0.550**	0.001*	−0.121*	−0.422*	0.080*	−0.548**	0.405*

*identifies statistically insignificant correlations ($P > 0.05$); **identifies statistically significant low correlations (< 0.700, $P < 0.05$); bold type identifies high correlations (0.700–0.749); *bold italicized type* identifies very high correlations ($\geq$0.750).
Data for algae is not shown because it showed no significant correlations with any water quality parameters. Variables for which relationships to dose or any of the toxicity metrics were not statistically significant are not shown.

Conclusions

Coagulation has been considered as a means to target phosphorus and fine particle removal from stormwater in the Tahoe Basin. These constituents are considered the main culprits in the decrease in lake water clarity. However, concerns exist over the use of chemical coagulants and potential deleterious effects on the lake ecosystem. This study investigated toxicity of raw and coagulant-treated stormwaters under optimal and overdosed conditions. This study was the first to assess stormwater toxicity in the context of optimal and suboptimal dosing using streaming current detectors.

Raw stormwater from Stag was chronically toxic to the zooplankton species *C. dubia* and to fathead minnow larvae. Optimal doses of coagulants reduced toxicity at Stag and did not increase the toxicities of the other stormwaters. However, over-dosing of stormwater with PAX-XL9 significantly increased toxicity to zooplankton in most stormwater samples tested.

Toxicity of the coagulants at optimal and overdosed conditions varied based upon the stormwater source, so the interactive effects between coagulants and stormwater were considered in trying to understand the constituents causing toxicity. PH, alkalinity, total unfiltered aluminum, and DOC were all strongly correlated with coagulant dosing and with some toxicity metrics. From our study and a review of the literature, UAl(T) appears to be the most likely cause of toxicity to zooplankton, probably due to precipitation or polymerization and subsequent hypoxia or compromised ion regulation. UAl(T) concentrations in treated stormwaters at all dosing levels exceeded US EPA water quality criteria. In this study, increased toxicities tended to correspond to UAl(T) over 1 mg/L. However, aluminum toxicity is dependent on other factors such as pH, DOC, alkalinity, and hardness, so toxic levels will vary depending on these parameters.

We conclude that increases in toxicity to aquatic species due to dosing only occurred when stormwater was overdosed with coagulant. We strongly recommend that care be taken to keep coagulant dosing levels at or below optimal levels. The use of SCD in accurately determining optimal dosing levels may help to prevent over dosing.

Acknowledgments

This project was funded by a grant from the USDA Forest Service through the City of South Lake Tahoe. We thank Tim Delaney, formerly with the UC Davis Tahoe Research Group, for his remarkable efforts in completing field and laboratory work. We also thank current and past employees of the UC Davis Toxicology Lab, including Alyssa Gartung, Stephanie Fong, Linda Deanovic, and Daniel Markiewicz, who advised on and implemented the toxicity analyses. We appreciate the input from John Reuter of the Tahoe Environment Research Center for his help in envisioning this study. Finally, thanks to Steve Peck and Russ Wigart, former employees of the City of South Lake Tahoe, who worked hard to facilitate the implementation of these studies.

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