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Golden alga presence and abundance are inversely related to salinity in a high-salinity river ecosystem, Pecos River, USA

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ABSTRACT

Prymnesium parvum (golden alga, GA) is a toxigenic harmful alga native to marine ecosystems that has also affected brackish inland waters. The first toxic bloom of GA in the western hemisphere occurred in the Pecos River, one of the saltiest rivers in North America. Environmental factors (water quality) associated with GA occurrence in this basin, however, have not been examined. Water quality and GA presence and abundance were determined at eight sites in the Pecos River basin with or without prior history of toxic blooms. Sampling was conducted monthly from January 2012 to July 2013. Specific conductance (salinity) varied spatiotemporally between 4408 and 73,786 μS/cm. Results of graphical, principal component (PCA), and zero-inflated Poisson (ZIP) regression analyses indicated that the incidence and abundance of GA are reduced as salinity increases spatiotemporally. LOWESS regression and correlation analyses of archived data for specific conductance and GA abundance at one of the study sites retrospectively confirmed the negative association between these variables. Results of PCA also suggested that at <~15,000 μS/cm, GA was present at a relatively wide range of nutrient (nitrogen and phosphorus) concentrations whereas at higher salinity, GA was observed only at mid-to-high nutrient levels. Generally consistent with earlier studies, results of ZIP regression indicated that GA presence is positively associated with organic phosphorus and in samples where GA is present, GA abundance is positively associated with organic nitrogen and negatively associated with inorganic nitrogen. This is the first report of an inverse relation between salinity and GA presence and abundance in riverine waters and of interaction effects of salinity and nutrients in the field. These observations contribute to a more complete understanding of environmental conditions that influence GA distribution in inland waters.
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1. Introduction

A harmful alga of growing concern is *Prymnesium parvum*, or golden alga (GA). Although believed to have originated from coastal and estuarine environments (Nicholls, 2003), GA has invaded brackish inland waters throughout the world (Lutz-Carrillo et al., 2010). In the United States, GA has been reported in at least 23 states (Sager et al., 2008; Weber and Janik, 2010; Bertin et al., 2012; Hambright, 2012). The specific nature of the toxin(s) produced by GA is still unclear (Blossom et al., 2014), but they can be lethal to fish and other gilled aquatic organisms and have caused great ecological and economic harm to the affected areas (Ulitzur and Shilo, 1966; Southard et al., 2010).

The physicochemical properties of aquatic habitats can influence the abundance and toxicity of GA. A recent retrospective analysis of archived data for reservoir water quality concluded that salinity is a primary factor influencing the potential for toxic bloom formation (Patiño et al., 2014). The minimum salinity for toxic blooms to develop in reservoir waters is about 0.5–1.0 psu (Roelke et al., 2011; Patiño et al., 2014). In laboratory cultures, the optimum temperature for GA growth decreases as salinity decreases (Baker et al., 2007, 2009). Because of the low salinity

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of inland waters compared to estuarine-marine waters, this may explain why inland blooms typically occur at cooler temperatures (e.g. winter) (Roelke et al., 2011). The presence of hardness cations (Ca, Mg) enhances the potency of GA toxin extracts in laboratory bioassays (Yariv and Hestrin, 1961; Ulitzur and Shilo, 1964, 1966), and a positive relation between water hardness and GA-like ichthyotoxicity was reported in the field (VanLandeghem et al., 2012). Lowering the pH of ambient water or laboratory cultures during an active bloom reduces the toxicity of GA (Ulitzur and Shilo, 1964, 1966; Valenti et al., 2010); however, in the field, associations between ambient pH and GA abundance or toxicity vary among different watersheds (Roelke et al., 2011; VanLandeghem et al., 2012; VanLandeghem, 2013). The ability of GA to obtain nutrients via photosynthetic and heterotrophic mechanisms, known as mixotrophy, is likely to contribute to its invasive and bloom potential (Granéli et al., 2012). Total amounts of nitrogen and phosphorus as well as their ratio seem to influence toxic GA bloom formation (Hambright et al., 2010; Granéli et al., 2012). In the field, GA abundance seems to be positively associated with overall nutrient levels (Hambright et al., 2010).

Some water treatments (e.g., fertilization with inorganic nutrients) seem to have potential for controlling GA in small impoundments or mesocosms (Guo et al., 1996; Kurten et al., 2007, 2010; Barkoh et al., 2010; Grover et al., 2013), but these treatments have not been fully tested in larger lentic systems (lakes, reservoirs) or in flowing waters. In addition, potential negative effects of water chemistry manipulation on natural ecosystem functions are not fully understood. While the available evidence suggests that GA abundance and toxicity are associated with certain aspects of water quality (see preceding paragraph), results of a recent study indicated that patterns of bloom formation, dynamics, and their consequent ecological impacts can vary greatly among basins (VanLandeghem et al., 2013). Thus, basin-specific information on the associations between water quality and GA is necessary to better understand mechanisms of GA dispersal and growth and for successful management.

This study focuses on the Pecos River basin, one of the saltiest river systems in North America. The river flows approximately 1500 km in a southeast direction across arid and semiarid landscapes from the Sangre de Cristo Mountains in northern New Mexico to its confluence with the Rio Grande in west Texas. The first reported GA bloom in North America occurred in the Pecos River in 1985 (James and De La Cruz, 1989). Since then, toxic blooms have occurred throughout much of the basin killing millions of fishes and other aquatic organisms (James and De La Cruz, 1989; Rhodes and Hubbs, 1992; Southard et al., 2010). Golden alga blooms have greatly affected the aquatic biodiversity of some reaches of the river (Rhodes and Hubbs, 1992; Linam and Kleinsasser, 1996) and pose a threat to the state-listed Pecos Pupfish (*Cyprinodon pecosensis*), a species endemic to the Pecos River (Dixon, 1996; Echelle et al., 1997). Compared to most other inland aquatic ecosystems, the high salinity of the Pecos River has been hypothesized to provide habitat that is especially favorable to GA dispersal and growth (Rhodes and Hubbs, 1992; Gregory and Hatler, 2008). Despite the severe ecological impacts recorded in the Pecos River as well as its historical importance as an early stage during the invasion of inland waters of the western hemisphere by GA, environmental factors associated with GA occurrence in this basin have not been examined.

The objective of this study is to determine the association between ambient conditions, especially water variables related to salinity and nutrients, and GA presence and abundance in the Pecos River basin. Selected sites were sampled through an 18-month time-span to account for seasonal and phenological events. Results of this study are anticipated to provide information that will further current understanding of GA dispersal and growth in inland waters.

2. Materials and methods

2.1. Study area

Eight sites were selected for this study including four in New Mexico and four in Texas (Fig. 1; for site coordinates, see Supplemental Table 1). Sites were chosen on the basis of their accessibility and records of GA blooms. The New Mexico sites are, in a downstream direction, Lea Lake (Lea), Devil's Inkwell Lake (Inkwell), Brantley Lake (Brantley), and a Pecos River free-flowing site just downstream of a bridge on New Mexico State Highway 31 (HW31) (Fig. 1). Lea is a gypsum sinkhole lake with no history of GA blooms. It provides habitat for the state-listed Pecos Pupfish (S. Denny, personal observations). Inkwell is also a gypsum sinkhole but with a history of GA blooms beginning in May 2006 (S. Denny, personal observations). Lea and Inkwell are both located in Bottomless Lakes State Park near Roswell, New Mexico. Brantley is a large (1619 hectares) reservoir near Carlsbad, New Mexico, that has been affected by GA blooms since 2003. Golden alga was first identified at HW31 in September 2003 and its highest abundance at this site was recorded in February 2004 (269,000 cells/mL; S. Denny, personal observations).

The Texas sites are, in a downstream direction, Red Bluff Reservoir (Red Bluff), Salt Creek (tributary that confluences with Pecos River downstream of Red Bluff), a Pecos River free-flowing site near Coyanosa, Texas (Coyanosa), and a Pecos River free-flowing site near Girvin, Texas (Girvin) (Fig. 1). Red Bluff had its first reported GA-related fish kill as early as 1985 (Southard et al., 2010). Salt Creek, a saline tributary to the Pecos River, is thought to be the last place in Texas that has a population of genetically pure Pecos Pupfish (Hoagstrom and Brooks, 1999). The site examined in

Fig. 1. Study sites (stars) in the Pecos River basin, and weather stations (crosses) used for collecting precipitation data. The Pecos River originates in the Sangre de Cristo Mountains in New Mexico (NM), and confluences with the Rio Grande in Texas (TX) at the international border with Mexico (framed map).

Salt Creek has no history of GA blooms and is free-flowing. Coyanosa had its first recorded appearance of GA in 2006 [Texas Parks and Wildlife Department (TPWD), 2013]. In the last site, Girvin, GA has been recorded since the fall of 2006 (M. Scott, TPWD, personal communication). From the Bottomless Lakes State Park in New Mexico to Girvin, Texas, sampling sites for this study stretch over 400 river kilometers.

2.2. Sampling schedule and water variables measured

All sites were sampled every month from January 2012 to July 2013 for temperature (°C), specific conductance (μS/cm), pH, dissolved oxygen (DO; mg/L), and GA abundance (cells/mL). Additional variables were measured or estimated during the odd-numbered months of the year; these included turbidity (Nephelometric Turbidity Unit; NTU), fluoride (mg/L), chloride (mg/L), sulfate (mg/L), hardness (total, calcium, magnesium; as $CaCO_3$ equivalents), alkalinity (mg/L; as $CaCO_3$ equivalent), pheophytin (μg/L), extracted chlorophyll *a*, *b*, and *c* (μg/L), in vivo chlorophyll *a* (relative fluorometric units), organic nitrogen (mg/L), inorganic nitrogen (mg/L), total nitrogen (mg/L), organic phosphorus (mg/L), inorganic phosphorus (mg/L), and total phosphorus (mg/L). Each sampling event in the odd-numbered months (e.g., January, March, et cetera) lasted two days; New Mexico sites were sampled on the first day and Texas sites on the second by Texas Tech University personnel. In the even-numbered months (e.g., February, April, et cetera), personnel from TPWD and New Mexico Game and Fish sampled the sites in their respective states within the same week. Sampling times typically lasted from mid-morning to mid-afternoon.

2.3. Field measurements and sample processing

Temperature, specific conductance, pH, and DO were measured in situ at the time of sampling using a YSI 556 multi-parameter probe (5563-4; Yellow Springs Instrument Company, Inc., Yellow Springs, OH, USA) calibrated daily according to protocols and standards recommended by the instrument manufacturer. Turbidity was measured on site by optical density (Oakton T-100 turbidimeter, EW-35635-00; Oakton Instruments, Vernon Hills, IL, USA), and in vivo chlorophyll *a* by fluorescence (Aquafluor Fluorometer, 998-0851; Turner Designs, Sunnyvale, CA, USA) in duplicate grab samples each. A calibration kit with four standards (800, 100, 20.0, and 0.02 NTU) (13-300-252; Fisher Chemical) was used for calibrating the turbidimeter and a solid secondary standard (8000-952; Turner Designs) was used for calibrating the fluorometer. In vivo chlorophyll *a* measurements served as back up for in vitro (extracted) measurements of chlorophyll *a* that fell below limits of detection (see Section 2.4), as the fluorometer method is more sensitive at low chlorophyll *a* concentrations (Turner Designs, 2005).

Grab water samples were taken at each sampling site for laboratory analyses of GA (duplicate), specific ions (duplicate), and nutrients (single sample per analyte). Samples for nutrient analyses (see Section 2.4) were collected in bottles containing sulfuric acid (except for bottles designated for the orthophosphate procedure) and shipped in wet ice (0 °C) overnight to Tarleton Institute for Applied Environmental Research (TIAER) in Stephenville, Texas. Grab samples for GA counts and extracted chlorophyll *a* measurement were collected at an approximate depth of 25 cm using plastic bottles, and those for chlorophyll *a* were filtered (single filter per site) in the field (250–500 mL) using a portable filtration apparatus and 47-mm GF/B glass fiber filters (1821-047; Whatman, Maidstone, Kent, UK). Filters were stored with dry ice in the field and subsequently in an ultracold freezer at −70 °C until processed. Grab samples for GA counts were placed on wet ice and counted within 48 h. Pilot assessments indicated that GA counts are stable within this time frame.

2.4. Laboratory analyses

Alkalinity and hardness were measured using a digital titrator (HACH model 16900; HACH, Loveland, CO, USA). Alkalinity analysis was based on method 8203, total hardness on method 8213, and calcium hardness on method 8204; magnesium hardness was calculated by subtracting calcium hardness from total hardness (HACH, 2013a). Fluoride and sulfate analyses were conducted using a colorimeter (HACH model DR/890). Fluoride analysis was based on method 8029 and sulfate analysis on method 8051 (HACH, 2013b). Chloride was measured with a YSI Professional Plus probe (Yellow Springs Instrument Company, Inc.) precalibrated with a chloride standard solution (1000 mg/L; HACH). Most samples had to be diluted with deionized water to bring chloride concentrations within the appropriate measurement range of the equipment.

The method used for GA cell counts was as described by Southard (2005), where GA cells are enumerated with a hemocytometer. The detection limit for this method is approximately 1000 cells/mL. Extracted chlorophyll *a*, *b*, and *c* and pheophytin were measured using method 10200H (trichromatic method and monochromatic method) described in APHA (1998). Positive and negative controls were processed according to French (2010). The limit of detection and method detection limit (MDL) were calculated for extracted chlorophyll and pheophytin following the "statistical" approach (Anderson, 1989; Thomsen et al., 2003). The method detection limit was the limit of detection adjusted for extraction volume.

Unfiltered samples sent to TIAER were analyzed for nitrite-nitrate nitrogen [low level modification; SM 4500-NO3 F; MDL, 0.0044 mg/L], ammonia nitrogen (SM 4500-NH3 G; MDL, 0.008 mg/L), total Kjeldahl nitrogen (SM 4500-NH3 G; MDL, 0.148 mg/L), orthophosphate phosphorus (low level modification; SM 4500-PE; MDL, 0.0005 mg/L), and total phosphorus (low level modification; EPA 365.4; MDL, 0.002 mg/L). Organic nitrogen was calculated by subtracting ammonia nitrogen from total Kjeldahl nitrogen, and inorganic nitrogen was calculated by adding nitrite-nitrate nitrogen and ammonia nitrogen. Organic phosphorus was calculated by subtracting inorganic phosphorus, or orthophosphate phosphorus, from total phosphorus.

2.5. Precipitation

Precipitation data were collected from the National Oceanic and Atmospheric Administration (NOAA; http://www.ncdc.noaa.gov/cdo-web) for the study period from selected weather stations near the study sites (Fig. 1; for weather station coordinates, see Supplemental Table 1). These data were used to calculate cumulative precipitation for a period of 7 days prior to sample collection (7-day precipitation). Cumulative 7-day precipitation was used because Roelke et al. (2011), who examined the relation between 7-day, 10-day, 30-day, or 365-day cumulative inflow and GA bloom formation in the Brazos River basin, concluded that 7-day inflow showed the best relations.

2.6. Data processing and analysis

Fluorometric chlorophyll *a* readings taken in the field were corrected for turbidity (Turner Designs, 2012). These corrected fluorometer readings were linearly regressed against spectrophotometry readings, and the regression model ($y = 0.9628x + 0.0989$; $R^2 = 0.88$) was used to estimate extracted chlorophyll *a* values for samples whose concentration fell below detection limits. Some of

the nutrient values also fell below detection limits (nitrate-nitrate nitrogen, 4% of all samples; ammonia nitrogen, 26%; total Kjeldahl nitrogen, 7%; orthophosphate phosphorus, 17%; total phosphorus, 0%). Prior to statistical analyses, six values for each nutrient measurement below the detection limit were estimated using a distribution-based imputation method (MI Procedure, SAS software, Version 9.2, SAS Institute Inc. SAS/STAT®, Cary, NC, USA). The mean of these six estimated values was then used to replace each value below detection limits. Several extracted chlorophyll *a* values for January 2012 (5 samples) fell below detection limits and fluorometry-based measurements were not collected at this sampling event. For these cases, missing values were replaced in a similar manner as described for nutrient observations below detection limits.

Principal component analysis (PCA) reduces complex datasets of potentially correlated, multiple variables into a lower number of uncorrelated, canonical variables without much loss of information. This analysis is especially useful for exploring patterns in the data (Ringnér, 2008) and was used to determine patterns in water quality grouped according to various criteria. There were many observations where GA abundance was undetectable (zero; see Section 3.1). Zero-inflated Poisson (ZIP) regression analysis was used to examine specific associations between water quality variables (predictor variables) and GA presence/absence and abundance (response variables). ZIP regression is a mixture model that combines a logit model for modeling the presence/absence of an organism with excess zero counts, and a Poisson method for modeling non-zero abundances of the organism (Wenger and Freeman, 2008).

Data collected in the odd-numbered months contained incomplete records (values for nutrients, chlorophyll *a* and major ions were not available for the odd-numbered months). Default specifications for PCA based on raw data inputs with missing values result in case-wise deletion of data. To avoid loss of information, we estimated a correlation matrix based on pair-wise observations from all monthly samplings and all variables, and this matrix was used in the PCA as described by Jolliffe (2002). Eigenvalues, scree plots, and percent variances were used to evaluate the number of principal components (PCs) to be retained in the analysis. Component loadings $\geq|0.40|$ were used to determine the predominant water quality variables in each retained PC (Manly, 1994). In addition, results of PCA were used to assist with the selection of variables for ZIP regression. PCAs were conducted using the PRINCOMP Procedure in SAS.

Two separate ZIP regressions were performed due to the different sampling schedule for the nutrient variables. The first analysis modeled GA presence and abundance against specific conductance, temperature, and pH for all monthly samples. The second analysis was conducted for samples collected only in the odd-numbered months. For this analysis, GA presence and abundance were modeled against inorganic nitrogen, inorganic phosphorus, organic nitrogen, and organic phosphorus in addition to specific conductance, temperature, and pH. For a given sample size, the stability of regression models generally decreases as the number of variables (estimates) in the model increases. For this reason and to maintain model performance, a number of variables were not included in the ZIP regression; these included variables that were highly correlated with other explanatory variables already in the models, and variables deemed of low importance by the PCA (when GA presence/absence was used as grouping variable). Fluoride, chloride, sulfate, and hardness were not included in the models due to their high correlation with specific conductance (see Section 3.1). Similarly, total phosphorus, total nitrogen, chlorophyll *a*, and turbidity were strongly correlated with the organic nutrient fractions, and were not included (see Section 3.1). Precipitation was not included because it did not have a strong component loading in the PCA (all $<|0.40|$), suggesting it was of little importance (see Section 3.2). While pH had a low component loading on all first three PCs (see Section 3.2), it was included in the models because this variable has previously been suggested to be an important factor influencing GA abundance and toxicity (Valenti et al., 2010; Prosser et al., 2012; Roelke et al., 2012). One of the selected PCs (PC3) was predominated by variables exhibiting seasonal variability; namely, temperature, alkalinity, and dissolved oxygen (see Section 3.2). Because temperature had the highest component loading on PC3, it was retained for the ZIP analysis but alkalinity and dissolved oxygen were not. All water quality variables were standardized to mean of 0 and variance of 1 prior to analysis in order to produce standardized regression coefficients. These analyses were conducted using the COUNTREG Procedure in SAS.

Correlation analyses were conducted in STATISTICA, version 10 (StatSoft, Inc. Tulsa, Oklahoma, USA) to examine bivariate associations. Plots of all associations were generated to evaluate patterns of data distribution and presence of outliers. Only those correlations significant at $p < 0.05$ and of moderate magnitude or greater ($r \geq |0.40|$) were interpreted.

2.7. Post hoc analyses

Coyanosa was the only site with previously reported toxic blooms of GA that had undetectable cell counts during the course of the present study (see Section 3.1). This finding prompted further examination of historical relations between salinity and GA abundance at this site. Archived data for specific conductance (1987–2013) and GA abundance (2006–2011) at Coyanosa were obtained from databases maintained by the Texas Commission on Environmental Quality (http://www80.tceq.texas.gov/SwqmisPublic/public/default.htm; Segment ID, 2311; Station ID, 13260) and TPWD, respectively. Golden alga abundance data from the present study (2012–2013) was also used in these analyses. Time-series plots of specific conductance (1987–2013) and GA abundance (2006–2013) were graphically examined, and LOWESS regression fitting (default specifications in STATISTICA; smoothing parameter, 0.15) was used to help identify time-dependent fluctuations. Also, the strength of the relation between specific conductance and GA abundance was quantitatively determined with Spearman rank correlation analysis using the median of available values for each variable in each year. Medians were chosen as representative yearly values because the sampling dates for the two variables generally did not coincide. The period of record for correlation analysis was 2006–2013, as GA was first recorded at Coyanosa in 2006.

3. Results

3.1. General observations

A total of 151 samplings were conducted during the entire study and were used to describe basic water quality (Table 1). A total of 79 samplings were conducted in the odd-numbered months and were used to describe selected major ion and nutrient profiles at the sampling sites (Tables 2 and 3). The Salt Creek site was not sampled in January 2012 due to accessibility problems.

Golden alga was not detected in Lea, Salt Creek, and Coyanosa at any time during the study period, and GA was undetectable multiple times at the other sites (Table 4). Sites with the highest incidence of GA presence were Inkwell, Brantley, HW31 and Red Bluff, and these sites also showed at least one bloom event during the study period (GA abundance $\geq$10,000 cells/mL; Roelke et al., 2007; Schwierzke et al., 2010) (Table 4). Golden alga was observed at similar overall rates of occurrence in winter (December–February), spring

Table 1
Basic water quality and 7-day (pre-sampling) cumulative precipitation [median and lower (Q25) and upper quartiles (Q75)] in the Pecos River basin grouped by study site and season (period of record, January 2012–July 2013). All monthly data were used to generate the values. Winter, December–February; spring, March–May; summer, June–August; fall, September–November.

Grouping variable	N	Temperature (°C)			Specific conductance (μS/cm)			Dissolved oxygen (mg/L)			pH			7-day precipitation (mm)		
		Q25	Median	Q75	Q25	Median	Q75	Q25	Median	Q75	Q25	Median	Q75	Q25	Median	Q75
Site																
Lea Lake	19	14.4	17.9	23.7	11,590	11,740	11,997	5.9	7.1	7.6	7.5	7.6	7.8	0.0	0.5	5.6
Devil's Inkwell Lake	19	9.5	16.0	23.5	7780	8004	8434	6.3	8.3	9.8	7.8	7.9	8.0	0.0	0.5	5.6
Brantley Lake	19	10.7	18.6	24.5	4706	4963	5899	6.8	8.4	9.8	7.9	8.0	8.2	0.0	1.3	10.2
Highway 31	19	12.7	20.6	24.7	5550	6030	6560	5.4	7.5	9.7	7.5	7.8	8.0	0.0	0.5	11.2
Red Bluff Reservoir	19	11.7	15.2	24.0	14,860	15,960	17,950	6.3	7.3	9.3	8.0	8.2	8.3	0.0	1.3	6.4
Salt Creek	18	11.6	20.2	25.5	21,800	34,488	52,338	5.5	6.8	8.0	7.9	8.3	8.5	0.0	1.9	6.4
Coyanosa	19	15.8	23.2	29.4	20,730	22,911	25,567	7.0	9.2	10.1	7.7	7.8	8.0	0.0	0.0	0.0
Girvin	19	16.6	21.9	29.6	23,004	25,908	27,937	9.1	9.8	12.7	7.9	8.1	8.5	0.0	2.0	24.4
Season																
Winter	39	7.9	11.4	13.4	6036	12,092	21,370	7.9	9.3	10.7	7.7	7.9	8.1	0.0	0.0	8.4
Spring	48	15.0	18.7	21.9	7084	13,059	25,419	6.9	8.3	9.7	7.8	8.0	8.2	0.0	0.0	3.0
Summer	40	24.6	26.5	28.1	7569	12,236	26,470	5.4	6.7	7.7	7.7	8.0	8.2	0.0	2.4	10.2
Fall	24	12.8	20.3	22.4	7799	14,290	20,617	6.3	7.4	8.5	7.7	7.9	8.2	0.0	3.8	14.2

(March–May), and summer (June–August) and was lowest in fall (September–November) (Table 4). The highest GA abundance recorded was 27,000 cells/mL at HW31. Although bioassays of toxicity were not performed, fish-kills in the field were not observed during this study.

Specific conductance varied spatiotemporally from a low of 4408 μS/cm to a high of 73,786 μS/cm. Plots of specific conductance and GA presence/absence or abundance revealed a highly skewed distribution of GA toward the low end of the range of specific conductance values (Fig. 2), and 4 of the 5 bloom events (≥10,000 cells/mL) occurred below the overall median level of specific conductance (12,250 μS/cm). The fifth bloom event occurred at a specific conductance of 15,975 μS/cm, which is below the overall upper quartile (22,877 μS/cm) and well below the highest specific conductance observed (73,786 μS/cm). Golden alga was never detected at specific conductance >~30,000 μS/cm.

Standard water quality observations grouped by site revealed generally higher but more variable levels of specific conductance and some of the anions and hardness cations in Texas sites relative to New Mexico sites, especially in Salt Creek (Tables 1 and 2). Organic and total phosphorus also seemed to be higher in Texas sites than New Mexico sites, and total and organic nitrogen as well as chlorophyll *a* were notably highest at HW31 and Red Bluff (Table 3). Winter was associated with lower temperatures and generally higher levels of DO, turbidity, and inorganic nitrogen (Tables 1 and 3). Organic nitrogen was highest during summer and fall, and chlorophyll *a* was highest in summer and lowest in winter (Table 3). No other general patterns in water quality variables were apparent. Most values (>75%) of extracted chlorophyll *b* and *c* and of pheophytin fell below their detection limit and are not reported.

Specific conductance strongly correlated with all anions and hardness cations measured (Supplemental Table 2). Inorganic nitrogen was negatively correlated with specific conductance, individual ions, and temperature. Turbidity was positively correlated with chlorophyll *a*, organic and total nitrogen, and organic and total phosphorus. Other than its correlation with turbidity, total phosphorus was only correlated with organic phosphorus (Supplemental Table 2). Among the nutrients, only organic and total nitrogen correlated with chlorophyll *a* (Supplemental Table 2). Inorganic phosphorus and 7-day precipitation did not correlate with any other variable (Supplemental Table 2). Scatterplots of all bivariate associations did not reveal the presence of non-monotonic (e.g., unimodal) associations or outliers that would have affected the results of correlation or PCA analyses (data not shown).

There was a large overlap in chlorophyll *a* values between grab samples with GA (range, 1.70–69.01 μg/L) and without GA (1.01–25.34 μg/L), and the highest chlorophyll *a* value observed in this study (69.01 μg/L) coincided with a relatively low GA abundance (3000 cells/mL). Excluding cases where GA was undetectable (see next paragraph), correlation analysis indicated that chlorophyll *a* and GA abundance are not strongly associated (Spearman $r = 0.25$, $p > 0.05$).

Seventy-six percent of samples (115 of 151 cases) had GA abundance values below detection limit (<1000 cells/mL) and were classified as "GA absent." This condition, however, should be interpreted with caution as it could include an unknown number of cases with low numbers of cells.

3.2. Principal component analysis

Results of the PCA based on the correlation matrix for all data collected during this study yielded three PCs with eigenvalues greater than 1 that together explained 61.9% of the total variance (Table 5). The scree plot indicated that additional PCs with eigenvalues > 1 contributed relatively little to the cumulative variance explained (data not shown), and were therefore not interpreted. Salinity-related variables including specific conductance, anions and hardness cations predominated PC1. Inorganic nitrogen had a loading >|0.40| but was of lower magnitude and inversely related to the salinity-related variables (Table 5). Total and the organic fractions of nitrogen and phosphorus, turbidity, and chlorophyll *a* predominated PC2. Temperature had the highest component loading in PC3, followed by inversely related loadings for alkalinity, inorganic nitrogen, DO, and organic nitrogen and phosphorus (Table 5). These observations indicate that salinity-related variables (PC1) explain most of the variance structure in the data, followed by nutrients and primary productivity (PC2), and season (PC3; e.g., temperature).

Case coordinates on the PCA biplots are based on the correlation matrix for the entire database (all months and variables), but only those cases with values available for all variables (odd-numbered months) can be plotted (Fig. 3). When data on biplots of PC1 and PC2 were labeled by site, a clear separation between New Mexico and Texas sites was observed along PC1, with New Mexico sites having lower values of specific conductance and other salinity-related variables (Fig. 3B). Some degree of individual site separation was also apparent, especially in New Mexico. Generally, the separation of New Mexico sites occurred along the nutrient gradient; from high to low, HW31 > Brantley > Inkwell > Lea. The

Table 2
Turbidity, alkalinity, and major ion concentrations [median and lower (Q25) and upper quartiles (Q75)] in the Pecos River basin grouped by study site and season (period of record, January 2012–July 2013, odd-numbered months). Hardness and alkalinity values are expressed as calcium carbonate equivalents. Winter, December–February; spring, March–May; summer, June–August; fall, September–November.

Grouping variable	*N*	Turbidity (NTU)			Chloride (mg/L)			Fluoride (mg/L)			Sulfate (mg/L)			Ca hardness (mg/L)			Mg hardness (mg/L)			Total hardness (mg/L)			Alkalinity (mg/L)		
		Q25	Median	Q75	Q25	Median	Q75	Q25	Median	Q75	Q25	Median	Q75	Q25	Median	Q75	Q25	Median	Q75	Q25	Median	Q75	Q25	Median	Q75
Site																									
Lea Lake	10	1.2	1.6	2.1	2985	3052	3190	2.6	2.7	2.8	1963	2147	2258	2055	2104	2155	473	514	580	2555	2644	2668	163	168	170
Devil's Inkwell Lake	10	1.8	2.2	3.2	1627	1677	1711	2.6	2.8	2.8	2326	2392	2555	2283	2355	2420	728	778	890	3043	3170	3235	92	109	112
Brantley Lake	10	8.1	14.0	20.3	628	947	1081	2.2	2.2	2.3	1625	1636	1769	1293	1357	1445	550	604	620	1878	1954	2058	103	119	137
Highway 31	10	5.2	8.9	12.3	1221	1291	1451	1.8	2.0	2.0	1449	1541	1608	1075	1160	1308	715	799	903	1848	1969	2080	95	116	153
Red Bluff Reservoir	10	6.1	8.6	11.9	4322	4695	4836	2.5	2.8	3.0	2832	3225	3353	2070	2325	2370	1470	1553	1675	3540	3865	4053	86	93	108
Salt Creek	9	2.7	3.3	4.3	6425	11,441	14,864	3.2	4.3	5.4	2209	3635	4935	1715	2355	2960	1340	1880	3165	3055	4235	6100	75	98	110
Coyanosa	10	6.7	10.6	20.7	6308	6769	7972	4.2	4.9	5.8	3109	3625	4368	2050	2385	2675	1533	2008	2410	3570	4423	5110	107	124	141
Girvin	10	3.8	5.4	23.2	7038	7323	8060	5.0	5.4	5.7	3852	4104	4732	2075	2284	2350	2033	2657	3020	4108	4943	5370	73	151	179
Season																									
Winter	15	2.4	7.4	22.1	1451	3366	6301	2.3	2.7	3.9	1625	2357	2952	1368	2035	2155	635	785	1520	1978	2958	3570	111	147	169
Spring	32	2.3	4.5	7.9	1451	3444	7319	2.5	2.7	4.5	1907	2331	3830	1546	2199	2343	698	1015	2203	2389	3150	4675	101	115	151
Summer	16	3.2	7.0	12.3	1591	3947	8577	2.5	3.0	5.6	1961	2695	4498	1702	2294	2513	683	1250	2798	2459	3400	5428	87	93	120
Fall	16	2.4	5.2	13.7	1563	3946	6405	2.4	2.9	4.8	1854	2535	3424	1567	2199	2378	686	1142	1950	2454	3402	4144	85	111	152

Table 3
Nutrients and chlorophyll *a* [median and lower (Q25) and upper quartiles (Q75)] in the Pecos River basin grouped by study site and season (period of record, January 2012–July 2013, odd-numbered months). Winter, December–February; spring, March–May; summer, June–August; fall, September–November.

Grouping variable	*N*	Organic N (mg/L)			Inorganic N (mg/L)			Total N (mg/L)			Organic P (mg/L)			Inorganic P (mg/L)			Total P (mg/L)			Chlorophyll a (μg/L)		
		Q25	Median	Q75	Q25	Median	Q75	Q25	Median	Q75	Q25	Median	Q75	Q25	Median	Q75	Q25	Median	Q75	Q25	Median	Q75
Site																						
Lea Lake	10	0.081	0.282	0.601	0.076	0.243	0.325	0.338	0.524	0.859	0.010	0.036	0.049	0.000	0.002	0.003	0.011	0.040	0.052	1.4	1.6	2.4
Devil's Inkwell Lake	10	0.246	0.422	0.771	0.061	0.103	0.160	0.278	0.559	0.894	0.015	0.028	0.038	0.001	0.002	0.005	0.016	0.031	0.045	1.4	2.1	2.9
Brantley Lake	10	0.314	0.494	0.878	0.039	0.203	0.530	0.541	1.004	1.562	0.015	0.038	0.062	0.001	0.001	0.008	0.016	0.039	0.067	4.0	5.8	8.4
Highway 31	10	0.871	1.737	2.031	0.131	0.755	2.992	2.169	2.489	3.990	0.040	0.048	0.081	0.003	0.005	0.008	0.045	0.060	0.096	16.9	22.6	42.0
Red Bluff Reservoir	10	1.419	1.563	1.925	0.048	0.150	0.371	1.666	1.897	2.236	0.046	0.075	0.090	0.002	0.002	0.004	0.050	0.077	0.114	11.5	13.7	16.1
Salt Creek	9	0.558	0.875	1.358	0.021	0.033	0.060	0.780	1.252	1.563	0.020	0.063	0.127	0.000	0.002	0.004	0.032	0.070	0.129	1.6	2.1	4.2
Coyanosa	10	0.538	0.707	0.922	0.049	0.073	0.111	0.579	0.974	1.265	0.031	0.064	0.085	0.001	0.001	0.002	0.031	0.065	0.091	2.0	2.7	4.2
Girvin	10	0.900	1.233	1.316	0.021	0.030	0.221	0.929	1.301	1.423	0.025	0.050	0.113	0.001	0.002	0.003	0.028	0.052	0.115	3.3	4.0	6.2
Season																						
Winter	15	0.465	0.797	1.293	0.370	0.720	1.097	0.862	1.644	2.563	0.016	0.062	0.096	0.000	0.001	0.002	0.018	0.063	0.099	1.4	2.6	7.2
Spring	32	0.273	0.648	1.264	0.031	0.081	0.278	0.440	0.690	1.582	0.030	0.047	0.088	0.002	0.003	0.007	0.031	0.059	0.092	1.9	4.3	8.1
Summer	16	0.397	1.152	1.455	0.040	0.064	0.116	0.456	1.287	1.571	0.026	0.054	0.083	0.001	0.002	0.005	0.030	0.057	0.088	2.9	8.3	13.9
Fall	16	0.823	1.423	1.808	0.023	0.069	0.187	0.895	1.493	1.891	0.019	0.034	0.049	0.001	0.002	0.004	0.020	0.035	0.051	2.1	4.1	16.7

Table 4
Summary statistics for golden alga abundance in the Pecos River basin grouped by study site or season (period of record, January 2012–July 2013). Mean, median, minimum (min), and maximum values (max) of abundance (cells/mL) were calculated from monthly measurements made during the entire study period (January 2012–July 2013). Percent values of occurrence (presence) and bloom formation (≥10,000 cells/mL) are also shown. Winter, December–February; spring, March–May; summer, June–August; fall, September–November.

Grouping variable	*N*	Mean	Median	Min	Max	% Samples with GA present	% Samples ≥10,000 cells/mL
Site							
Lea Lake	19	0	0	0	0	0.0	0.0
Devil's Inkwell Lake	19	1789	0	0	10,000	36.8	5.3
Brantley Lake	19	2000	0	0	19,000	36.8	5.3
Highway 31	19	3526	1000	0	27,000	52.6	10.5
Red Bluff Reservoir	19	1737	1000	0	10,000	52.6	5.3
Salt Creek	18	0	0	0	0	0.0	0.0
Coyanosa	19	0	0	0	0	0.0	0.0
Girvin	19	105	0	0	1000	10.5	0.0
Season							
Winter	39	974	0	0	10,000	25.6	2.6
Spring	48	1250	0	0	19,000	29.2	2.1
Summer	40	1725	0	0	27,000	20.0	7.5
Fall	24	292	0	0	3000	16.7	0.0

same biplot with data labeled by GA presence/absence showed that most GA detections were observed in the lower-salinity (New Mexico) sites (Fig. 3A), thus confirming the results of graphical analyses (Fig. 2). Also, while GA occurred at a relatively wide range of nutrient concentrations in the lower-salinity sites (except Lea), all GA occurrences in the higher-salinity sites occurred at the mid-to-high nutrient levels (Fig. 3A; note the absence of GA incidences in the lower right quadrant of the plot). Lea was notable in that it had the lowest overall concentration of nutrients (especially organic nitrogen; see Table 3) and showed no occurrences of GA. The three bloom events observed during odd-numbered months happened at low-to-mid salinity and mid-to-high nutrient levels (Fig. 3A). No additional patterns in data distribution were observed in biplots of PC1 and PC3 (data not shown).

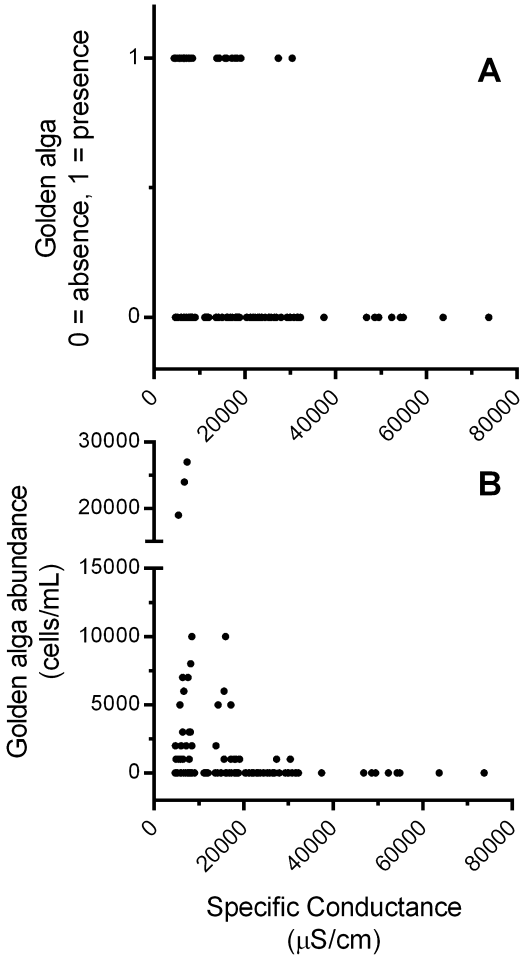

Fig. 2. Scatterplot of golden alga presence/absence (A) and abundance (B) against specific conductance measured monthly at all study sites over the sampling period (January 2012–July 2013).

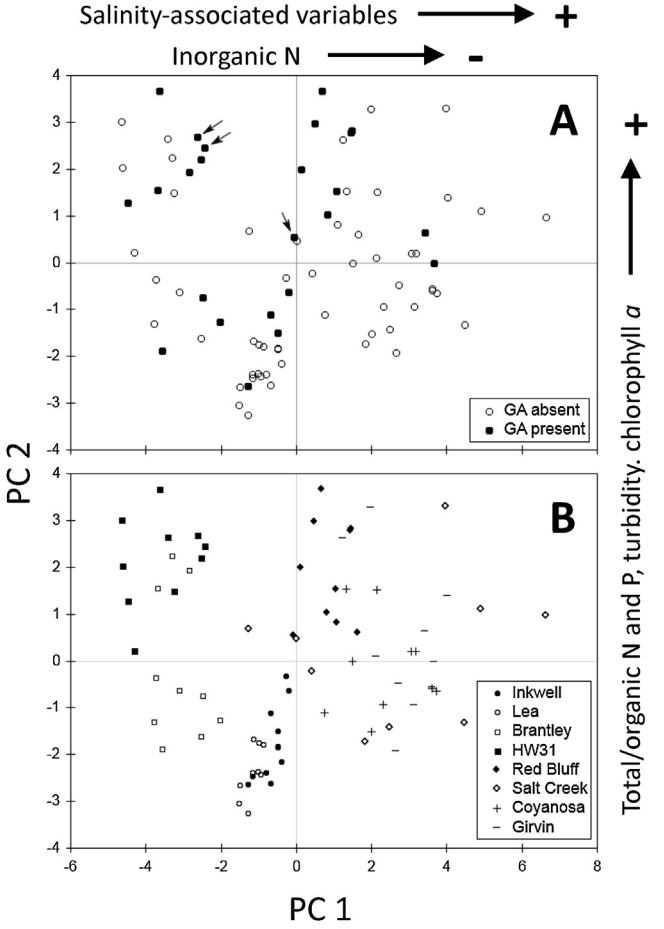

Fig. 3. Biplots of principal components 1 and 2. Grouping variables for this analysis were golden alga (GA) presence/absence (A) and sampling site (B). Data associated with bloom events (≥10,000 cells/mL) are indicated with black arrows. Variables with factor loadings > |0.40| and the direction (sign) of their gradient are summarized on the top and right sides of the plots.

Table 5
Component loadings, eigenvalues and total and cumulative variance for the first three components of principal component analysis of water quality variables in the Pecos River basin (period of record, January 2012–July 2013). The analysis was based on the correlation matrix for all data and variables. Component loadings ≥|0.40|are bolded.

Variable	Principal component		
	1	2	3
Total hardness	**0.98**	0.03	0.00
Sulfate	**0.97**	0.01	0.03
Specific conductance	**0.95**	0.06	0.03
Chloride	**0.93**	0.05	0.11
Fluoride	**0.92**	−0.08	0.05
Magnesium hardness	**0.89**	0.32	−0.04
Calcium hardness	**0.85**	−0.27	0.05
Total nitrogen	−0.22	**0.82**	−0.04
Organic nitrogen	0.11	**0.80**	**−0.41**
Chlorophyll *a*	−0.28	**0.67**	−0.39
Turbidity	−0.04	**0.67**	0.14
Total phosphorus	0.30	**0.62**	0.39
Organic phosphorus	0.32	**0.59**	**0.41**
Inorganic phosphorus	−0.06	0.32	−0.07
7-day precipitation	0.03	0.30	−0.13
Temperature	0.21	−0.13	**−0.72**
Alkalinity	−0.22	−0.13	**0.62**
Inorganic nitrogen	**−0.53**	0.32	**0.53**
Dissolved oxygen	0.17	−0.11	**0.42**
pH	0.17	0.00	−0.15
Eigenvalue	6.8	3.5	2.1
Total variance (%)	34	17.4	10.4
Cumulative variance (%)	34	51.5	61.9

3.3. Zero-inflated Poisson regression analysis

In samples from all months, the binary component of ZIP regression analysis indicated that specific conductance had a significant, negative association with GA presence, while temperature and pH were not significantly associated (Table 6). The Poisson component of the ZIP model indicated that specific conductance and pH both had significant, negative associations with GA abundance (Table 6).

In samples from the odd-numbered months, specific conductance also showed a significant negative association with GA presence, and organic phosphorus had a significant positive association (Table 7). When GA was present, GA abundance showed a significant negative association with specific conductance, pH, and inorganic nitrogen and a significant positive association with organic nitrogen (Table 7).

The negative association between pH on GA abundance in both ZIP regression analyses may have been the artifact of two outliers; namely, two of the five observed blooms (>10,000 cells/mL) occurred at the HW31 site, which had one of the lowest median pH values of all sites (7.8). When these two cases (pH ≈ 7.5) were removed, the estimated effect of pH on abundance became non-significant (all-month estimate = 0.30, $p = 0.12$; odd-numbered-month estimate = 0.07, $p = 0.80$). Thus, pH did not appear to influence GA presence or abundance.

Table 6
Parameter estimates from zero-inflated Poisson regression analysis. This analysis was used to model the effects of water quality on golden alga presence and abundance for samples collected from all months. The analysis is a combination of a logit model used to model golden alga presence and a Poisson model for non-zero abundances. Significant *p*-values are highlighted in bold font.

Model	Parameter	Estimate ± SE	*t*-value	*p*-value
Binary	Intercept	−1.25 ± 0.23	−5.37	**<0.001**
	Specific conductance	−0.77 ± 0.24	−3.17	**0.0015**
	Temperature	−0.25 ± 0.21	−1.19	0.2334
	pH	0.21 ± 0.21	0.79	0.4271
Poisson	Intercept	1.28 ± 0.13	10.13	**<0.001**
	Specific conductance	−0.34 ± 0.12	−2.88	**0.0039**
	Temperature	0.14 ± 0.08	1.73	0.0844
	pH	−0.53 ± 0.13	−4.04	**<0.001**

Table 7
Parameter estimates from zero-inflated Poisson regression analysis. This analysis was used to model the effects of water quality on golden alga presence and abundance for samples collected from odd-numbered months. The analysis is a combination of a logit model used to model golden alga presence and a Poisson model for non-zero abundances. Significant *p*-values are highlighted in bold font.

Model	Parameter	Estimate ± SE	*t*-value	*p*-value
Binary	Intercept	−0.36 ± 0.49	−0.74	0.4611
	Specific conductance	−1.18 ± 0.51	−2.29	**0.0219**
	Temperature	0.24 ± 0.44	0.54	0.5876
	pH	0.86 ± 0.61	1.41	0.1578
	Inorganic nitrogen	0.33 ± 0.59	0.54	0.5902
	Organic nitrogen	0.61 ± 0.36	1.7	0.0892
	Inorganic phosphorus	−0.36 ± 0.52	−0.68	0.4943
	Organic phosphorus	1.36 ± 0.65	2.09	**0.0367**
Poisson	Intercept	0.61 ± 0.22	2.73	**0.0064**
	Specific conductance	−0.76 ± 0.20	−3.74	**<0.001**
	Temperature	−0.19 ± 0.13	−1.48	0.1386
	pH	−0.81 ± 0.18	−4.45	**<0.001**
	Inorganic nitrogen	−0.62 ± 0.20	−3.09	**0.002**
	Organic nitrogen	0.29 ± 0.10	2.84	**0.0045**
	Inorganic phosphorus	−0.21 ± 0.19	−1.11	0.2655
	Organic phosphorus	−0.02 ± 0.14	−0.16	0.8697

3.4. Coyanosa

Specific conductance at Coyanosa was relatively variable in the period between 1987 and 2010, but a transient increase was evident in 2002–2003 followed by relatively low levels in 2004–2010 (Fig. 4). An increasing monotonic trend was observed beginning in ~2009 that became very steep in 2011–2013 (Fig. 4). The scatterplot of available GA data indicated that abundance levels generally were low in 2006, increased in 2007–2008, declined but were still present in 2009–2010, and were undetectable in 2011–2013 (see also Table 4). Correlation analysis of yearly medians of specific conductance and GA abundance

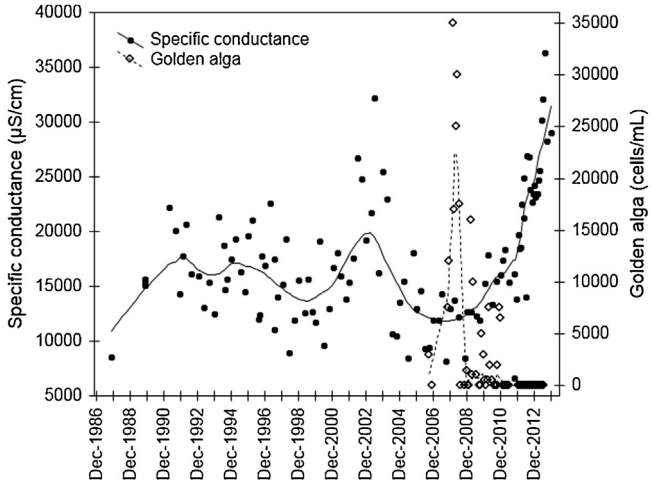

Fig. 4. Scatterplot of specific conductance (October 1987–December 2013) and golden alga abundance (October 1987–July 2013) against date of measurement at the Coyanosa site. LOWESS regression lines were added to each plot to help visualize patterns of change (smoothing parameter, 0.15). Data for specific conductance were obtained from the Texas Commission on Environmental Quality and for golden alga, from Texas Parks and Wildlife Department and present study (see text).

(2006–2013) indicated a strongly negative and highly significant association (Spearman $r = -0.90$, $p = 0.0024$).

4. Discussion

Salinization has deleterious effects on freshwater habitats and organisms (Cañedo-Argüelles et al., 2013) and can also facilitate colonization (invasion) by species from marine or saline ecosystems, especially in inland water bodies with high nutrient levels (Lee et al., 2013). The Pecos River is among the saltiest river systems in the southwestern United States (Yuan and Mayer, 2012). While a large portion of the dissolved solids in the Pecos River basin originates from natural sources, anthropogenic activities have also contributed to increased stream salinity (Yuan and Mayer, 2012). It has been suggested previously that the relatively high salinity of the Pecos River provides habitat that is particularly well suited for GA growth and toxic bloom formation (Rhodes and Hubbs, 1992; Gregory and Hatler, 2008). This suggestion is conceptually supported by results of laboratory studies showing that growth of GA in cultures is reduced as salinity falls below critical levels (Baker et al., 2007, 2009), and by field studies in other river basins reporting positive associations between salinity and GA abundance or toxicity; e.g., Red River (Texas/Oklahoma, USA; Hambright et al., 2010), Brazos River (Texas, USA; Roelke et al., 2011), and Colorado River (Texas, USA; VanLandeghem, 2013). In the present study, however, results of graphical, PCA, and ZIP regression analysis clearly indicated the existence of an inverse relation between salinity and GA presence as well as abundance in the Pecos River. Rates of GA presence and levels of abundance were highest at the low end of the range of salinities measured over space and time, and 4 of the 5 bloom events (abundance $\geq$10,000 cells/mL) were below the overall median level of specific conductance (12,250 μS/cm). The absence of GA was not confined to Salt Creek, a highly saline (median, 34,488 μS/cm) tributary with no prior history of GA blooms. Other sites of high salinity, including the mainstem site of Coyanosa (median, 22,911 μS/cm), with an earlier history of toxic blooms, did not have detectable levels of GA during the present study period.

A detailed examination of archived records for Coyanosa supported the conclusion of an inverse association between salinity and GA in the greater Pecos River basin. Although the exact date of its first appearance at Coyanosa is uncertain, GA was first recorded in 2006 when specific conductance was relatively low. Graphical examination of scatterplots and LOWESS regression lines indicated that GA abundance reached the highest levels ever recorded at this site in 2007–2008 while salinity remained low, declined as specific conductance began to increase in 2009–2010, and became undetectable in 2011–2013 coincidentally with a sharp increase in specific conductance. Quantitative support for these graphical observations was provided by correlation analysis of yearly medians for specific conductance and GA abundance (2006–2013), which demonstrated a strong, negative association between the two variables.

The negative association between salinity and GA occurrence observed in this study was unexpected because salinity in the Pecos River is generally below levels observed in coastal and marine environments, where GA is believed to have originated (Nicholls, 2003). However, this finding is not necessarily inconsistent with previous observations of positive associations between salinity and GA. The Pecos River basin is generally more saline (in present study, 4408–73,786 μS/cm) than river systems where the relation was found to be positive [e.g., <6000 μS/cm in the Red River (Hambright et al., 2010); <7000 μS/cm in the Colorado River (VanLandeghem, 2013)]. Thus, a plausible scenario that incorporates both patterns of observations is that the salinity-GA association is positive at relatively low salinity but reverses to negative at higher salinity. This scenario is consistent with results of a study of saline lakes in the People's Republic of China, where golden alga cell abundance in individual lakes was positively associated with lake salinity up to salt concentrations of 8 parts per thousand (approximately 14,000 μS/cm), but negatively correlated at higher salinities (Gou, 1983; cited in Guo et al., 1996). The limited amount of information presently available makes it difficult to consider mechanisms for this proposed reversal. One possible explanation is that among the dissolved solids in Pecos River water may be factors that inhibit GA growth, such that as salinity increases so does the concentration of inhibitory factors. Another possibility is that GA populations in the Pecos River have experienced physiological adaptations to their inland environment that affected their tolerance to salinity, making them less euryhaline. To address these two scenarios, it would be useful to determine if salinity-GA relations in other high-salinity inland waters are similar to those observed in the Pecos River, and if these patterns can be replicated with Pecos River strains of GA in the laboratory. Still another possible scenario is that the abundance of salinity-tolerant zooplankton grazers increases with salinity in the Pecos River, thus leading to a decline in GA abundance. Although zooplankton were not examined in the present study, other studies have demonstrated inverse associations between GA and zooplankton (Michaloudi et al., 2009; Schwierzke et al., 2010).

Earlier studies reported positive associations between GA abundance (and toxicity) and chlorophyll *a* and nutrient concentrations (Granéli et al., 2008; Hambright et al., 2010), especially during GA bloom events in winter (Hambright et al., 2010). Generally consistent with the earlier reports, results of PCA suggested a positive association between total and organic nutrient levels and GA presence in the Pecos River. More specifically, PCA biplots indicated that when salinity is relatively low, such as in the New Mexico sites, GA is present at a relatively wide range of total and organic nutrient and chlorophyll *a* levels whereas at higher salinity, such as in the Texas sites, GA was observed only at mid-to-high nutrient and chlorophyll *a* levels. Furthermore, ZIP regression suggested that GA presence is positively associated with organic phosphorus, and that when GA is present, its abundance is positively associated with organic nitrogen but negatively associated with inorganic nitrogen. The former observation is consistent with the worldwide trend of increased frequency and intensity of algal blooms due to eutrophication in both inland and marine systems over the last ~200 years, with inland systems generally impacted due to elevated phosphorus levels (de Jonge et al., 2002). The observed relations between GA and organic and inorganic nitrogen are also consistent with findings from previous field and laboratory studies. In the Colorado River basin of Texas, for example, reservoirs frequently impacted by GA were characterized by high levels of organic nitrogen and low levels of inorganic nitrogen (VanLandeghem, 2013). In the laboratory, GA is able to fulfill its nitrogen requirement by utilizing organic forms of nitrogen (Lindehoff et al., 2011), and other studies also have shown that ichthyotoxicity of GA cultures is high when grown under inorganic nitrogen-limited conditions (Granéli et al., 2012). Overall, the present observations are consistent with current understanding of the importance of salinity and nutrients, but also suggest the existence of an interaction between salinity and nutrients on GA incidence and abundance in the Pecos River.

Of the two gypsum sinkhole lakes examined in this study, Inkwell and Lea, GA was observed only in Inkwell during the study period and only Inkwell has a prior history of GA occurrence. Results of PCA and ZIP regression both suggested that Inkwell has higher overall levels of organic nutrients than Lea; however, closer inspection of their descriptive statistics indicated that organic

nitrogen may have been primarily responsible for this differential, and that inorganic nitrogen levels are lower in Inkwell. These findings at the gypsum sinkhole lakes are consistent with, and provide a specific example for, the general results of ZIP regression indicating a wider Pecos River basin association between GA abundance and organic (positive) and inorganic (negative) nitrogen. In addition, results of PCA and examination of descriptive statistics indicated that Inkwell has slightly lower, overall levels of salinity-related variables than Lea, a trend which is also consistent with an inverse salinity-GA relation. However, the overall differences between these two sites are greater for nutrients than for salinity-related variables, and therefore differences in nutrient concentrations may better explain the presence of GA in Inkwell but not in Lea.

Results of ZIP regression initially appeared to show a negative association between pH and GA abundance (in samples where GA was present), but this observation was attributable to two outlier values. Previous studies of the association between ambient pH and GA abundance/toxicity have yielded inconsistent results. Several field and laboratory studies documented a positive association between GA toxicity and pH (Ulitzur and Shilo, 1964; Valenti et al., 2010; Roelke et al., 2012), and Prosser et al. (2012) reported bloom inhibition at pH levels of 7.5 and below but bloom formation at pH of 8.5 in mesocosms. Conversely, other field studies reported either a weak negative association between pH and GA-like toxicity (VanLandeghem et al., 2012) or no association between pH and either GA abundance or toxicity (VanLandeghem, 2013). Patiño et al. (2014) concluded that while it is clear that pH can acutely regulate GA toxicity, the importance of pH as a natural driver of GA growth and toxicity in the field is uncertain or could vary among systems.

A recent analysis of archived data for the Brazos and Colorado River basins in Texas showed that GA-impacted reservoirs have much higher sulfate levels than non-impacted reservoirs (Patiño et al., 2014). This observation led to the formulation of an untested hypothesis of a possible role for sulfate as nutrient (sulfur) source for GA growth in reservoirs impacted by this alga (Patiño et al., 2014). Relatively high levels of total hardness also have been associated with GA habitat or bloom occurrence in other river basins impacted by GA (VanLandeghem et al., 2012; VanLandeghem, 2013; Patiño et al., 2014). In the present study, sulfate and hardness levels at all study sites and sampling times were considerably higher than those observed in GA-impacted reservoirs of the Brazos and Colorado Rivers (Patiño et al., 2014), and both water quality variables were highly correlated with specific conductance. Therefore, no specific associations between these water variables and GA could be detected in this study.

Seven-day cumulative precipitation was not associated with GA occurrence in the PCA, and extending the period of cumulative precipitation to 30-days prior to sampling yielded the same observations (data not shown). It should be noted, however, that surface water salinity can be influenced by rainfall in the Pecos River. In at least one of the sampling sites of this study (Salt Creek), salinity was transiently reduced following a rainfall event during the study period (data not shown).

5. Conclusions

Results of this study provided the first documentation of a negative association between salinity and GA abundance in a river ecosystem, and are consistent with previous findings in lacustrine environments (Gou, 1983; cited in Guo et al., 1996). Together with earlier reports of a positive association at lower salinities, the present observations indicate that salinity-GA associations are not straightforward in inland waters and that there may be upper (present study; Gou, 1983; cited in Guo et al., 1996) as well as lower (Roelke et al., 2011; Patiño et al., 2014) salinity barriers to dispersal or growth. The basis for the negative association between GA and salinity observed in the Pecos River is uncertain and requires further study.

While there are ongoing efforts to reduce natural salt inputs into the mainstem of the Pecos River for purposes other than alga control (Gregory et al., 2013), it seems unlikely that these programs will suffice to reduce salinity to the relatively low level necessary to minimize or prevent GA blooms in inland waters (<0.5–1 psu; Roelke et al., 2011; Patiño et al., 2014). A complementary strategy for GA control in the Pecos River would be to reduce nutrient inputs, especially because GA presence at high salinity (>~15,000 μS/cm) only occurred when total and organic nutrient concentrations were also elevated. Irrigated croplands can be extensive in some areas of the Pecos River basin (NMDGF, 2006; Thompson, 2008; Gregory et al., 2013) and the application, or more effective use, of nutrient-reducing technologies may help reduce eutrophication as well as the risk of GA blooms. The positive association between organic nitrogen and GA abundance suggests that reductions in nitrogen inputs may be more effective at curbing the risk of toxic GA blooms in the Pecos River basin than reductions in phosphorus; however, reductions in phosphorus may also create conditions unfavorable to GA colonization, as the presence of GA was positively related to organic phosphorus levels. Whether efforts to control eutrophication in inland waters should focus on reducing inputs of phosphorus, nitrogen, or both has been a topic of longstanding debate (Canfield, 1983; Conley et al., 2009). Overall, the results of this study provide information that may be useful for developing water quality criteria to reduce the frequency or prevent the further spread of GA blooms in the Pecos River and perhaps other river basins.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.hal.2014.06.012.

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