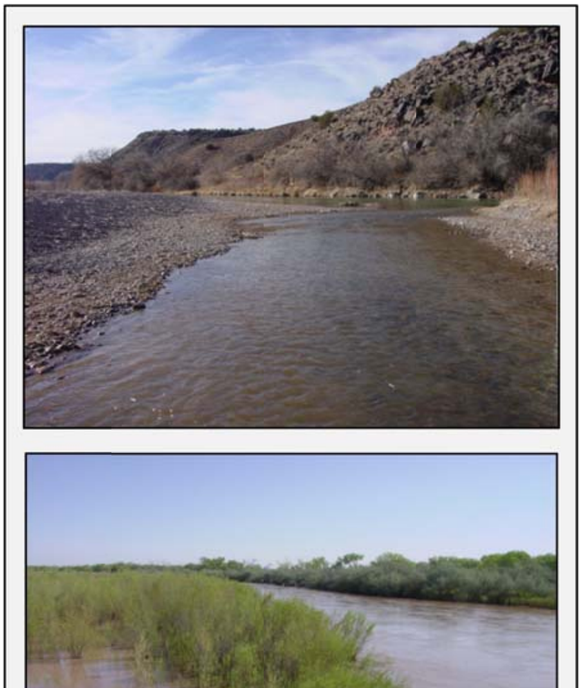
Final Report 2011

Effects of nutrient availability on periphyton growth and diversity in the middle Rio Grande: top-down and bottom-up factors

Prepared for:
Middle Rio Grande Endangered Species Collaborative Program



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Effects of nutrient availability on periphyton growth and diversity in the Middle Rio Grande: Top-down and bottom-up factors

Final Report
Contract: 07-CS-40-8204
2 July 2007 – 30 April 2011

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Middle Rio Grande Endangered Species Act Collaborative Program
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Draft: 30 April 2011, comments received 26 May 2011, revised 1 June 2011

Executive Summary

In lotic and lentic ecosystems, primary producers serve as a food resource for higher trophic levels. In the middle Rio Grande, invertebrate and fish grazers, including the federally endangered Rio Grande silvery minnow (*Hybognathus amarus*), are dependent on attached algae (periphyton) as a food resource. However, the relationships between environmental factors and algal biomass/community structure in the middle Rio Grande are poorly understood. In many aquatic systems, primary production is often limited by nutrient availability; it is not known how nutrient levels affect algal food resources for grazers in the Rio Grande or whether other factors limit productivity (e.g. high turbidity and decreased light penetration). Seasonal changes in precipitation also influence environmental parameters, including nutrients; periphyton may be limited by increased nutrient concentrations and turbidity levels.

The research presented here is three-fold:

1. We examined the longitudinal relationship between periphyton and environmental parameters, including nitrogen (N) and phosphorus (P). Seasonally at five locations, from Angostura to Bosque del Apache, we collected physical, chemical, and biological (i.e., algal) data from November 2007 to July 2010. Multiple physical and chemical parameters varied significantly both seasonally and among locations. Generally, turbidity was much lower at upstream locations than at downstream locations and tended to be lower in winter/spring than in later summer. Substrates varied by site with generally more sand in the northern reaches and more silt at Bosque del Apache, in part related to tributary inputs. Also, nutrient concentrations (NO_3-N and PO_4-P) and anions all differed significantly among locations and seasons. Concentrations were consistently low at Angostura and Bosque del Apache, but varied seasonally at the other locations that were more heavily influenced by urban and agricultural inputs. By contrast, algal biomass (measured as chlorophyll *a*) was consistently low with some significant variation among sites and seasons. However, there were marked differences among locations in diatom community structure – upstream sites tended to have more epilithic and alkaliphilic diatom taxa that prefer lower turbidity, whereas downstream sites contained taxa tolerant to poor water conditions and high turbidity that live on sediment substrates.

2. Nutrient-diffusing substrates (NDS) were used to investigate effects of nitrogen and phosphorus availability on algal biomass and species composition in the middle Rio Grande. Algal biomass was high on all nutrient treatments (control, N, P, N+P) but not significantly different from each other, largely due to light limitation caused by consistently high turbidity levels. Similarly, it was difficult to detect differences in the abundance of invertebrate grazers among nutrient treatments, with only the density of one caddisfly, *Nectopsyche*, being significantly different among treatments.

3. Seasonally, shifts in turbidity (related to tributary flows) played a major role in shaping algal communities. In the summer months, high turbidity associated with tributary inputs created a light-limited environment where primary production was limited to a littoral zone “bathtub ring.” **We tested the idea of a “bathtub ring” of primary production in the Rio Grande related to turbidity and light availability.** In 2010, we conducted detailed transverse transects across reaches of river to document the relationships among depth, algal parameters and invertebrate parameters in different seasons (spring, summer, fall). These data can be used to map relationships between estimated fish densities and the

presence of good food sources (high algal abundance and high invertebrate densities. In periods of relatively high flow (April and November), there was some evidence of a bathtub ring of primary production with higher chlorophyll *a* concentrations and greater diatom densities in water less than 12 cm depth. However, in periods of relatively low flow (July) there was no bathtub ring as production occurred across the entire transect when the water was shallow and clear.

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List of Important Acronyms/ Special Terms Used in Report

AFDM – Ash-free dry mass (a measure of organic biomass)

N – nitrogen

NDS – nutrient-diffusing substrate

P – phosphorus

TN – total nitrogen

TP – total phosphorus

Introduction

In the middle Rio Grande, primary producers play a critical role in the aquatic food web. Many invertebrate and fish grazers, including the federally endangered Rio Grande silvery minnow (RGSM; *Hybognathus amarus*), are dependent on attached algae (periphyton) as a food resource (Pease et al. 2006). Previous research has verified that all life stages of the RGSM ingest diatoms and other algal groups during at least part of the year (Shirey 2004, Pease et al. 2006). Diatoms are generally a more nutritious food source due to their high lipid content, while cyanobacteria are less palatable and often contain toxin (Steinman 1996). Therefore, shifts in algal community composition can affect food quality for grazers.

The relationships between environmental factors and algal biomass/community structure in the middle Rio Grande are currently poorly understood. In aquatic systems, primary production is often limited by nutrient availability (usually nitrogen and/or phosphorus). Currently, it is not known whether nutrient levels affect algal food resources for fish grazers in all reaches of the Rio Grande. Other factors such as light limitation (resulting from increased turbidity) and temperature may also play roles in determining patterns of primary productivity. Seasonal changes in precipitation also influence environmental parameters, including nutrients and turbidity, which can subsequently influence periphyton growth and community structure. River flow dynamics differ significantly among seasons and among years. High flows can result in increased scouring and decreased substrate availability for algal growth. From a management perspective, research has not determined if habitat restoration efforts for the RGSM are creating appropriate habitat for periphyton communities.

Project Justification

This research identifies nutrient availability for algal periphyton biomass and species composition in the middle Rio Grande. This baseline research addresses the potential of anthropogenic impacts and habitat restoration projects to provide sufficient nutrients to support food supply (i.e., periphyton) for grazers such as the RGSM. More broadly, this study examined the temporal and spatial relationships between periphyton and environmental factors in this aridland river. **First, longitudinal surveys documented spatial and seasonal changes in relationship between periphyton and environmental parameters, including nitrogen (N) and phosphorus (P) along a longitudinal gradient. Secondly, a detailed experiment was conducted to investigate the role of N and P availability and how these nutrients and grazers affect biomass and species composition of algae in the middle Rio Grande. Finally we have examined the fine-scale spatial patterns of periphyton and invertebrates along the littoral zone of the river; this information maps the potential food resources for the RGSM and other grazers.**

The bottom-up influence of nutrient enrichment generally shows increased algal biomass and shifts towards greater diversity and a community composed of tolerant and nutrient-loving taxa (Lowe et al. 1986, Tank and Dodds 2003) . Combining nutrient enrichment with top-down grazing influence from fish, snails or macroinvertebrates often show varying periphyton responses from strong top-down effects due to grazing to stronger stimuli from nutrient enrichment (Stewart 1987, Rosemond et al. 1993). In our nutrient enrichment experiment, **we expected that stronger bottom-up effects would result in an increase in periphyton biomass, reflecting a response related to limitation or co-limitation by one or**

more nutrients. If grazing was a stronger factor than nutrient enrichment, periphyton biomass would be limited in nutrient treatments where grazers were present.

In general, it has been shown that rivers in the southwestern US are nitrogen-poor with few nutrient inputs (Grimm et al. 1981, Passell et al. 2005). Although it is difficult to infer historical nutrient levels, some information can be gleaned from fish gut content analysis of RGSM collected in 1874 (Cowley et al. 2006). This analysis indicated that diets were dominated by diatoms from nutrient-loving epipelic diatoms (i.e., those that grow on sediment), which provides evidence that the floodplain may have been wider than it is currently and provided areas for grazing by fish (Cowley et al. 2006). This historic floodplain also provided a connection to the river via nutrient cycling in an arid landscape that otherwise provides little nutrient input. While the diatom information infers that historically there were higher levels of nutrients and higher sediment deposition in the floodplain compared to the modern river, these data were not appropriate to hypothesize on nutrient levels in the main channel.

Today, nutrient concentrations are heavily influenced by anthropogenic inputs. Wastewater treatment effluents contribute the majority of phosphorus and nitrogen. Additionally, fish grazers are restricted to diets dominated by smaller, epipsammic diatoms (i.e., those that grow on sand), (based on gut content, Cowley et al. 2006). This indicates that fish are no longer grazing in the floodplain areas and have been forced to forage in the main channel (where there is generally less fine sediment accumulation due to channel incision).

The study presented here examined the temporal and spatial distributions of periphytic taxa that may play a role as food resources for RGSM. Furthermore, the algal flora of the arid southwest U.S. is not well understood and this work will provide baseline information about the natural history of algal taxa and increase our understanding of the primary producer role in aridland river systems.

Project hypotheses and objectives

In a system with naturally low nutrient levels like the middle Rio Grande (Passell et al. 2005), we expect that low nutrient availability (N and/or P) in the upper reaches of the river system will limit primary production and biomass of benthic algae. Work on streams in the Gila watershed and Zuñi Mountains has shown that NO_3-N, rather than PO_4-P, was consistently a limiting factor for primary production (Grimm et al. 1981, Coleman and Dahm 1990). The Rio Grande watershed has predominantly volcanic soils, which tend to be phosphorus-rich (Triska et al. 2006), (Passell et al. 2005), we expect that low nutrient availability (N and/or P) in the upper reaches of the river system will limit primary production and biomass of benthic algae. Work on streams in the Gila watershed and Zuñi Mountains has shown that NO_3-N, rather than PO_4-P, was consistently a limiting factor for primary production (Grimm et al. 1981, Coleman and Dahm 1990). The Rio Grande watershed has predominantly volcanic soils, which tend to be phosphorus-rich (Triska et al. 2006) and may provide sufficient PO_4-P for algal production. Therefore, it is likely that in the middle Rio Grande, **nitrogen may be the limiting factor** for primary producers in areas upstream of the Albuquerque area wastewater treatment plants.

Nutrient and turbidity levels tend to be relatively higher downstream from Albuquerque compared to upstream, due to inputs from wastewater treatment facilities and from landscape runoff. Wastewater treatment effluent appears to be the major source of nutrient loading to the main river channel in the Rio Grande (Van Horn et al. 2006, Zeglin et al. 2006), so nutrients may not be limiting to algae directly downstream from the wastewater facilities. In contrast, it has been demonstrated the agricultural fields along the Rio Grande serve as sinks for total dissolved nitrogen; water in agricultural return channels has lower nitrogen levels than the water diverted from the Rio Grande to fields for irrigation (Oelsner et al. 2007). Therefore, sources of nutrients may be attributed to wastewater treatment effluent. Additionally, it is more likely that **light becomes limiting to primary producers with increases in turbidity** downstream from Albuquerque. Further downstream, nutrients may be absorbed by sediment and biota, causing a decline in water column nutrient levels (Newbold et al. 1982).

The research presented here is composed of a quarterly longitudinal survey, a nutrient enrichment experiment and transverse food resource surveys in the middle Rio Grande. The longitudinal survey provides informational about temporal and spatial shifts and relationships between nutrient levels and the biomass and diversity of algae. The nutrient enrichment experiment examined the effects of nitrogen and/or phosphorus availability on algal biomass and community composition. The experiment was conducted in combination with the exclusion of large-bodied algal grazers. Macroinvertebrates grazers potentially limit some biomass accumulation but nutrients generally play a stronger role in shaping periphyton communities. Finally, the transverse surveys provided high resolution information on patterns of food resource availability in the MRG.

This three-tiered study examined the following questions:

1. Is there a relationship between periphyton biomass and species composition and environmental parameters in any given reach in the middle Rio Grande?
2. Along a longitudinal reach, is there a relationship between periphyton biomass and species composition and environmental parameters in the middle Rio Grande?
3. Do seasonal patterns in environmental variables affect periphyton biomass and species composition?
4. Does increased nutrient availability affect algal biomass and change species composition?
5. Is grazing a strong factor in determining periphyton communities? How do factors of top-down and bottom-up control affect primary producers?

Background

Periphyton as a food resource

Diet of the Rio Grande silvery minnow: Research indicates that the RGSM has a primarily herbivorous diet composed of benthic algae. For example, larval and juvenile fish in the middle Rio Grande consume benthic algae as part of their diet during high flow periods in spring (Pease et al. 2006). However, the isotopic analysis presented in that study did not separate diatoms from other common algal divisions such as filamentous chlorophytes and cyanobacteria.

Further research on minnow diets verifies the consumption of periphyton as a major portion of nutrition. Gut content analysis of adult *Hybognathus amarus* shows a dominance of diatom frustules in the guts (Shirey 2004, Cowley et al. 2006). Green algae and several taxa of cyanobacteria, including *Merismopedia* and *Anabaena*, were also identified from guts in *H. amarus* (Shirey 2004). Gut content analysis is likely to show disproportionately higher percentages of diatoms compared to other algal taxa because diatoms have cell walls composed of biogenic silica, allowing the frustules to pass through guts intact. Other algal taxa have cell walls composed of less durable substances, such as cellulose, which are more easily digested and often unidentifiable in the gut (Gelwick and Matthews 2006).

Magaña (2007) also found that RGSM prefer diatoms in a series of food preference studies. However, results from food preference studies are often not verified with gut analysis, or skewed by failure to select representative food resources (i.e., cyanobacteria, chlorophytes) (Steinman 1996). The genus *Hybognathus* has pharyngeal teeth and pharyngeal taste buds, which may allow the fish to selectively filter diatoms (Hlohowskyj et al. 1989). It is likely that herbivorous fish do not selectively graze only diatoms, but may also get nutrition from other periphytic taxa (i.e., Shirey 2004). Diatoms are generally considered to be a superior food source with high lipid content, while cyanobacteria are less palatable (Steinman 1996). Additionally, shifts in diatom species composition can result in changes of overall lipid content (Sicko-Goad and Andresen 1991). Therefore, shifts in algal community composition can affect food quality and quantity for grazers.

Periphyton dynamics

Periphyton as environmental indicators: Diatoms and other algae often live within narrow environmental conditions, making them important environmental indicators in aquatic and terrestrial ecosystems (Lowe 1974). Conductivity, pH, dissolved oxygen, turbidity/light availability, salinity, flow, and microhabitat are all known to affect periphyton growth, production, and species composition (Van Dam et al. 1994, Potapova and Charles 2002, Pan et al. 2004, Potapova and Charles 2005). Rapid response time to environmental change (often in days) and ease of collection make diatoms robust environmental indicators of aquatic ecosystems, including rivers and streams (Stevenson and Pan 1999). For example, light limitation can shape species assemblages, with species-specific adaptation to low light levels (Greenwood and Rosemond 2005). Periphyton communities respond to environmental factors by changes in biomass, shifts in taxa at different taxonomic levels (division, genera, or species shifts), or changes in photosynthetic stress. Understanding the diversity and role of diatoms and other algae in aridland rivers is crucial to our understanding of how management in the middle Rio Grande watershed affects this riverine ecosystem.

Endemism in aridland algae: Algal taxonomic studies of arid lands in the southwestern U.S. are limited (Czarnecki and Blinn 1978, Czarnecki et al. 1981, Spaulding et al. 2002), but such studies document a flora containing both tolerant, cosmopolitan taxa and species that are regionally endemic to the southwestern U.S. Adaptation to new or variable habitats has been documented in many ecosystems (e.g., Kociolek and Spaulding 2000, Bixby et al. 2005a). Evolving to fit into a new environment would be especially important for periphyton in the middle Rio Grande, which is a flood-pulsed system with high salinity concentrations and high water temperature.

Factors influencing periphyton biomass and community composition

Nutrients in the Rio Grande: Nutrients are one of the main determinants affecting algal growth and species composition. Termed a “bottom-up” factor, nutrient levels often control periphyton biomass and species composition. Nutrients can be obtained by periphyton communities from the water column or can be available from sediment through absorption (Vadeboncoeur et al. 2006). Patchiness in algal communities is also closely linked with heterogeneous nutrient distributions in the water and sediment interfaces (Coleman and Dahm 1990). Often nutrients can be co-limiting. In temperate lakes that are usually P-limited, research has shown that the system may be co-limited by both N and P (Elsner et al.

1988). It is possible that this situation may also be true for the oligotrophic northern reaches of the Rio Grande.

The geomorphology of the middle Rio Grande changes from a more channelized river north of Albuquerque to a sandy bottom riverbed with a wide floodplain in the southern reaches of the study site. This change is reflected in the dominant sediment type in the river bottom. This geomorphology may affect how nutrients can be retained and cycled. Overall, it is unclear if rates and patterns of nutrient processing in the Rio Grande are similar to other large river systems in which low nutrient retention is found (Alexander et al. 2000).

Both irrigation return flows and wastewater treatment plant effluent are likely to affect nutrient loading in the middle Rio Grande. Wastewater treatment effluent in the middle Rio Grande consistently contributed the largest source of nitrogen loading to the river (Oelsner et al. 2007). In comparison, agricultural return flows has been shown to have much lower nitrogen concentrations than diversion water during both wet and dry years (Oelsner et al. 2007), indicating agricultural lands may act as a sink for nitrogen

Turbidity and light availability: In some reaches of the middle Rio Grande, primary production is most likely limited by light availability in the water column. Chlorophyll *a* measurements taken from the river thalweg show extremely low levels of algal biomass (chl *a* < 0.005 mg/L) (D. Van Horn and C. Dahm (UNM), unpublished data). Furthermore, there is no diurnal O_2 signal, supporting the hypothesis that photosynthesis is minimal in the water column (D. Van Horn and C. Dahm (UNM), unpublished data). Similar conditions exist in an aridland Australian river with high turbidity levels (Secchi depth 6-15 cm) (Bunn et al. 2003). In that study, the primary production was restricted to a "bathtub ring" along the shallow, littoral margins of the river where some light is available (Bunn et al. 2003). Turbidity data collected along the littoral zone of the middle Rio Grande show a similar type of ecosystem with high turbidity readings (~25-240 N.T.U.) (Eichhorst et al. 2006).

Interdependence of light and nutrients can change the predicted response of organisms. In short-term nutrient enrichment additions, heavy shading can negate the effects of elevated nutrient levels (Bernhardt and Likens 2004, Greenwood and Rosemond 2005). However, research has also demonstrated distinct periphyton community shifts between high and low nutrient streams in stream systems with low light (in this case, because of dense canopy cover) and naturally high levels of nutrients (Mosisch et al. 2001, Bixby et al. 2005b).

River morphology and seasonal changes: Seasonal differences in flow and changing hydroperiod cycles may influence growth and species composition of primary producers by altering substrate availability in the floodplain (see below) and increasing the effects of scouring (Biggs and Hickey 1994). In aridland streams and rivers, the temporal and spatial fluctuation in flow and stream channel can vary substantially (Stanley et al. 1987). Nutrient concentrations are diluted during high flow, which could result in decreased periphyton growth. In contrast, Pease et al. (2006) show that ephemerally flooded backwaters and channels with low flow are utilized by very high densities of larval and juvenile fish for nurseries and feeding. Isotope data confirm that epibenthic algae are temporally important as a food resource for grazers during high flow (Pease et al. 2006).

Substrate availability for periphyton: Microhabitats, such as sediment, sand and woody debris (e.g., tumbleweeds) provide different conditions for periphyton growth and species diversity; this is reflected in differences in chlorophyll *a* (Vadeboncoeur et al. 2006) and community structure (Stevenson and Hashim 1989, Potapova and Charles 2005). In one study, chlorophyll levels were 10× higher in sediments compared to on hard substrates, possibly because of nutrient sorption to the sediment (Vadeboncoeur et al. 2006). Lotic systems are especially patchy in terms of habitat quantity and quality (Pringle et al. 1988). For example, differences in light availability (related to riparian cover and water column turbidity (because of local sediment mixing) may play important roles in shaping algal communities. Finally, epipsammic diatoms tend to be smaller in size because shifting sand may crush their frustules. Generally, larger growth forms of diatoms and other algae characterize epipelic communities. This may imply that better grazer food resources may be available in epipelic habitats.

Top-down effects from grazers: Top-down factors such as grazing by fish and invertebrates can also alter periphyton productivity, growth and community composition (i.e., Steinman et al. 1987, Feminella and Hawkins 1995, Ranvestel et al. 2004). Generally, a reduction in grazers results in periphyton biomass increases and shifts in growth forms (to more upright taxa) (i.e., Steinman et al. 1991, Connelly et al. 2008)). Additionally, several studies indicate that top-down predation pressure from fish can alter abundances of invertebrate populations and algal standing crops in a three-tiered food web. For example, the presence of fathead minnows was associated with lower abundances of many invertebrate fauna in wetlands (Hanson et al. 2005). Rio Grande silvery minnows are likely to consume periphyton, as well as some aquatic invertebrates and detritus, so their presence may have significant effects on the local food web.

Methods

Longitudinal survey

Survey locations

Field research was conducted in the middle Rio Grande from the Angostura Diversion Dam downstream to Bosque del Apache, north of Elephant Butte Reservoir Figure 1). Five locations along the Rio Grande were monitored seasonally (four times per year). The locations were chosen based on access and location relative to wastewater treatment effluent discharge and irrigation return drains.

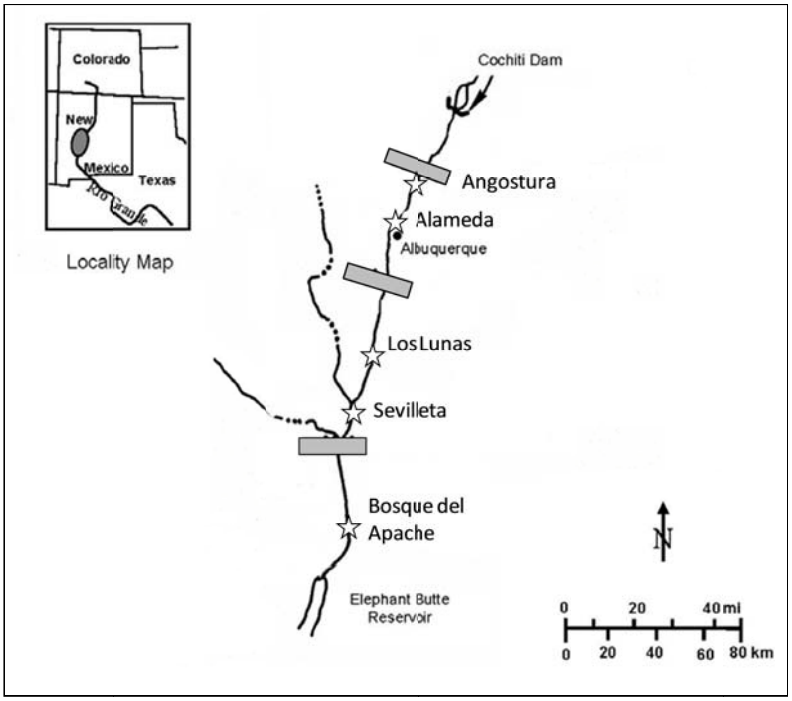


Figure 1. Locations for longitudinal sampling on the middle Rio Grande (indicated with stars). From upstream to downstream: Angostura, Alameda, Los Lunas, Sevilleta, and Bosque del Apache. Grey boxes indicate diversion structures.

1. Angostura directly below the diversion dam, River Mile 209.7 (middle Rio Grande Conservancy District). This location is upstream from Albuquerque area wastewater treatment effluent.

2. Alameda bridge crossing, New Mexico State Highway 528, River Mile 192.2 (City of Albuquerque) (downstream from the Rio Rancho wastewater treatment plant effluent).

3. Los Lunas, two miles above the Peralta wasteway input, River Mile 161.4 (middle Rio Grande Conservancy District). This location is downstream from the Los Lunas WWTP effluent, and is noted to have some of the highest nutrient loadings in the middle Rio Grande (Van Horn et al., 2006).

4. Sevilleta National Wildlife Refuge, Bernardo, River Mile 120.0 (U.S. Fish and Wildlife Service), south of the confluence with the San Francisco drain (from west, contains water from the Rio Puerco) and the La Joya drain (from east, continuous flows). Our site is at the confluence with the Rio Salado and downstream from several irrigation ditch returns.

5. Bosque del Apache National Wildlife Refuge, River Mile 79.1 (U.S. Fish and Wildlife Service). Downstream from San Acacia Dam (influences of inputs from the low flow conveyance channel).

Longitudinal Survey Methods

Physical and Chemical Parameters

Each location along the Rio Grande was sampled for physical, chemical and biological (i.e., algal) parameters on a quarterly basis. We were unable to sample all parameters at sites during some sampling periods (e.g., spring sampling periods) because of high water; in these situations, water and physical measurements were collected but no biological data was collected that required entering the river. At each location, three subsites (usually backwaters or pools) were selected in the river in low flow areas where algae could colonize on substrates. This multiple site sampling design accounted for variability in the river because of differences in shading, habitat, and mixing within the water column. These subsites changed from season to season depending on river flow and geomorphology. Both sides of the river were sampled (as required in the original RFP) in some cases but often it was logistically impossible to cross the river at certain locations and seasons.

Physical and chemical measurements were taken in backwaters and pools. Water depth and a brief habitat description were recorded at each site. Water temperature (°C), specific conductance (µS/cm), pH, dissolved oxygen (mg/L), and salinity (ppt) were measured using a multiparameter water quality meter (YSI Model 85D). Turbidity (NTU) (as a surrogate for light attenuation) was measured using a portable turbidity meter (La Motte 2020e). Velocity (m/s) was measured using a Marsh–McBirney Flo-Mate water velocity meter. Water samples were collected in replication ($n = 3$) from the water column from each of the three sites at each location, filtered in the field or in the lab at the University of New Mexico using a 47mm diameter Millipore membrane filter (0.45 µm pore size) and a Swinnex filter apparatus and syringe. These samples were later analyzed for anions. Unfiltered water samples were also collected for analysis of total nitrogen (TN), total phosphorus (TP), and ammonium (NH_4). Filtered and unfiltered water samples were frozen at the lab until analysis was conducted.

In addition to analyzing nutrient levels in the water column, sediment samples were collected at each subsite and analyzed for TN and TP, as an indication of nutrient availability for algal communities through sorption from sediment. These samples were collected as bulk samples in clean wide-mouth bottles and transported to the University of New Mexico and frozen until analysis.

Anions (nutrients) were analyzed from replicate filtered water samples at the University of New Mexico Biology Annex Analytical Laboratory. PO_4-P (µg/L), NO_3-N (µg/L), Cl^- (mg/L), Br^- (µg/L), and SO_4 (mg/L) were analyzed using a Dionex DX-100 Ion Chromatograph, using Chromeleon 6.60 software (AWWA 1998, USEPA 1997). NH_4-N (µg/L) was analyzed using a colorimetric spectrophotometric method (AWWA 1998, Technicon Industrial Systems, 1973).

Total P and TN were extracted from unfiltered water by oxidation with persulfate and boric acid (Stelzer and Lamberti 2001) and then analyzed using a Technicon AutoAnalyzer.

Total N from sediments was analyzed by combustion with a Thermoquest CE Instruments NC2100 Elemental Analyzer. Total P was extracted from sediments using combustion, followed by HCl addition. Samples were analyzed using a Technicon AutoAnalyzer. We have stopped doing TN and TP collection and analysis from sediment after 2008 because of the lack of correlation with algal variables.

Unmarked distilled water blanks and lab standards were included in all analyses for machine and sample calibration.

Dominant substrate types were also determined at each location at each sampling period. Using the soil analysis methods described in Day (1965), the sediment samples from each subsite at all five sites were suspended with a metaphosphate detergent. A colloid hydrometer was used to take measurements at two minutes and again at two hours to determine the percent composition of clay, silt, and sand of each sediment sample.

Periphyton Parameters

Benthic periphyton were quantitatively sampled from epipelic/epipsammic (sediment/sand) and epilithic (i.e., rock) habitats, depending on availability of substrate. Three replicate epipelic/epipsammic samples were collected from each subsite using a 0.5 cm core made from a modified 60 ml syringe. Epilithic samples were scrubbed from 2-3 rocks from each subsite (only at Angostura), and then surface area was calculated for each rock. Sampling of benthic periphyton from the surface of submerged tumbleweed was planned, but tumbleweeds occurred rarely and seasonally and did not represent an important substrate for periphyton.

Ash free dry mass (AFDM) was measured from a subsample from each replicate. Each AFDM replicate was oven-dried (60^oC, overnight), weighed, ashed (540^oC, 2hr) in a muffle furnace and then reweighed. AFDM was calculated as the difference between dried and ashed weights.

Chlorophyll *a* was analyzed as a second measure of algal biomass. Chlorophyll *a* was extracted from a subsample from each replicate core sample (~2g sediment), by immersing the sediment in ethanol (95%, 10mL) which was then heated (70^oC, 5min) (Sartory and Grobbelaar 1984). The supernatant was analyzed using a HP 8452A diode spectrophotometer. Chlorophyll *a* content was calculated from optical densities measured at 660nm and 750nm pre- and post-acidification (Sartory and Grobbelaar 1984).

An additional epipelic/epipsammic or epilithic sample was collected from each site and preserved in 10% formalin to be used for diatom identification. We have focused on diatom taxa which are the dominant taxa in the algal community and responded strongly to the environmental variables. To determine diatom richness (number of taxa) and species abundance (cells/mm^2), 2 mL aliquots from each sample were processed using a method developed for sediment samples (30% hydrogen peroxide and concentrated nitric acid) (Stoermer et al. 1995). These samples were then rinsed six times with distilled water to remove oxidation by-products. Processed samples were evaporated onto coverslips and mounted to microscope slides with Naphrax mounting medium, making permanent slides. Specimens along transects were examined under oil immersion at 1250× magnification using phase and brightfield optics on a Zeiss Universal Research microscope. 500 valves were enumerated from each sample. In samples with extremely low diatom densities, counting ceased after 10 transects. Diatoms were enumerated and identified to the species level. Identification of taxa was based on taxonomic literature including work from the southwestern U.S. (NAQWA data, NM region, D.F. Charles, personal comm., Czarnecki and Blinn 1978, Czarnecki et al. 1981). Digital images of each taxon were recorded and compiled in a database developed in Filemaker Pro 10 (Filemaker 2009) . Duplicate slides and periphyton subsamples will be accessioned in the newly formed algal collection at the Museum of Southwestern Biology (MSB).

Because only a subset of algae was processed and analyzed, duplicate periphyton samples have been processed from 5% of the samples collected (from the longitudinal survey) and processed and analyzed using standard methods. Quality control was monitored by calculating a percent community similarity index for proportional data from two duplicate diatom slides; the similarity index should be greater than 75% to be considered good replication. These duplicate slides have been assessed for variability related to microhabitats in the reach, sample preparation, and analytical variability (Stevenson and Bahls 1999).

Nutrient-diffusing substrate experiment

Nutrient diffusion substrates (NDS) were used in combination with electrical grazer exclusion to investigate bottom-up and top-down effects on periphyton biomass and species composition.

NDS experiment location

After two attempts to conduct this experiment (October 2007, July 2008), we conducted a moderately successful NDS experiment in July 2009 at the Sevilleta National Wildlife Refuge upstream from the confluence of the Rio Salado. River flows were moderate and the redesigned arrays (see below) allowed the experiment to accommodate changes in flow.

NDS construction

The nutrient-diffusing substrates were made from inverted terracotta saucers (4" dia.). Terracotta provides a suitable substrate with a textured surface for algal colonization. Four treatments were assigned to the saucers – control, N, P, and N+P. The saucers were filled with agar and a combination of KNO_3 (= NO_3-N), KH_2PO_4 (= PO_4-P), both NO_3-N and PO_4-P or neither (as a control) (modified from Tank and Dodds 2003, Pringle and Triska 2006, Richards et al. in review). Filled saucers were attached to a piece of Plexiglas which had holes drilled into each corner. Each saucer was randomly placed in a frame built from PVC pipe, with control treatments furthest upstream to avoid any nutrient contamination (Figure 2) although contamination from cross-over treatment is unlikely (Tank et al. 2006). The frame was then placed into the river and held in place with T-posts (Figure 3). A thin layer of sediment and sand was soon deposited on the saucers, mimicking an epipelic/epipsammic habitat. There were four replicate frames for the non-exclusion treatment. Four replicate samples of each nutrient treatment (control, N, P, N+P) were collected weekly and processed for algal parameters and invertebrates.

The design of the frame was modified for the grazer exclusion treatment. Invertebrates and fish were excluded from the saucers using an electrical field. Exposed electrical wires were distributed between the saucers on the PVC frame and then attached to a 12V solar-powered fence charger (Pringle and Blake 1994, Moulton et al. 2004). However, because of the high conductivity of the river, the solar fence chargers shorted out when the entire frame (16 saucers) was electrified. To compensate for the effect of high conductivity, the area electrified by each fence charger was reduced to two saucers. These two saucers were one control saucer (no nutrients) and one NDS (N+P), rather than including a control and three nutrient treatments (N, P, N+P). Because there were only two saucers, saucers from the exclusion were only collected on the final week of the experiment rather than every week during the experiment. Four replicate samples of each treatment (control, N+P) were collected from the non-grazer treatment.

In previous attempts to conduct this experiment, changing water levels deleteriously affected the NDS saucers. Significant declines in water depth left the saucers exposed and led to the algal communities drying out. Significant increases in water depth also affected algal production; generally high levels of turbidity inhibited light attenuation so saucers that were too deep under water also had poor algal production. The redesigned experiment allowed the PVC structure to float just below the waterline (Figure 3). Foam swim noodles were attached to the PVC arrays; the saucers remained submerged but close enough to the water surface for enough light for algal photosynthesis.

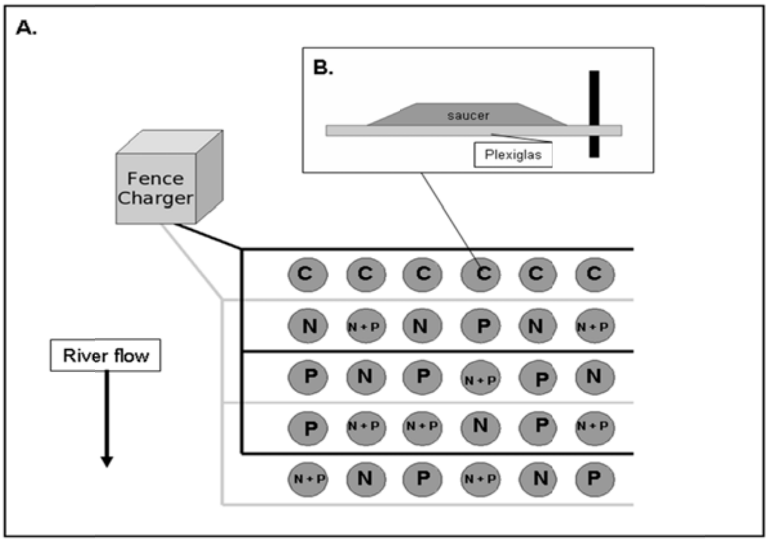


Figure 2. Schematic diagram of nutrient-diffusing substrate experiment (grazer exclusion component). A. Proposed experimental setup with electric grazer exclusions powered by fence charger; B. Enlarged view of nutrient-diffusing saucer. The actual design included only 16 saucers on the grazed arrays and two saucers in the non- grazed arrays.

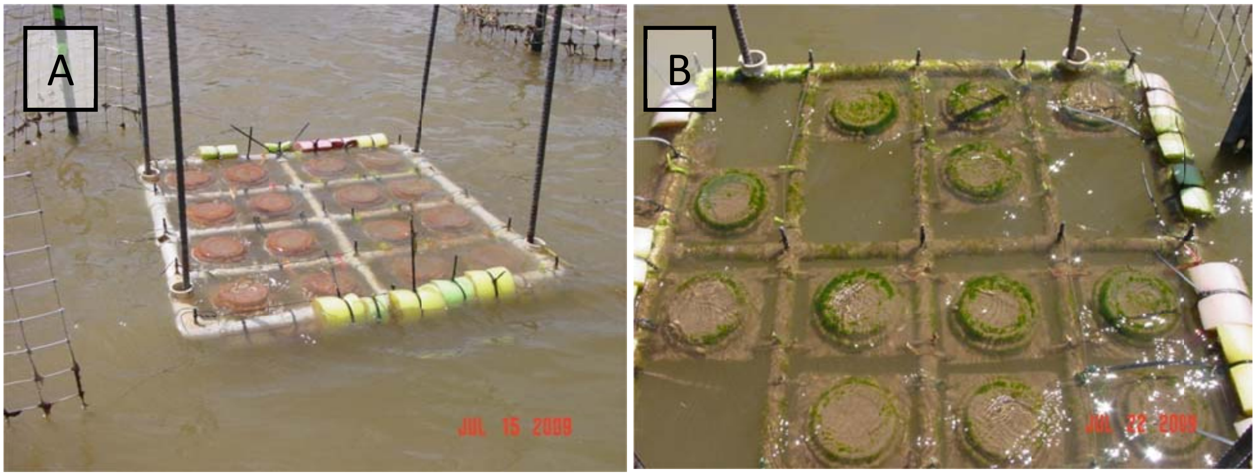


Figure 3. Nutrient-diffusing substrates in the Rio Grande near the Sevilleta National Wildlife Refuge. A. Sixteen saucers in an array without grazer exclusion, week 1. B. Array without grazer exclusion after samples were collected at week 2, demonstrating algal biomass.

NDS Sampling

For an outline of sampling techniques used each week, refer to Table 1.

Physical and chemical parameters were measured at the experimental location each week (pH, temperature, specific conductivity, dissolved oxygen, water depth, turbidity, and water velocity). Replicate water samples (n = 3) were also collected for ambient water chemistry in the river (PO_4-P, NO_3-N, NH_4-N, Cl^-, Br^-, and SO_4). Collection and analysis methods are described in the longitudinal monitoring section above.

Each week, four replicates of each treatment (control, N, P, N + P) of the non-grazed experiment were randomly selected and removed from the experiment. In the final week, saucers were also collected from the grazer experiment. In the field, before the saucers were scrubbed, invertebrates were hand-picked from the NDS and preserved in formalin. In the laboratory, invertebrate samples were enumerated and identified to the lowest possible taxonomic level (generally, microcrustacea were identified to order and insects were identified to family) (Smith 2001, Merritt et al. 2008). Voucher specimens of invertebrates will be accessioned in the Arthropod Division at the Museum of Southwestern Biology.

Each collected saucer was then scraped with a toothbrush and rinsed into a graduated cylinder using distilled water. A subsample was taken for AFDM and chlorophyll *a.* Samples for algal species identification were collected every week and preserved with 10% formalin. However, only samples from week 4 (the week of likely maximum colonization) are currently being analyzed. Collection and analysis methods are described in the longitudinal monitoring section above.

Table 1. Sampling schedule for nutrient-diffusing substrate experiment

sample week	physical/chemical	nutrients	chl a	algal species	invertebrates
5 July 2009, start experiment	X				
15 July 2009, Week 1	X	X	X	X[1]	X
22 July 2009, Week 2	X	X	X	X[1]	X
29 July 2009, Week 3	X	X	X	X[1]	X
5 August 2009,Week 4	X	X	X	X[1, 2]	X

[1] For accession at the Museum of Southwestern Biology

[2] For community analyses

Transverse survey

Transverse surveys were conducted at Los Lunas and Bosque del Apache in April, July and November 2010. However, no samples were collected at Bosque del Apache in July (river was dry) or November (discharge was too high). Data are only presented from the Los Lunas surveys.

For each survey, three transects were selected. Each transect was deliberately selected to include a shallow zone (at the edge of the river) and a deeper zone, usually with higher flow velocities in the deeper zone. Physical and chemical parameters were measured and recorded downstream from the three transects before sample collection began, using the same protocols as for the longitudinal survey.

Samples were collected across each transect from the shallow, inundated edge (depth = 0cm) to the main channel (maximum depth = 80 cm). At depths less than 30cm, samples were collected for every 5cm change in water depth or at 2m intervals along the transect (whichever occurred first). At depths greater than 30cm, samples were collected every 2m, regardless of changes in depth.

At each sample point in the transect, three physical variables were recorded: distance from the river edge (m), depth (cm) and flow velocity (m/s). Core samples were collected for chlorophyll *a* (3 cores) and diatom species identification (1 core). Sampling protocols were identical to those used in the longitudinal survey.

Invertebrate samples were also collected from each sample point. Epibenthic core samples were collected using a small PVC tube (7.5 cm diameter, 30 cm length) with a removable cap. A flange was attached 1cm from the base of the core to limit sample collection to the top 1cm of sediment. The epibenthic core was pressed into the sediment, then a spatula was slid underneath and the cap was closed. The core was lifted out of the river, emptied into a Whirl-Pak™ bag and preserved (10% formalin).

In the laboratory, samples for chlorophyll *a* and diatom genus identifications were prepared and analyzed using the same protocols as in the longitudinal survey. Three samples from each transect were identified for diatom genera identification; samples examined were determined by samples with the three highest concentrations of chlorophyll *a*. Usually, this was the three samples closest to the edge; the remaining samples had negligible concentrations of chlorophyll *a*.

Invertebrate samples were first washed through a fine-mesh sieve (47μm) to remove any fine silt, and then sorted under a dissecting microscope (magnification = 75X). Non-insect taxa were sorted to order (Smith 2001) and insect taxa were sorted to family (Merritt et al. 2008). Generally, insects were very small, early instars and it was not possible to identify individuals to genus.

Light extinction

Light intensity was quantified using a Licor LI 250A light meter. On five occasions during the longitudinal and transverse surveys, light readings (μmol Quanta s^{-1} m^{-2}) were taken at the water surface and then at 5cm intervals to measure decreasing light penetration with depth. Light extinction measurements were taken on several days to enable light extinction calculations at different turbidity levels.

Light intensity is negatively related to depth, as described by the Beer-Lambert Equation (Hauer and Hill 2006):

$I_z = I_0 e^{-kz}$ (Equation 1)

where I_z is the light intensity at depth z below the surface, I_0 is the surface light intensity and k is the vertical attenuation coefficient (rate of attenuation of downwelling light).

Vertical attenuation was calculated from light intensity data using a logarithmic transformation of equation (1):

$k_d = (\ln I_0 - \ln I_z)/z$ (Equation 2)

The relationship between vertical attenuation and turbidity was then examined using linear regression:

$k_d = a \times \text{turbidity} + b$ (Equation 3)

The turbidity data collected in earlier transverse surveys was then used to estimate k_d for each survey (April and July).

Photosynthesis occurs in the euphotic zone, above the euphotic depth (z_{eu}). The euphotic depth occurs where light intensity is reduced to 1% of that occurring at the surface. Z_{eu} was calculated for data from both calculated and estimated vertical attenuation coefficients:

$z_{eu} = \ln 100/k$ (Equation 4)

Statistical Analyses

SPSS (SPSS for Windows Release 16.0. SPSS Inc 2007) was used for all univariate statistical analyses. Data was transformed as necessary to meet assumptions of normality when using parametric statistical tests. Generalized linear models were used to test for differences among locations, years, and seasons within years. Wald χ-squared tests were used to test the models for each parameter. Additionally, linear regression was used to examine relationships among %TN, %TP and chlorophyll *a* in the longitudinal survey. Principal components analysis was performed using physiochemical data to build uncorrelated variables (principal components) that were then used in a linear regression to examine the relationship between the concentration of chlorophyll a and physiochemical variables. Data for chlorophyll a were first log-transformed to satisfy assumptions of normality and homogeneity of variance.

Data from the NDS experiment were tested for significant differences among sample week and nutrient treatment, or nutrient treatment and grazer exclusion; and data from the transverse survey were tested for significant differences among subsamples nested within survey months (treating transects as replicates). Linear regression was used to examine relationships among physical parameters in the transverse survey.

A Multidimensional Scaling ordination was utilized with the diatom community data to examine patterns based on spatial and temporal parameters. In addition, biological-environmental stepwise (BEST) analysis was conducted using all of the diatom data and eight environmental factors including physical parameters as well as nutrient measures. BEST uses rank correlations to find matches between two sets of multivariate data (in this case, diatom assemblage data and environmental data) and lists the variables that 'best' explain the data (Clarke and Gorley 2006). These analyses were completed using PRIMER software (PRIMER 6 for Windows, Primer-E Ltd 2006) (Clarke 1993).

Results

Longitudinal survey

General water quality

Quarterly surveys have been conducted thirteen times at five sampling locations. Sampling has been restricted during a number of sampling periods because of high water (e.g., spring sampling). Surveys were conducted in August 2007 but extremely high water levels and technical issues during method development resulted in an incomplete data set for that period. Most water quality parameters differed significantly among either season, location, or both (Table 2).

Turbidity was highly variable throughout the survey period, differing from less than 10 NTU (minimum = 3.55 NTU at Angostura in winter 2010) to over 4000 NTU at a number of sites during the summer (Figure 4). Turbidity was significantly different among sites ($P<0.001$) and seasons ($P < 0.001$) (Table 2) and was generally low at Angostura, and to some degree, Alameda, throughout the year compared to the downstream locations. Turbidity was generally highest in the summer months following monsoon rains which increased tributary inputs throughout the middle Rio Grande. Additionally, the variation in turbidity was extremely high at a number of sites (e.g. Sevilleta in summer 2007, 2008 and 2010), depending on the locations sampled at the site (pools, channel margin).

Water temperature differed significantly among seasons ($P < 0.001$) and among locations ($P < 0.001$) (Figure 4). Generally, water temperatures were lower at Angostura, which is immediately downstream from the cold hypolimnetic water releases from Cochiti reservoir. Temperatures were slightly higher at Los Lunas, Sevilleta and Bosque del Apache where the river is generally more shallow than at Angostura.

Flow velocity measured at individual sites was not significantly different among seasons or sites. Low-flow habitats with potentially higher algal biomass were specifically selected for surveys so this result does not necessarily reflect the flow in the main channel. In general, flow was relatively high in spring each year (Figure 4). The flows at individual sites remain relatively consistent at a given time of year throughout the river.

Dissolved oxygen (DO) levels were generally similar throughout seasons and locations. Generally, DO levels ranged from 3-15 mg/L (Figure 4) but there were no significant differences among seasons or locations. By contrast, pH did differ significantly among years ($P = 0.021$) and among seasons nested within year ($P < 0.001$).

Levels of salinity did not differ significantly whereas specific conductivity did ($P < 0.001$). In general, locations further upstream (Angostura, Alameda) had relatively low levels of specific conductivity compared to locations further downstream (Sevilleta, Bosque del Apache), and specific conductivity was generally lowest in spring.

Of all of the nutrients, only four differed significantly among locations (NO_3-N, chloride, sulfate, and ammonium) and one differed significantly among years (ammonium) (Table 2). Concentrations of these nutrients increase longitudinally – levels are relatively low at upstream locations (Angostura, Alameda, and Los Lunas) compared to downstream locations (Sevilleta, Bosque del Apache). The significant differences in ammonium concentrations seem to be driven by high levels of N-NH_4 (> 150 µm/L) at Bosque del Apache in spring 2009. Notably, there is a trend for NO_3-N and PO_4-P levels to increase in the cooler season and then decline in the warmer seasons at the southern locations (Los Lunas, Sevilleta, Bosque del Apache), but not at the northern locations (Angostura, Alameda) where NO_3 –N concentrations were always low (Figure 5). The trend is not as clear for the other anions (bromide, chloride, sulfate), although there are significant differences among seasons and locations.

No significant differences were detected for seasonal trends in the TN and TP concentrations in the water column (Table 2; Figure 6), although TN differed annually. Generally, TN concentrations varied throughout the year. TN was usually relatively lower at the downstream locations (Sevilleta, Bosque del Apache) compared to the upstream locations, although TN concentration at Angostura was very variable among seasons.

%TP and %TN measured from sediment samples collected in 2007-2008 were variable among seasons and locations but did not differ significantly (Table 2). Upstream locations (Angostura, Alameda) had relatively low levels of %TN compared to downstream locations. By comparison, Angostura generally had high levels of sediment %TP whereas Alameda had relatively low levels. The downstream locations (Sevilleta, Bosque del Apache) had intermediate levels of sediment %TP compared to the upstream locations. There was no strong relationship between chlorophyll *a* and %TN and %TP concentrations in the sediment (Figure 7). The relationship was not significant for %TN ($r = 0.146$, $P = 0.435$) or %TP (r =

0.151, P = 0.418). In the end, TP and TN from sediments were deemed biologically unimportant in this system and we have stopped analyzing sediment TN and TP after 2008 collections.

The sediment characterization shows location and seasonal differences in sediment type (Figure 8). In general, the northern locations (Angostura and Alameda) have substrates that are composed of more sand, compared to silt and clay. More silts and clays were collected in the winter months at these sites. At the middle locations (Los Lunas and Sevilleta), the percentages of sands are also high with little seasonality. At the southern location (Bosque del Apache), silts and clays are dominant during most sampling periods with a number of samples collected in the summer with a greater percentage of sand.

Chlorophyll *a* differed significantly among years and seasons (Figure 9, Table 2). Generally, chlorophyll *a* biomass was lower in summer months than in any other season. Highest concentrations of chlorophyll *a* were found at Angostura (particularly in Winter 2008 and 2009) and lowest further downstream at the Sevilleta and at Bosque del Apache, but there was very high variability. Ash-free dry mass also differed significantly among seasons and locations. Trends were difficult to detect – AFDM is heavily influenced by recent floods and flow conditions, which also differs among seasons and among years.

Table 2. Summary of results from generalized linear models of all water quality parameters, testing for differences among seasons and locations. Significant results (P<0.05) are shown in bold.

	Location			Year			Season(Year)			location * Season(Year)		
	Wald χ-Square	df	P	Wald χ-Square	df	P	Wald χ-Square	df	P	Wald χ-Square	df	P
depth (cm)	1.6	4	0.816	1.2	3	0.747	3.9	9	0.917	6.4	44	1.000
turbidity (NTU)	18.1	4	**0.001**	4.2	3	0.239	25.2	9	**0.003**	29.9	45	0.959
temperature (°C)	398.7	4	**<0.001**	547.1	3	**<0.001**	8172.6	9	**<0.001**	1038.7	48	**<0.001**
pH	1.0	4	0.915	9.8	3	**0.021**	35.9	9	**<0.001**	16.4	48	1.000
salinity (ppt)	0.7	4	0.952	0.1	3	0.986	0.5	9	1.000	1.5	48	1.000
specific conductivity (µS/cm)	439.3	4	**<0.001**	96.5	3	**<0.001**	587.7	9	**<0.001**	1335.1	48	**<0.001**
flow (m/s)	0.1	4	0.998	0.1	3	0.990	1.0	9	1.000	2.1	47	1.000
DO (mg/L)	0.0	4	1.000	1.0	3	0.812	2.4	9	0.984	1.0	44	1.000
NO$_3$-N (µg/L)	11.1	4	**0.026**	0.9	3	0.830	5.5	9	0.787	11.9	47	1.000
PO$_4$-P (µg/L)	0.9	4	0.928	0.1	3	0.995	0.3	9	1.000	0.9	47	1.000
bromide (µg/L)	0.3	4	0.990	0.5	3	0.923	0.5	9	1.000	0.5	47	1.000
chloride (mg/L)	23.7	4	**<0.001**	3.6	3	0.303	8.5	9	0.485	3.7	47	1.000
sulfate (mg/L)	9.5	4	**0.049**	3.4	3	0.327	3.1	9	0.961	4.5	47	1.000
ammonium (µg/L)	15.7	6	**0.016**	30.5	3	**<0.001**	6.3	6	0.393	51.0	43	0.188
water TN (mg/L)	4.0	4	0.405	0.8	2	0.669	23.8	6	**0.001**	20.8	27	0.794
water TP (mg/L)	0.7	4	0.952	0.1	2	0.975	1.1	6	0.983	4.7	27	1.000
sediment TN (%)	0.2	4	0.995	0.0	1	0.905	0.0	2	0.977	0.3	12	1.000
sediment TP (%)	0.0	4	1.000	0.0	1	0.996	0.0	2	1.000	0.0	12	1.000
chlorophyll *a* (mg/m^2)	8.1	6	0.231	11.8	3	**0.008**	26.2	8	**0.001**	44.5	44	0.449
AFDM (g/m^2)	847676.4	6	**<0.001**	95700.4	3	**<0.001**	492327.2	8	**<0.001**	1521822.0	44	**<0.001**

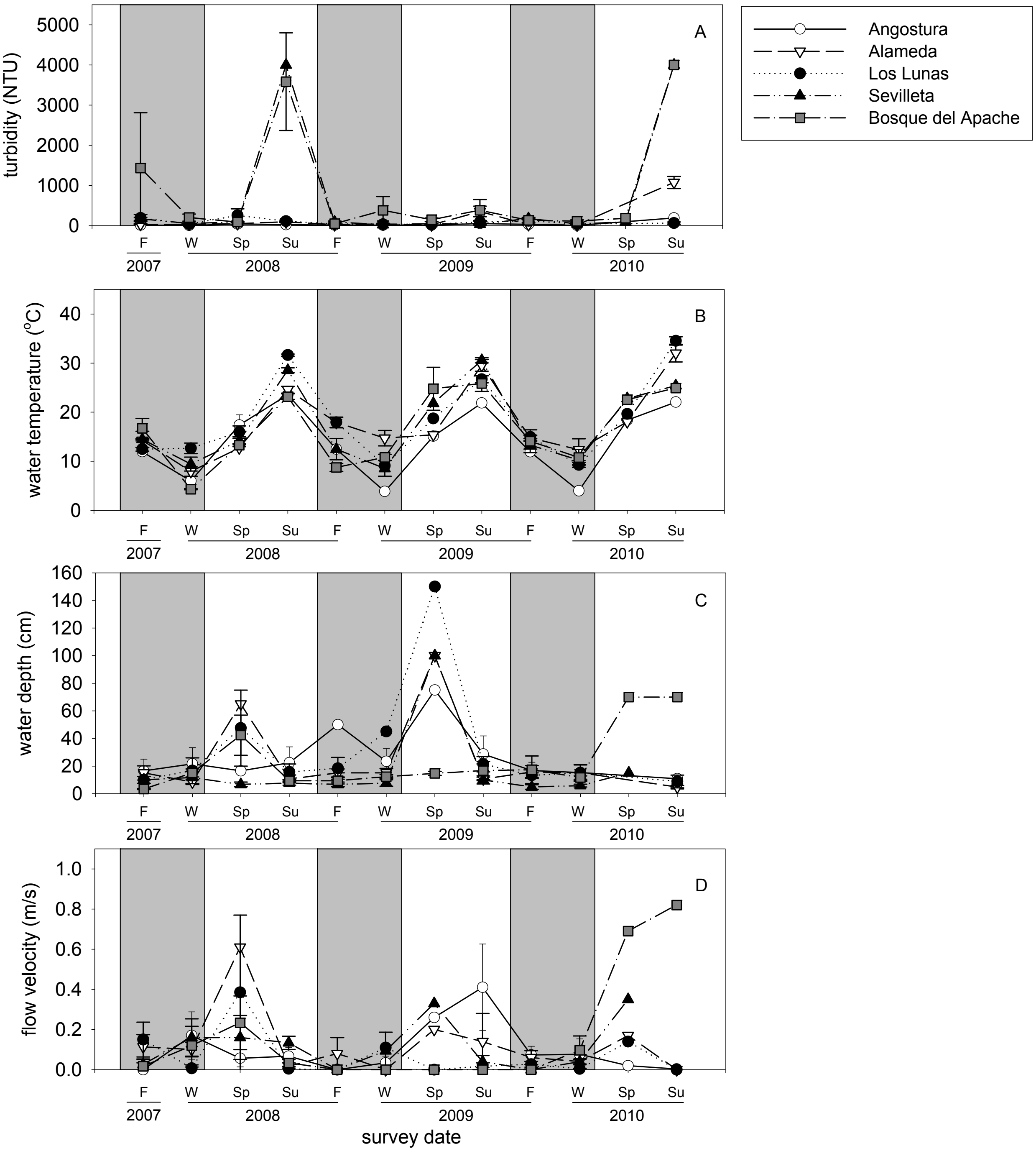


Figure 4. Mean (± SE) data for water quality variables measured at each of the five sampling sites on twelve sampling periods. Note log scale for turbidity. Grey bars indicate fall and winter samples.

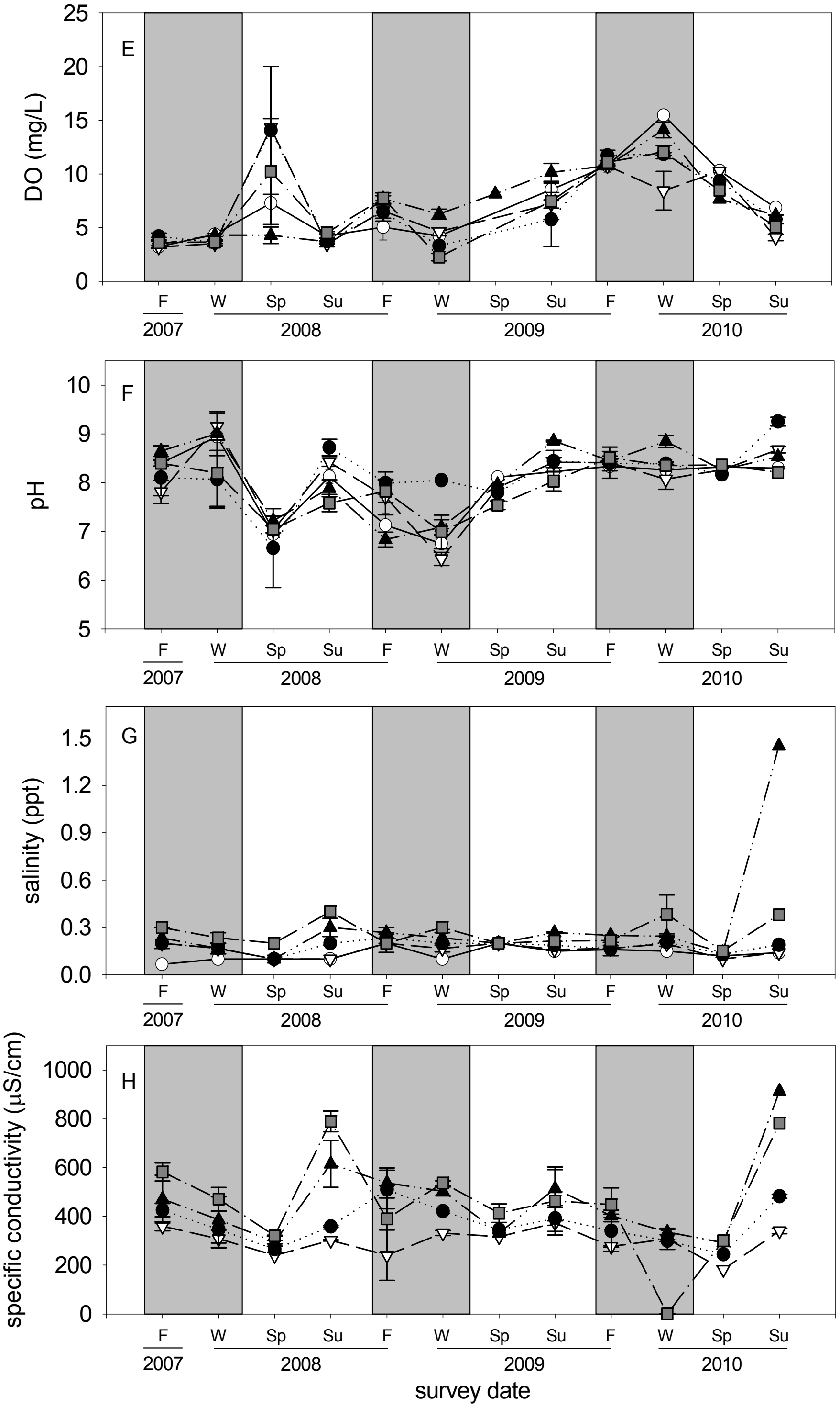


Figure 4. continued

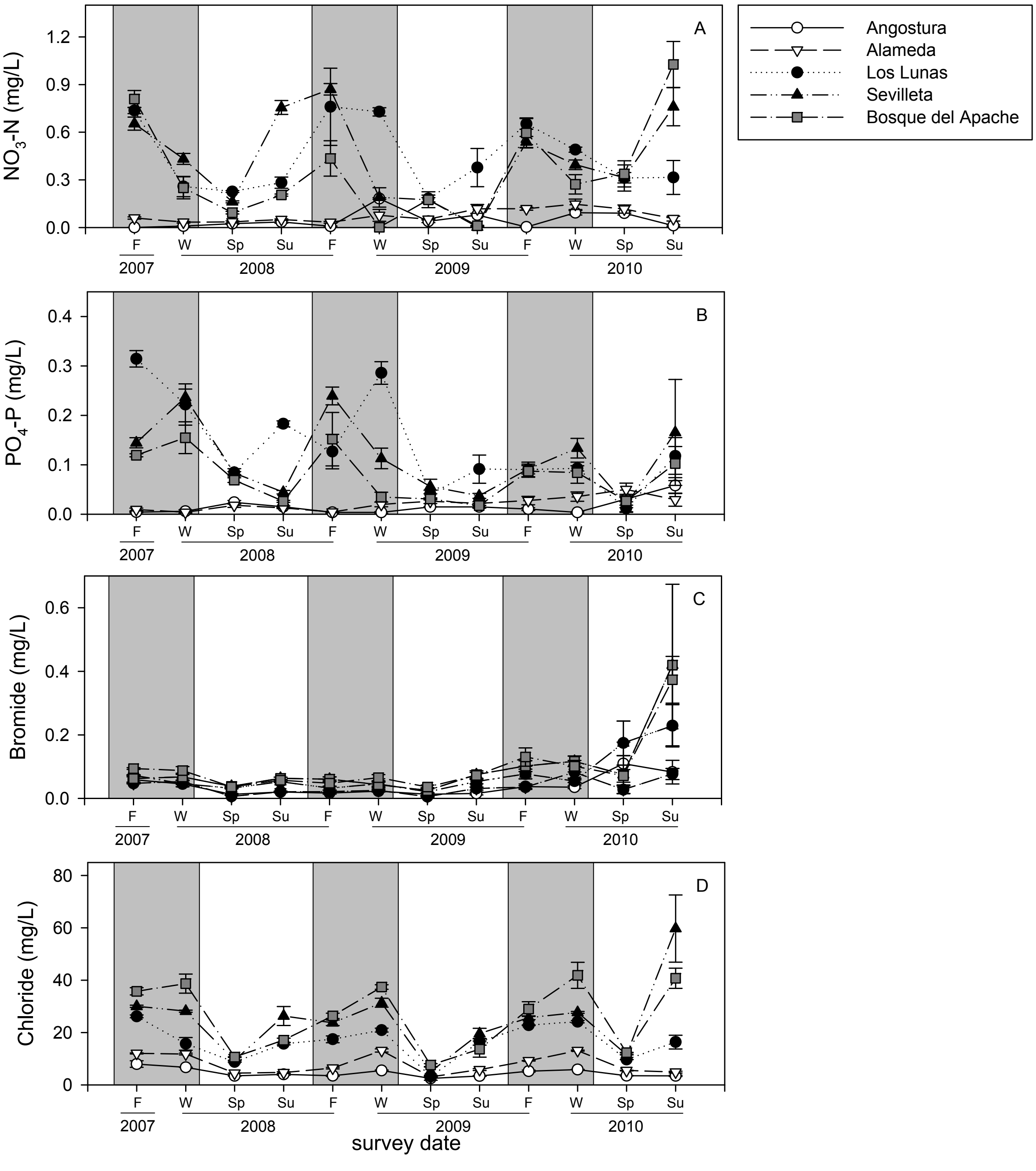


Figure 5. Mean (± SE) data for nutrient parameters analyzed from water samples at each of the five sampling sites on twelve sampling periods. Grey bars indicate fall and winter samples.

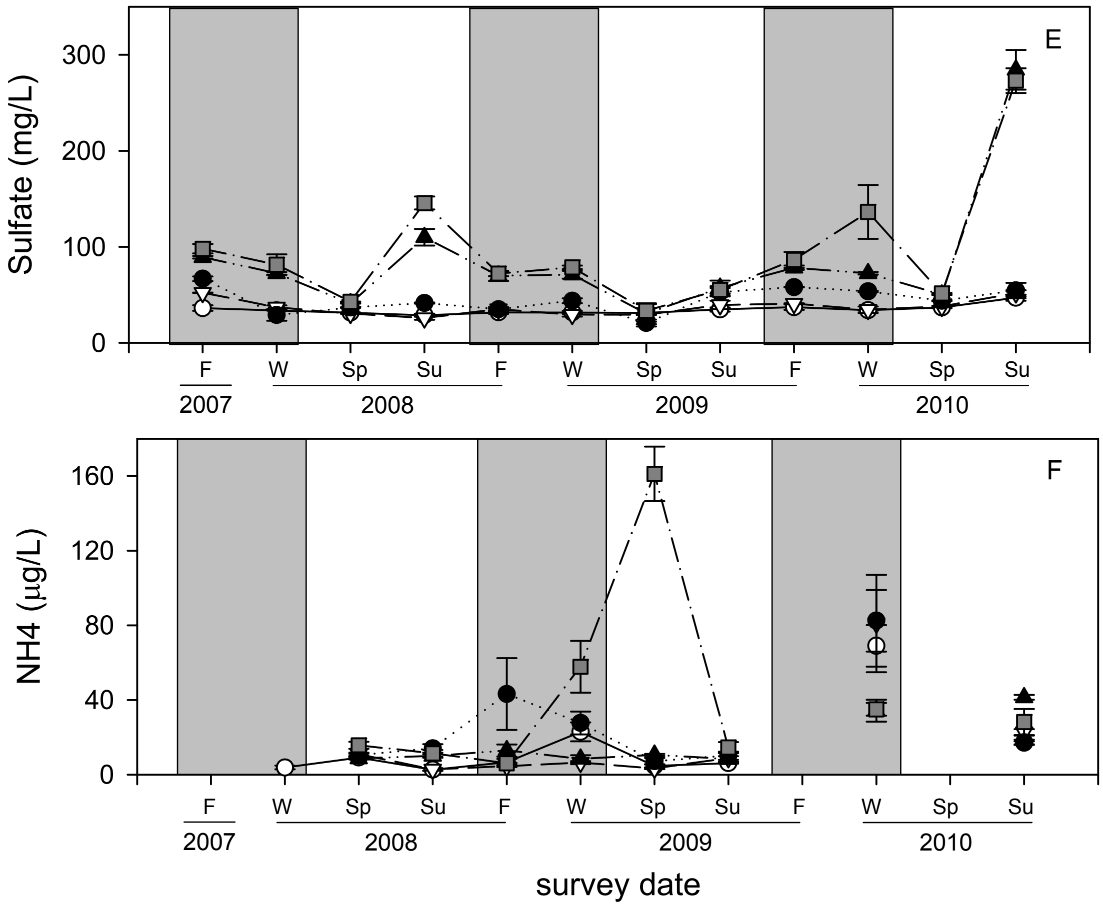


Figure 5 continued

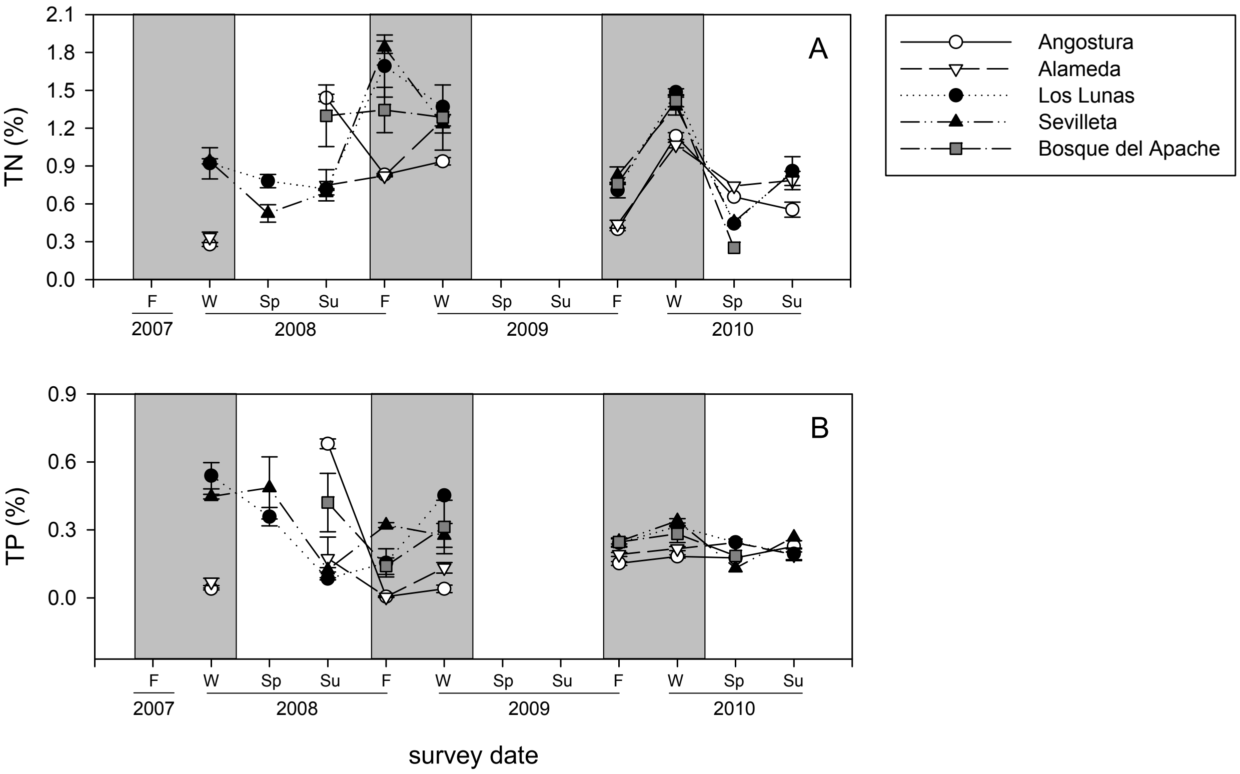
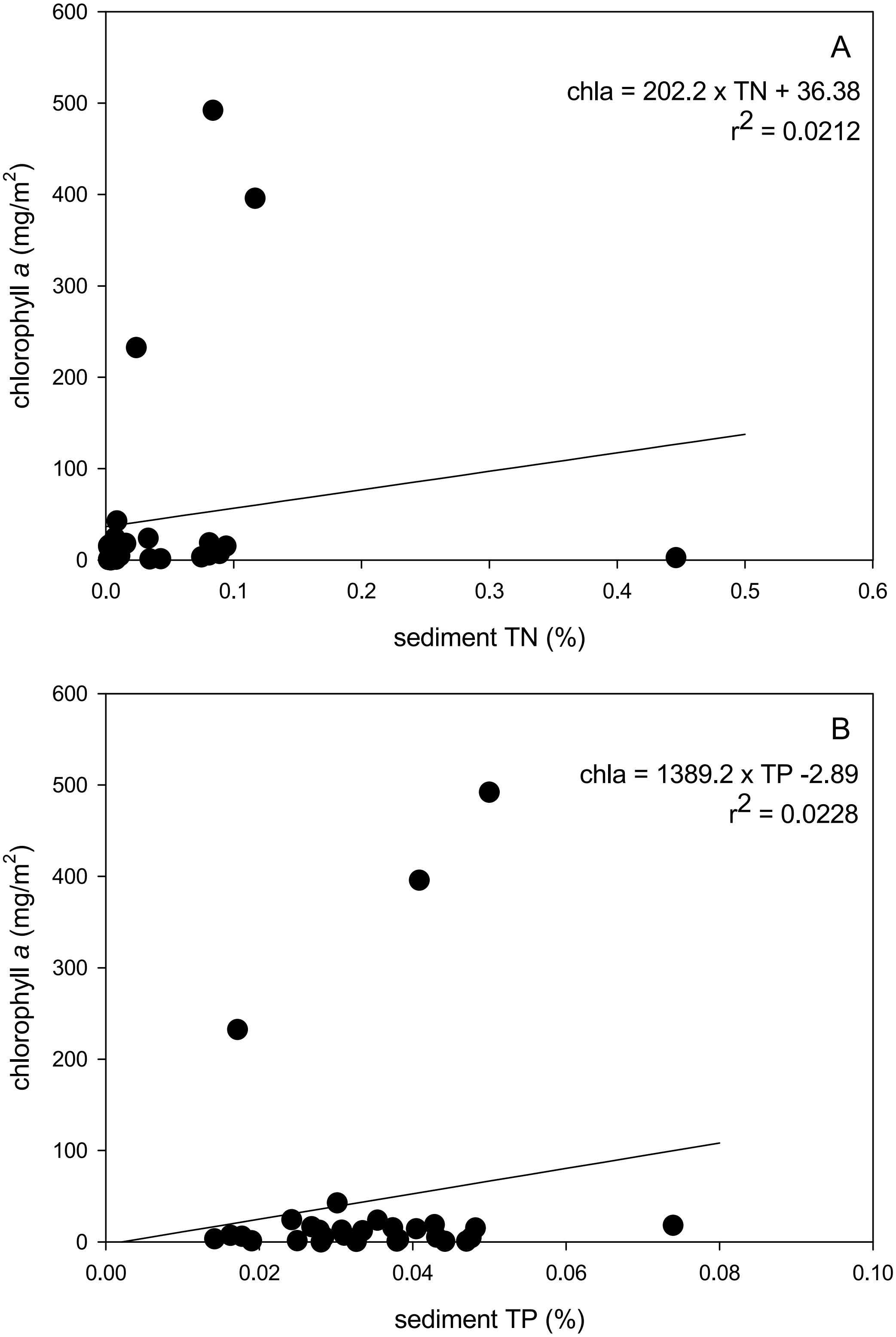


Figure 6. Mean (± SE) data for TN and TP measured from water samples at each of the five sampling sites on eleven sampling periods. Fall 2007 samples were not collected. Samples from spring and summer 2009 are missing. Grey bars indicate fall and winter samples.



2007 too

Figure 7. Relationship between chlorophyll *a* concentration and (A) TN and (B) TP measured from sediment samples, from surveys taken in 2008 across all locations and seasons.

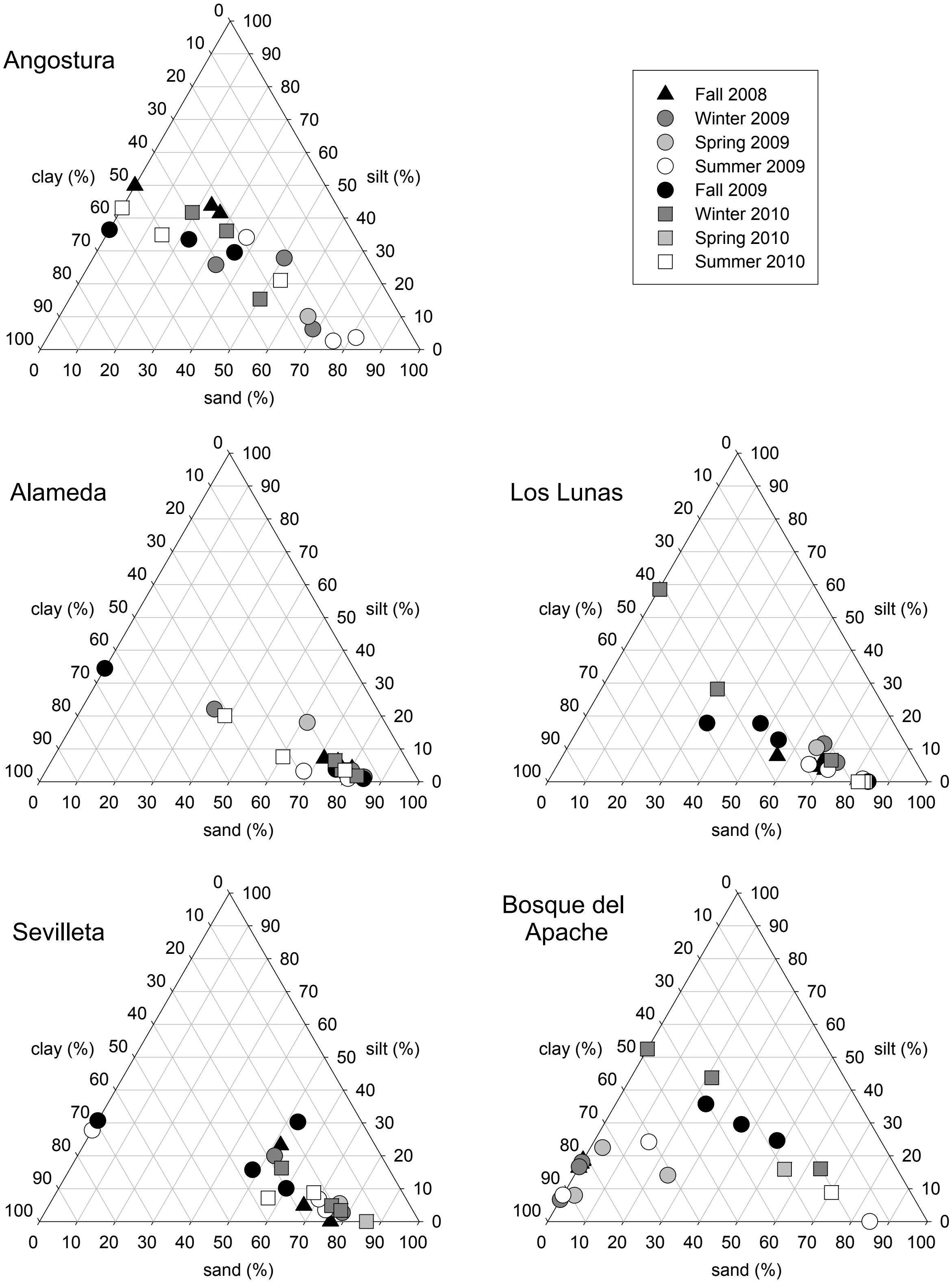


Figure 8. Sediment composition as percentages of sand, silt, and clays at eight sampling periods at five locations.

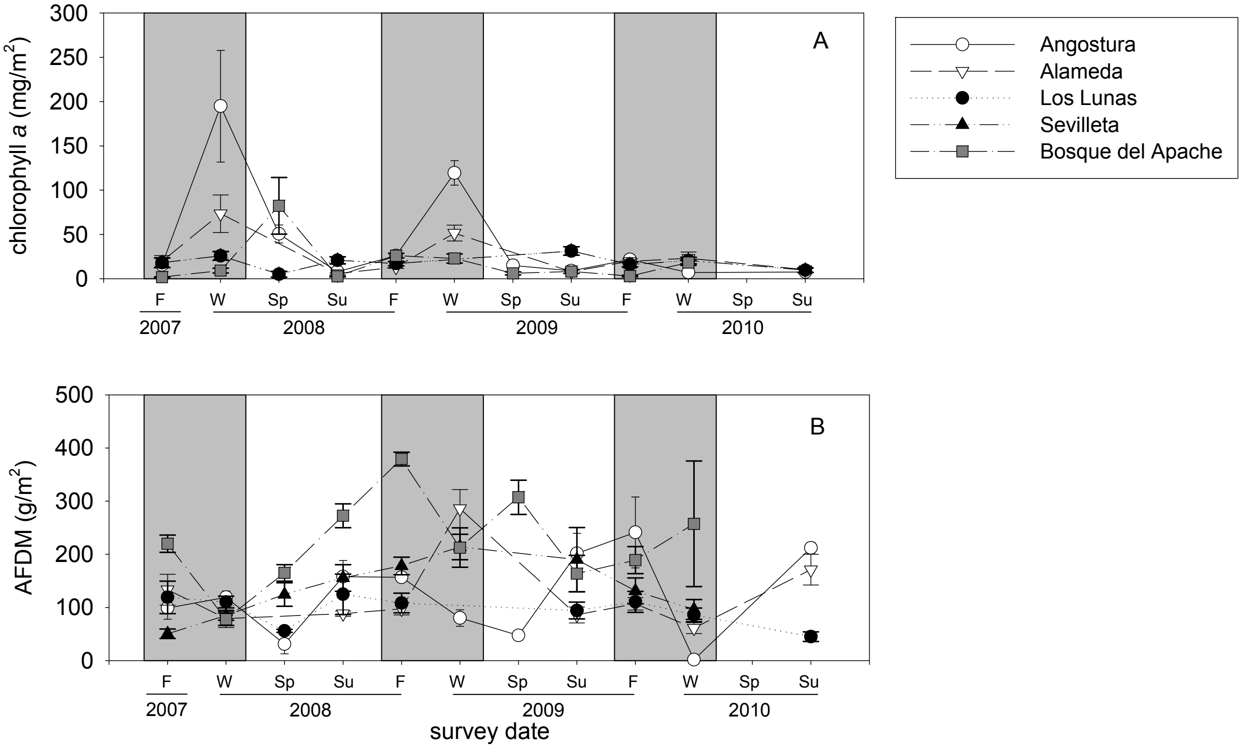


Figure 9. Mean (± SE) data for chlorophyll *a* and AFDM measured from benthic samples at each of the five sampling sites on twelve sampling periods. Grey bars indicate fall and winter samples.

The relationship between physiochemical variables and the concentration of chlorophyll *a* was investigated using principal components analysis and linear regression (Figure 10). The first two principal components explained 31.7% and 17.2% of variance. Principal component 1 (PC1) was associated with differences among locations and described a general gradient of nutrient concentration, from low concentrations at the northern sites to high concentrations at the southern sites (Figure 10A). PC2 was associated with differences among seasons (Figure 10B). For example, higher DO concentrations generally occurred in winter, when temperatures were lowest.

The relationship between chlorophyll *a* concentration and physiochemical variables is complex, but significant ($F_{4, 76} = 11.130$, $P < 0.001$):

$$\text{Log}_{10}\,[\text{chlorophyll } a] = -0.199(\text{PC1}) + 0.265(\text{PC2}) - 0.249\,(\text{PC3}) - 0.407\,(\text{PC4})$$

This indicates that highest concentrations of chlorophyll *a* generally occurred at the northern sites, where turbidity is relatively low, and generally occurred in winter and spring when there were relatively high concentrations of DO and nutrients (e.g. NH_4, TP and TN).

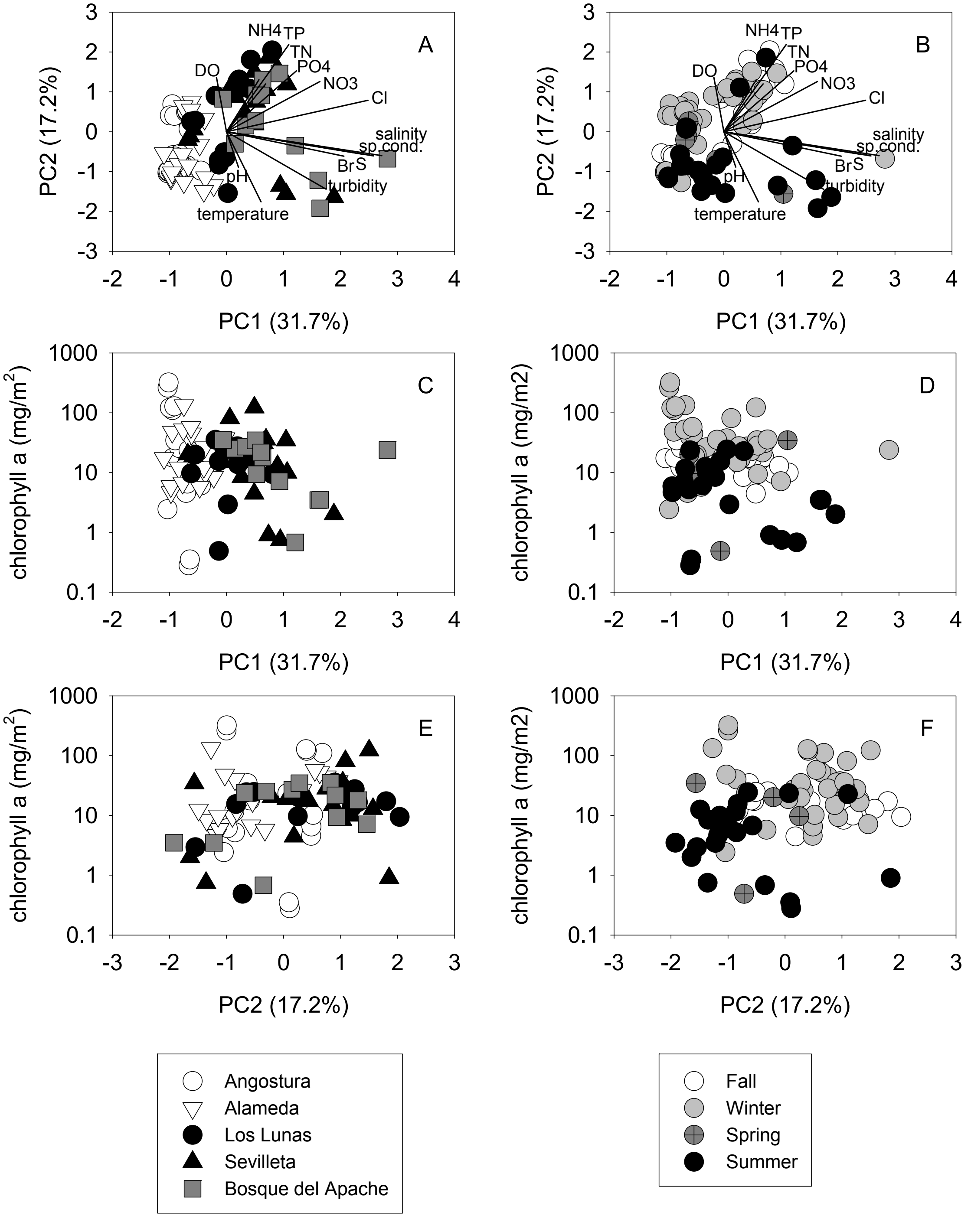


Figure 10. (A, B) Principal components analysis of physiochemical variables collected during longitudinal surveys 2007-2010. Loadings of individual variables are indicated by vectors. (C-F) Relationships between the first two principal components and the concentration of chlorophyll *a*. Plots on the left are identical to those on the right, but symbols represent location (left) or season (right).

Diatom community structure

To date, 297 diatom taxa have been recorded. This number is considerably higher than other published taxa lists from the middle Rio Grande (86 taxa, Magaña 2007) . This is likely related to extensive temporal sampling and a longitudinal gradient of biological chemical and physical parameters that create a heterogeneous environment. Species richness ranged from 36-82 taxa recorded at any given site (average species richness: Angostura 64; Alameda 69; Los Lunas 69; Sevilleta 69; Bosque del Apache 57). This taxa list included a number of diatoms recorded from the Rio Grande that may be undescribed and new to science. These new taxa are noted by "cf." if the specimen resembled a known taxon (i.e., *Navicula* cf. *cryptotenella*) or as numbered species (e.g., "sp. 1") if the specimen was completely unknown (Appendix 2). Species accumulation curves show that our seasonal sampling effort continues to sample new taxa (Figure 11). As sampling effort increases, gradually more and more of the taxa at a site will be enumerated, until only the rarest species remain unrecorded, increased effort will not increase the number of taxa recorded and the species accumulation curve will have reached an asymptote. Clearly, this curve has not yet reached an asymptote implying that we have not recorded all of the taxa at our sites yet. This species accumulation curve also highlights the high diversity of algal taxa in this arid system which has required repeated sampling to record all taxa in the system.

Diatom taxa can be divided into two groups: those that are characteristic of either the upstream sites (Angostura and Alameda) or downstream sites (Los Lunas, Sevilleta, and Bosque del Apache). This analysis showed that alkaliphilous *Epithemia sorex* and *Amphora pediculus* and cosmopolitan taxa *Cocconeis placentula*, *Planothidium lanceolatum*, and *Cocconeis pediculus* were most indicative of environmental conditions of the upper reaches of the middle Rio Grande, as well as being the most common taxa throughout the upper reaches of the river as well. In the southern sites, the dominant taxa were *Navicula* cf. *symmetrica, Nitzschia palea, Nitschia perminuta, Surirella minuta, and Surirella angusta*, taxa more characteristic of higher nutrients and silt substrates.

A Multidimensional Scaling (MDS) ordination indicates that the diatom community was highly variable among sites (Figure 12). Results indicate that there were no significant differences in diatom community structure that could be attributed to spatial and temporal patterns (ANOSIM: location, R = -0.028, P = 0.544; season, R = -0.078, P = 0.658). Small, negative values for the R-statistic indicate that there was a high level of variability among samples. However, BEST analysis did detect a correlation between diatom community structure and some physiochemical variables, ignoring the effect of site or season (Table 3). The strongest correlation was with nitrate, turbidity, and pH. However, this outcome must be interpreted cautiously as the R values were not high, implying that there were other factors that contributed to the diatom community variation.

The quality control measures taken in the analysis show that the percent similarity community index to be 80.5% among duplicate slides counted.

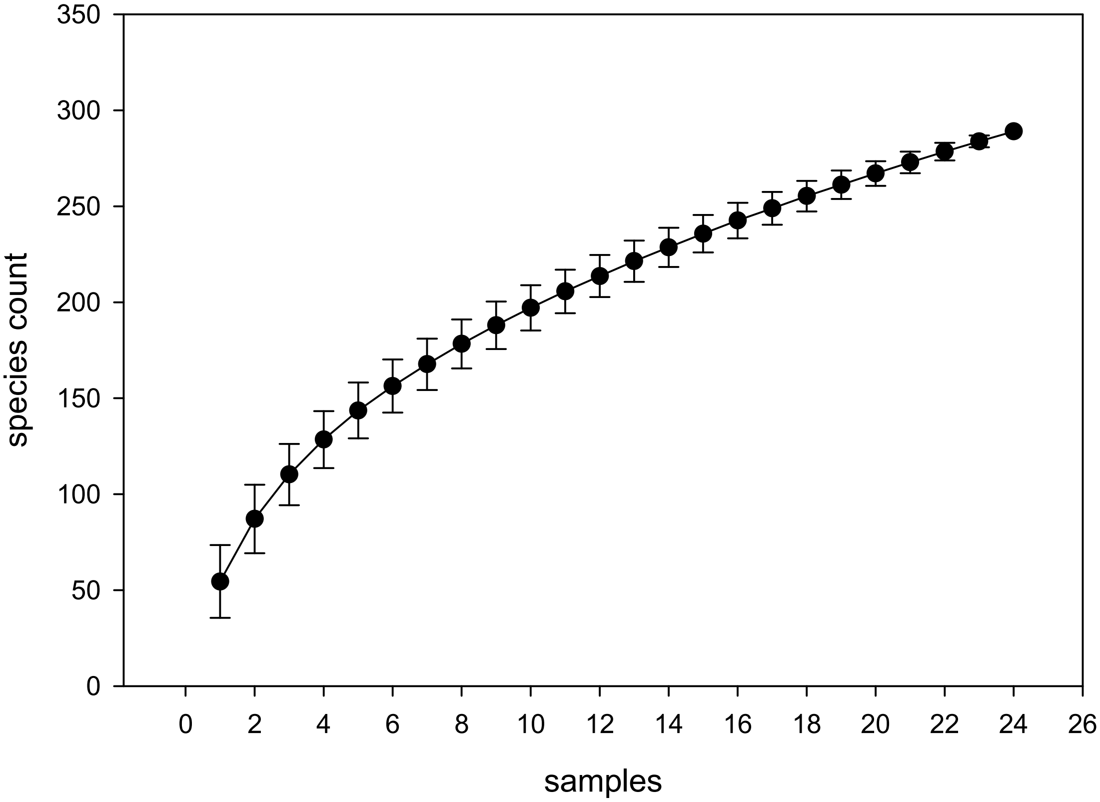


Figure 11. Species accumulation curve (number of samples versus species count) modeled as a measure of sampling effort and species richness.

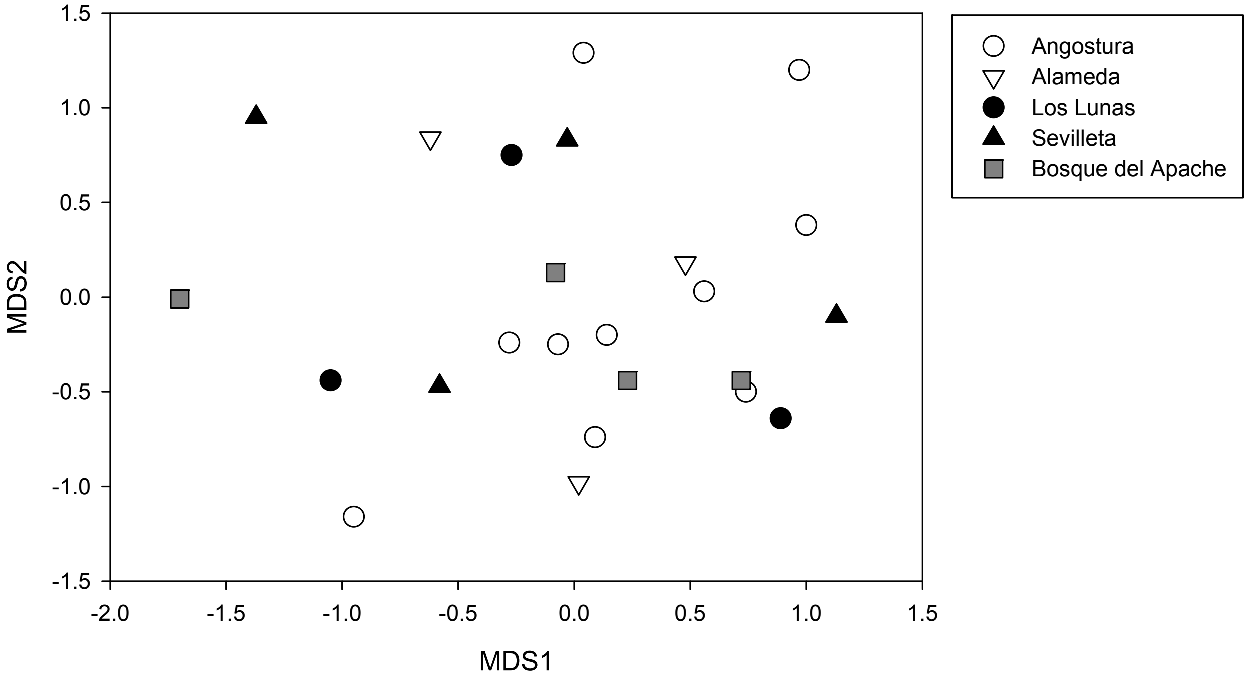


Figure 12. Multidimensional Scaling ordination of all diatom taxa across the five survey sites, 2007-2010 (stress = 0.18).

Table 3. Results from BEST analysis, which matches diatom assemblage data and multivariate environmental data based on rank correlations. R indicates the strength of the matching between diatom assemblages and environmental data. X indicates which variables contribute to the match.

variables (n)	R	NO_3	turbidity	pH	temperature	salinity	SO_4
3	0.393	X	X	X			
4	0.379	X	X	X	X		
3	0.376		X	X	X		
2	0.366		X		X		
2	0.364		X	X			
5	0.360	X	X	X	X	X	
4	0.360		X	X	X	X	
4	0.357	X	X	X		X	
5	0.350	X	X	X	X		X
3	0.348		X	X		X	

NDS experiment

Nutrient effects over time

Water quality was relatively consistent throughout the sampling period of the NDS experiment. Flow velocity was moderate throughout the entire experiment, while water temperature was warm (23-24°C) and DO levels were close to 100%. Specific conductivity was somewhat variable (~350-550µS/cm) and salinity was constant (0.25-0.28ppt). Turbidity remained relatively low (20-64NTU) and pH varied little (~8.6). Nutrients in the water column were consistent on a weekly basis although the NO_3-N concentration was relatively lower in week 2 than in any other sampling week (Table 4).

Chlorophyll *a* concentrations (as an analogue for primary producer biomass) were at the maximum level at week 2, whereas AFDM was at its highest at week 1 (Figure 13). Both chlorophyll *a* and AFDM differed significantly among sample weeks ($P<0.001$ for both parameters), but did not differ significantly among nutrient treatments (Table 5). Surveys of the diatom samples showed no differences among treatments (data not presented).

Invertebrate fauna was collected from the NDS experiment at week 4. Regardless of treatment, total abundance and taxonomic richness on individual saucers were highly variable, but generally low (37-212 individuals/NDS; 2-8 taxa/NDS) (Table 6). Chironomids were the most abundant taxa. Caddisflies (Trichoptera), mayflies (Ephemeroptera) and springtails (Collembola: Smicridea) also occurred frequently. Despite the variability, there were no significant differences among nutrient treatments for total abundance, taxonomic richness, or abundances of individual taxa (Table 7).

Table 4. Weekly ambient nutrient concentrations from the water column, nutrient diffusing substrate experiment.

	week 1	week 2	week 3	week 4
NO_3-N (µg/L)	111.3 ± 32.2	15.5 ± 5.1	300.9 ± 53.4	154.0 ± 31.5
PO_4-P (µg/L)	42.3 ± 8.7	76.2 ± 12.5	88.5 ± 17.7	66.9 ± 18.0
Bromide (µg/L)	62.5 ± 30.2	75.9 ± 18.4	79.2 ± 46.4	62.9 ± 11.5
Chloride (mg/L)	20.8 ± 3.2	19.9 ± 1.7	13.6 ± 1.8	16.0 ± 2.5
Sulfate (mg/L)	63.4 ± 10.2	58.5 ± 4.5	41.9 ± 7.9	52.3 ± 10.8
NH_4-N (µg/L)	3.8 ± 0.0	15.3 ± 2.9	1.6 ± 0.2	4.5 ± 0.6

Table 5. Effects of nutrient treatment over time (only testing non-electrified arrays).

	week		nutrients		week x nutrient	
	$F_{3,38}$	P	$F_{3,38}$	P	$F_{9,38}$	P
chlorophyll *a* (mg/m^2)	21.9	**<0.001**	2.6	0.068	1.0	0.425
AFDM (g/m^2)	14.3	**<0.001**	0.6	0.623	0.6	0.822

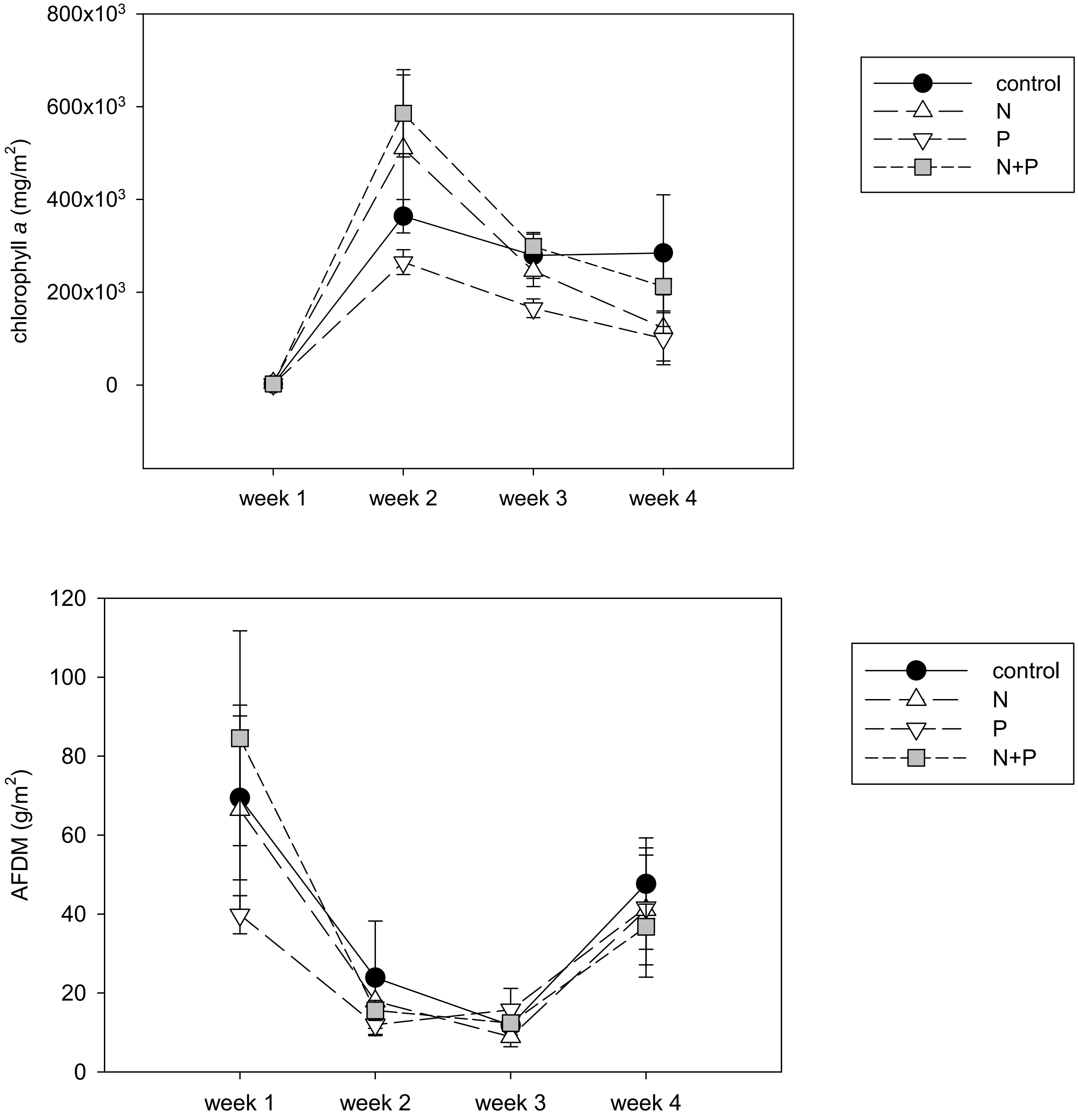


Figure 13. Mean (± SE) chlorophyll a concentrations (mg/m^2) and ADFM (g/m^2) by week, nutrient-diffusing substrate experiment.

Table 6. Mean (± SE) of total abundance and taxonomic richness of invertebrates by treatment at week 4, nutrient-diffusing substrate experiment.

	total abundance			taxonomic richness		
control	91.5	±	10.3	5	±	1.1
N	57.8	±	17.5	4.5	±	0.3
P	121	±	32.1	6.3	±	0.6
N+P	74.8	±	26.2	5.8	±	0.9

Table 7:. Effect of nutrient treatment (only testing non-electrified arrays) on invertebrate parameters at week 4, nutrient-diffusing substrate experiment.

	$F_{3, 12}$	P
Total invertebrate abundance	1.4	0.299
Invertebrate taxonomic richness	1.0	0.419
Chironomidae	1.4	0.281
Fallceon (Ephemeroptera: Baetidae)	0.0	0.987
Caenis (Ephemeroptera: Caenidae)	1.8	0.195
Nectopsyche (Trichoptera: Leptoceridae)	1.0	0.440
Smicridea (Collembola)	0.7	0.544

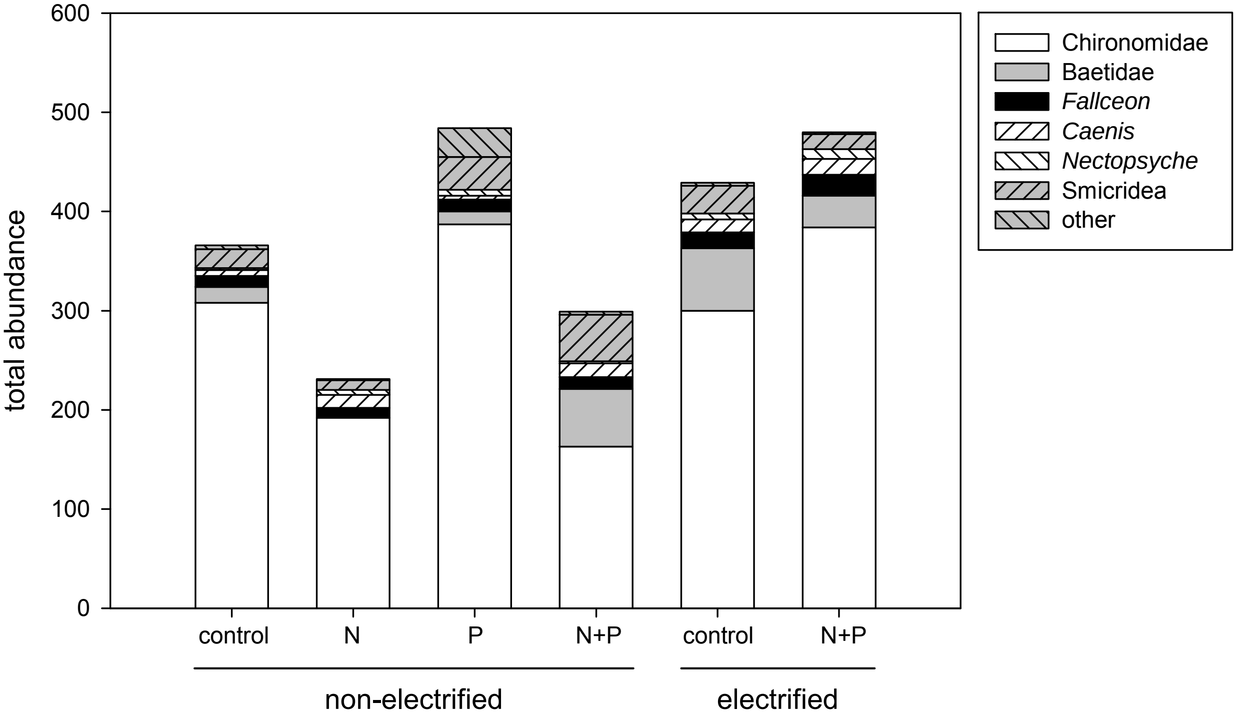


Figure 14. Summed invertebrate abundance data from all arrays at week 4, nutrient-diffusing substrate experiment

Nutrient x exclosure experiment

Chlorophyll *a* and AFDM were generally high on all NDS in the exclosure experiment, regardless of nutrient treatment or exclusion treatment. No significant differences were detected (Table 9). Similarly, invertebrate abundances were variable and no significant differences could be detected between nutrient or exclosure treatments (Table 9; Figure 14), with the exception of *Nectopsyche* (Trichoptera: Leptoceridae). *Nectopsyche* had slightly higher abundances on N+P saucers (1.5±0.7 individuals/NDS) than control saucers (1.0±0.5 individuals/NDS), and slightly higher abundances on arrays with electrical exclusion (2.0 ±0.7 individuals/NDS) than without (0.5±0.2 individuals/NDS). However, these results were biased by one electrified array that had unusually high abundances of *Nectopsyche* and probably do not represent a biological phenomenon.

Table 8. Effect of nutrient treatment and exclosure (only testing control and N+P saucers collected at week 4), nutrient-diffusing substrate experiment.

	nutrient		exclusion		nutrient x excl	
	$F_{1,10}$	P	$F_{1,10}$	P	$F_{1,10}$	P
chlorophyll *a* (mg/m^2)	0.0	0.960	0.6	0.460	0.3	0.576
AFDM (g/m^2)	0.0	0.952	0.0	0.980	0.3	0.577

Table 9. Effect of nutrient treatment and exclosure (only testing control and N+P saucers), nutrient-diffusing substrate experiment. Significant results are indicated in bold.

	nutrient		exclusion		nutrient x excl	
algal biomass	$F_{1,10}$	P	$F_{1,10}$	P	$F_{1,10}$	P
chlorophyll (mg/m^2)	0.0	0.960	0.6	0.46	0.3	0.576
AFDM (g/m^2)	0.0	0.952	0.0	0.98	0.3	0.577
invertebrate data	$F_{1,12}$	P	$F_{1,12}$	P	$F_{1,12}$	P
Total invertebrate abundance	0.0	0.928	2.0	0.186	0.5	0.510
Invertebrate taxonomic richness	0.2	0.648	0.0	0.878	0.2	0.648
Chironomidae	0.1	0.736	1.5	0.251	1.7	0.219
Fallceon (Ephemeroptera: Baetidae)	0.2	0.661	1.1	0.315	0.1	0.769
Caenis (Ephemeroptera: Caenidae)	1.8	0.206	1.2	0.295	0.4	0.555
Nectopsyche (Trichoptera: Leptoceridae)	23.5	**<0.001**	0.5	0.508	4.9	**0.047**
Smicridea (Collembola)	0.1	0.733	0.2	0.680	0.9	0.351

Transverse survey

General physical and chemical conditions differed among the survey times (Table 10). Water temperature was higher in July than in April or November, while specific conductivity was relatively high in November. Turbidity was lowest in July and highest in April.

The channel was wider in April and November than in July. Transects in July reached from one edge to the other, whereas transects in April and November were terminated in deep water in the main channel. Subsequently, fewer samples were collected across transects in July (Table 11). Water depth also differed among survey times (Figure 15, Table 12). There was a steep increase in water depth near the edge of the main channel (~1-4m from edge) in April and November, whereas it was consistently shallow across the whole transect in July (Figure 16). Flow velocity was greatest in mid-channel in April and November, and lowest in July when water depth was low across the whole channel. There was a positive correlation between depth and flow velocity in April ($r^2 = 0.336$, $P < 0.001$), July ($r^2 = 0.669$, $P < 0.001$) and November ($r^2 = 0.887$, $P < 0.001$).

Table 10. Summary of physical and chemical data collected at Bosque del Apache (BdA) and Los Lunas (LL) in conjunction with transverse surveys in April, July and November 2010. No biological data were collected at Bosque del Apache in July because the river was dry.

	April		**July**		**November**	
	LL	**BdA**	**LL**	**BdA**	**LL**	**BdA**
Temperature (°C)	13.59	15.17	34.14	na	12.67	10.13
DO (mg/L)	10.68	10.62	5.49	na	8.81	81.3
Specific conductivity (µS/cm)	345	306	478	na	3014	na
Salinity (ppt)	0.17	0.18	0.19	na	1.48	na
Turbidity (NTU)	463	327	81.2	na	144	3915
pH	8.23	8.32	9.24	na	8.68	8.7

Table 11. Number of subsamples collected from each transect at Los Lunas in April, July and November 2010.

	April	July	November
transect 1	10	5	13
transect 2	11	6	10
transect 3	13	5	9

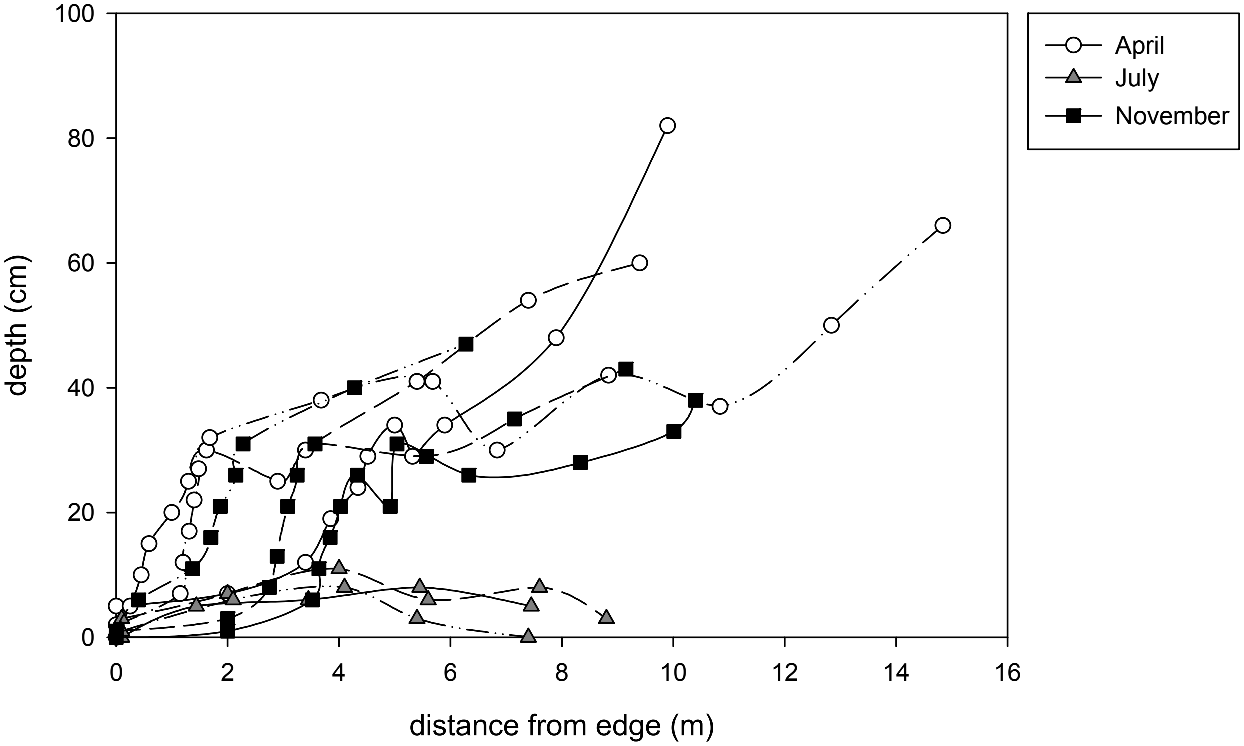
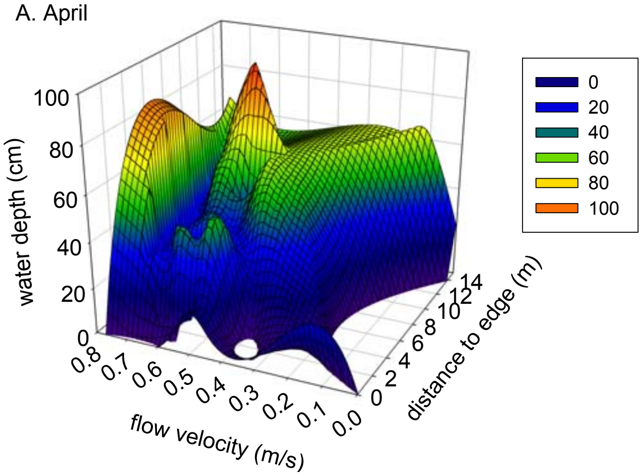


Figure 15. Depth profile of three survey transects (indicated by different line types) at Los Lunas in April, July and November 2010.

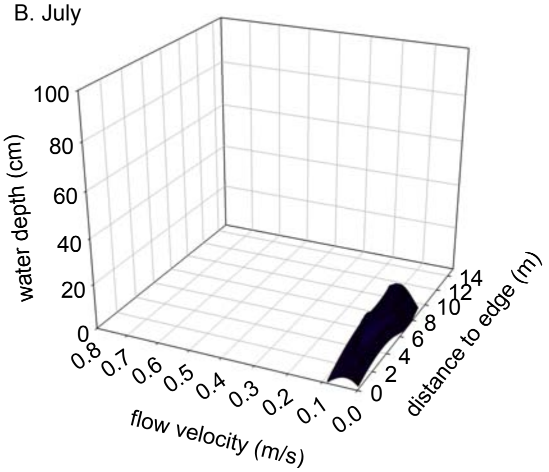
Table 12. Summary of results from generalized linear models of each variable measured at subsample points (df = 29) across transects at Los Lunas in April, July and November (month: df = 2). Significant results are indicated in bold.

	month		subsample(month)	
	Wald χ-Square	P	Wald χ-Square	P
distance from edge (m)	1.6	0.447	653.1	**<0.001**
water depth (cm)	4902.7	**<0.001**	11483.1	**<0.001**
flow velocity (m/s)	2.2	0.335	2.6	1.000
log chlorophyll *a* (mg/m^2)	9.6	**0.008**	5	1.000
log AFDM (g/m^2)	0.7	0.699	6.6	1.000
invertebrate density (indiv/sample)	4.3	0.119	0.8	1.000
log invertebrate richness (taxa/sample)	1.3	0.523	0.5	1.000
diatom density (cells/mm^2)	11.8	**0.003**	12.5	0.974
diatom richness (taxa/sample)	719.4	**<0.001**	904.4	**<0.001**

A. April



B. July



C. November

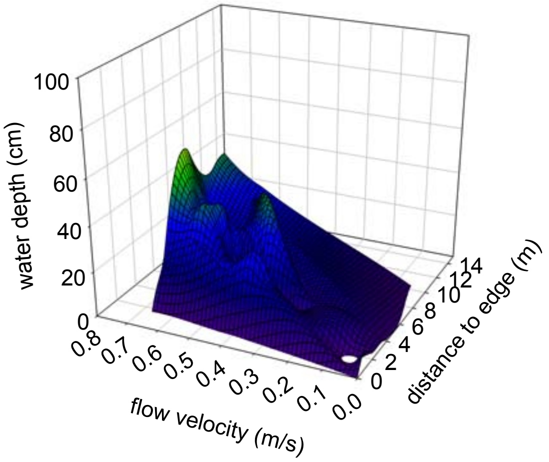


Figure 16. 3D mesh plots of basic physical parameters (distance to edge, flow velocity and water depth) at Los Lunas in April, July and November. Data are from all three transects at each survey time, and therefore represent a general view of the three parameters.

Concentration of chlorophyll *a* and density of invertebrate fauna was highly variable (Figure 17, Figure 18). Both of these biotic variables were greatest in July when the river was shallow and flow velocity was low. Significant differences were detected among survey months for chlorophyll *a* but not for invertebrate density (Table 12). Only five invertebrate taxa were collected (Chironomidae, Ceratopogonidae, Cyclopoida, Cladocera, Nematoda) and these were patchily distributed; no more than two taxa were collected from any subsample.

The density and taxonomic richness of diatoms differed significantly (Table 12). Densities were highest in July but richness was highest in November. Density of diatoms was highest in July (Figure 19), with maximum densities between 8000-12,000 cells/mm^2. Even in the shallow depths, the diatom densities were lower in April and November. The MDS plot supports the idea that there were differences among the survey months in the diatom community collected in the transverse survey (Figure 20). ANOSIM indicated that there were significant differences in community structure among survey months (Global R = 0.384, P = 0.001; Table 13). Pair-wise tests indicated that the diatom community in each survey month was significantly different to every other month. Bray-Curtis dissimilarity (measured between each pair of survey months) was relatively high, particularly for pair-wise tests including the April survey.

Two thresholds were detected for chlorophyll *a,* diatom densities, and invertebrate densities: water depth = 12cm and flow velocity = 0.2m/s. Concentration of chlorophyll *a* and density of invertebrate fauna were negligible above these thresholds and relatively high levels of both biotic parameters were only measured below the thresholds.

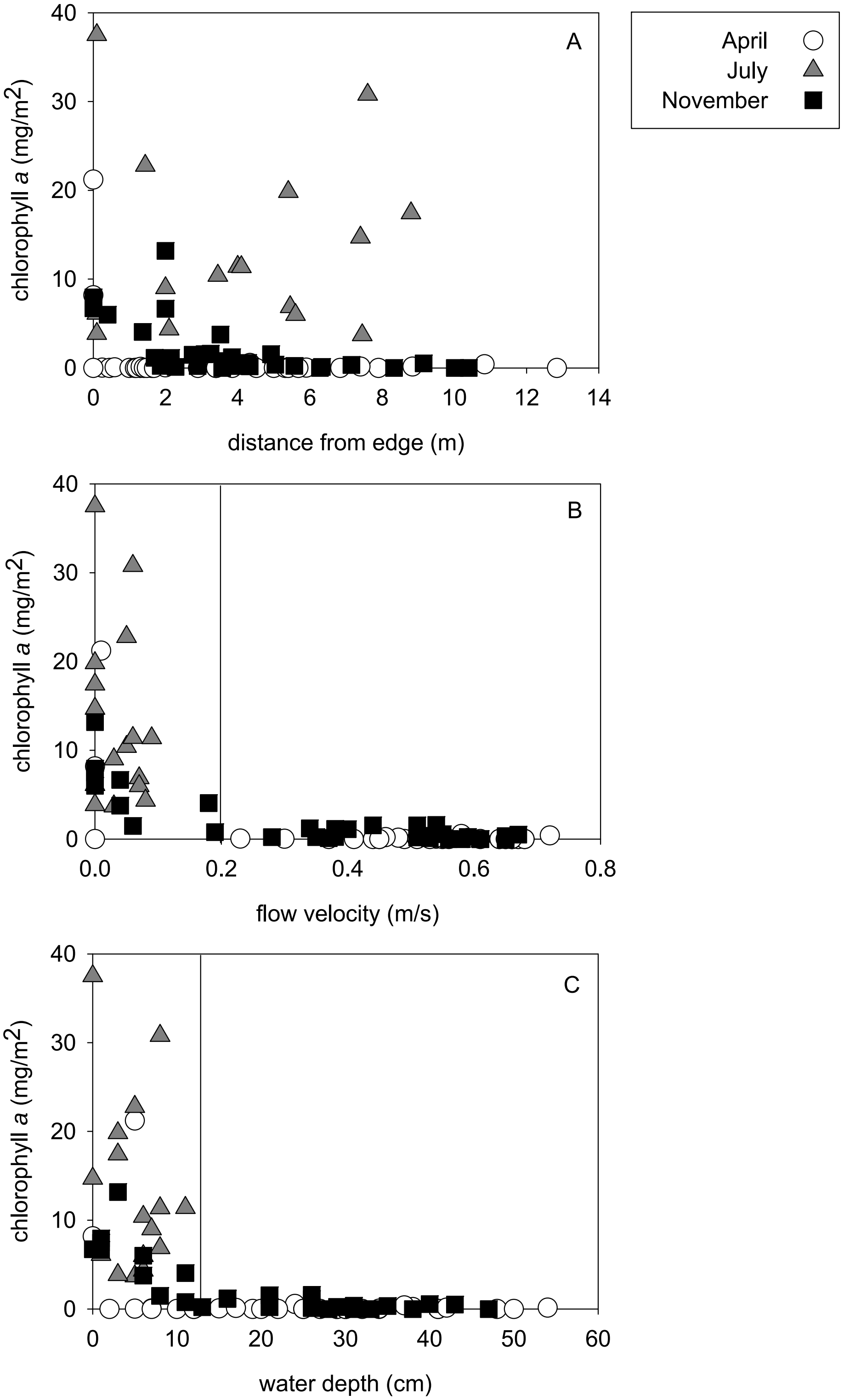


Figure 17. Concentration of chlorophyll *a* across sample transects in April, July and November in relation to (A) distance from the channel edge, (B) flow velocity and (C) water depth. Vertical lines indicate 'thresholds' of concentration of chlorophyll *a*.

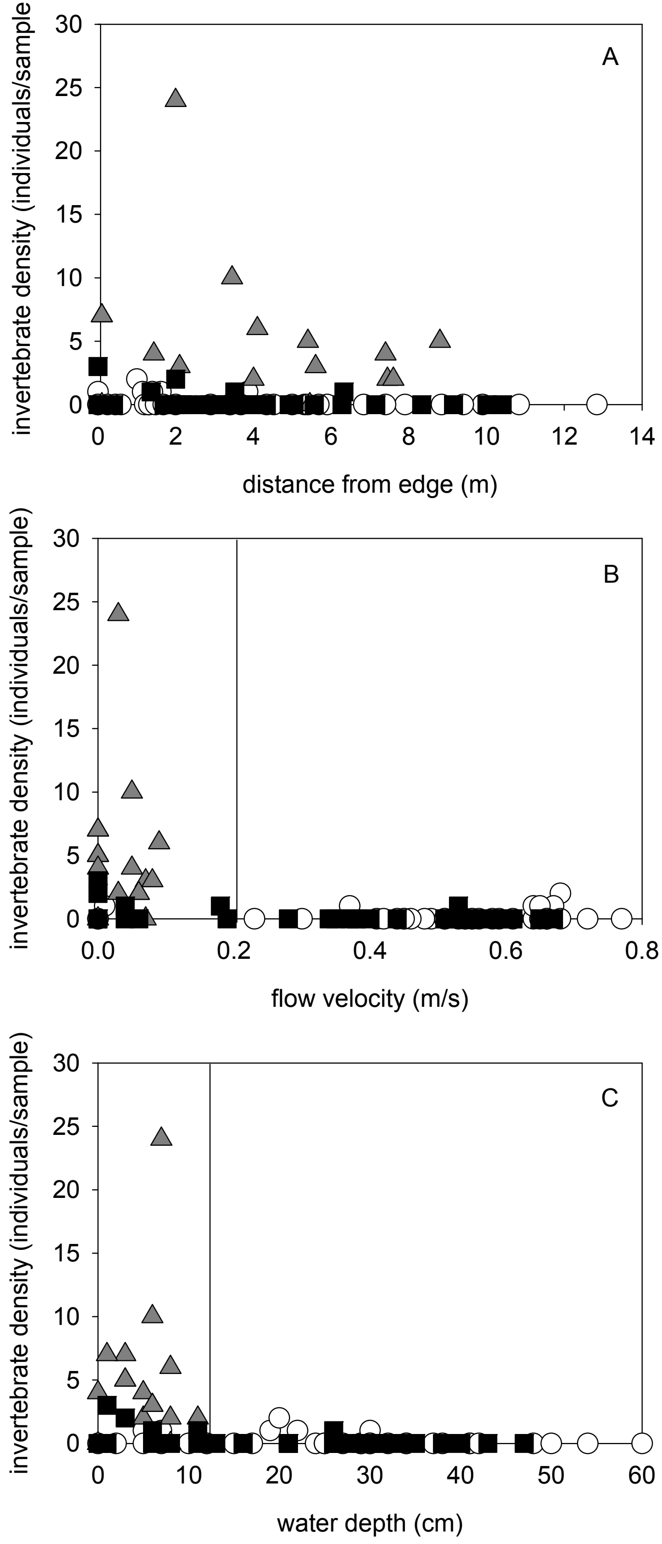


Figure 18. Density of invertebrate fauna across sample transects in April, July and November in relation to (A) distance from the channel edge, (B) flow velocity and (C) water depth. Vertical lines indicate ‘thresholds’ of invertebrate density.

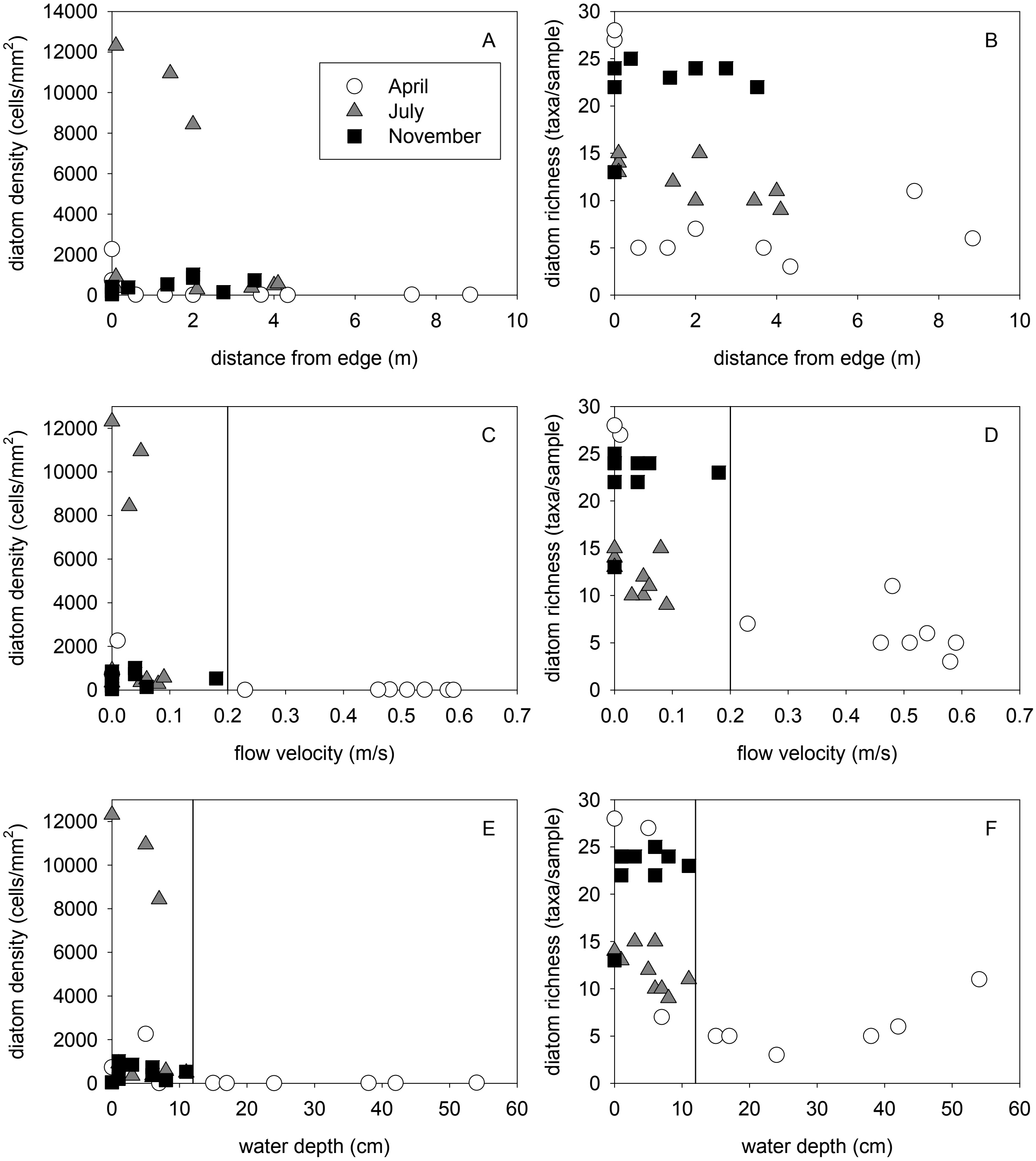


Figure 19. Density of diatoms (left) and taxonomic richness (right) recorded from individual subsamples, plotted with distance from the edge (A, B), flow velocity (C, D) and water depth (E, F). Vertical lines indicate 'thresholds' of diatom density, and are identical to those for invertebrate density.

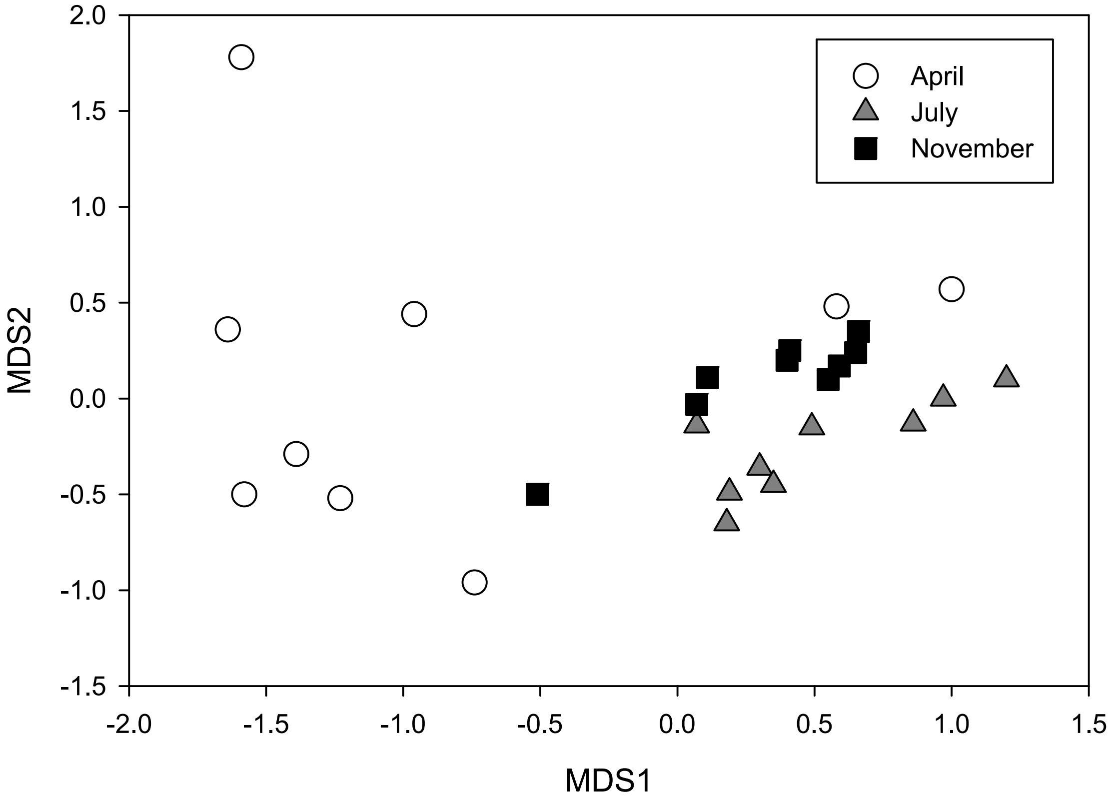


Figure 20. Multidimensional Scaling ordination of all diatom taxa indicating assemblage structure within survey months (stress = 0.08).

Table 13. Results from diatom community analyses (ANOSIM: Global R = 0.384, P = 0.001; and SIMPER), testing for differences among diatom communities collected at each sample month. See text for explanation.

pairwise test	R statistic	P	dissimilarity
April - July	0.485	0.001	76.73
April - November	0.408	0.002	73.59
July - November	0.329	0.002	53.00

Light extinction

Light intensity data were collected on five occasions during longitudinal and transverse surveys in July and November (turbidity: 174-3915NTU). Light intensity was recorded at 5cm intervals below the water surface and was used to calculate the mean vertical attenuation coefficient, k:

$k = 0.0208 \times \text{turbidity} + 10.004$

This equation was then used to calculate vertical attenuation in transverse surveys (Figure 21A). There was a strong correlation between vertical attenuation and turbidity ($r^2 = 0.9425$).

The vertical attenuation coefficient was used to calculate the depth of the euphotic zone during longitudinal and transverse surveys. The depth of the euphotic zone differed from 13cm (turbidity = 3915 NTU) to 3.28m (turbidity = 193 NTU) and was correlated with turbidity (Figure 21B, $r^2 = 0.6780$):

$z_{eu} = -0.612 \ln(\text{turbidity}) + 5.2907$

This equation can potentially be used to back-calculate depth of the euphotic zone given known turbidity levels.

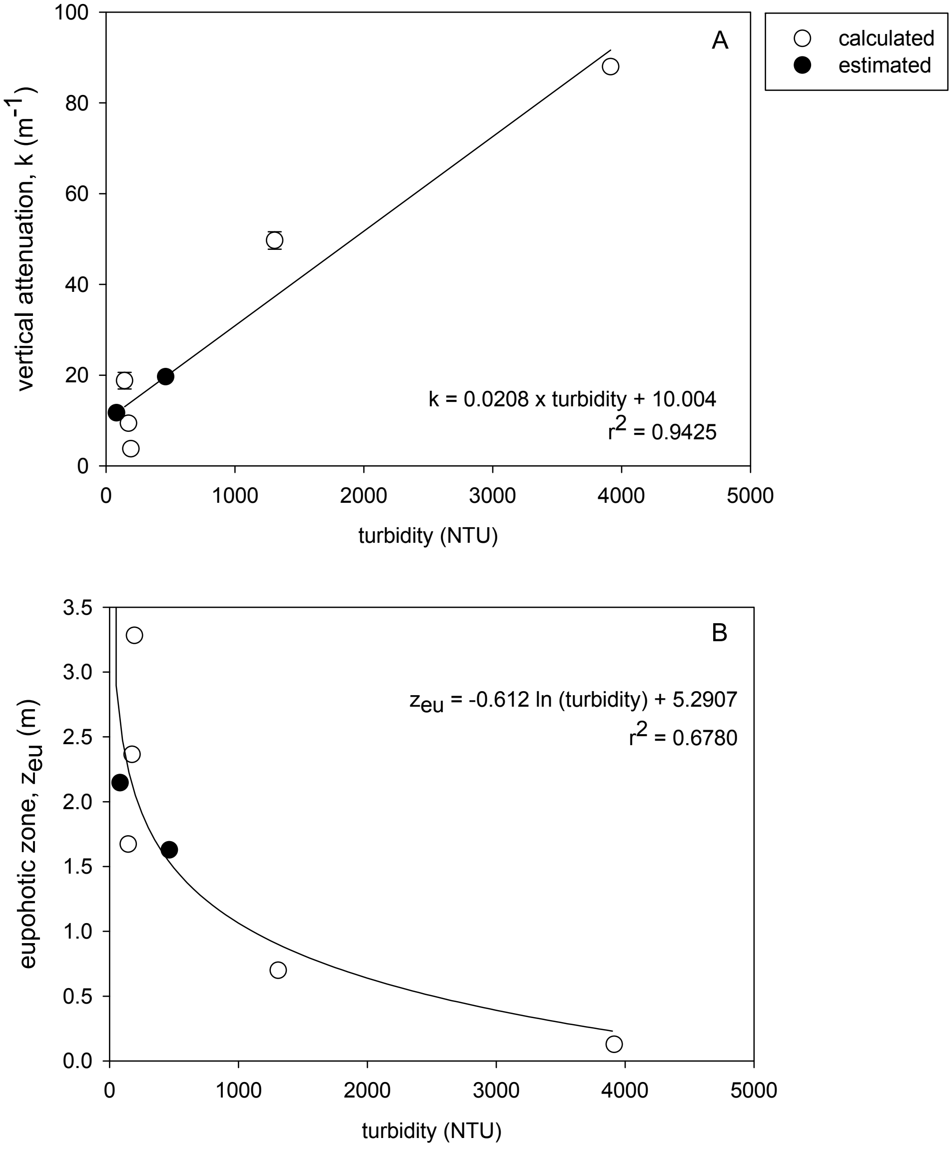


Figure 21. (A) Vertical attenuation coefficient and (B) depth of euphotic zone for data collected during longitudinal and transverse surveys. The equation for vertical attenuation was calculated using

longitudinal data only (and used to estimate vertical attenuation of transverse samples) whereas the equation for euphotic zone was calculated using both longitudinal and transverse data.

Discussion

Aridland rivers face decreased flows and more pronounced effects of anthropogenic factors over time as water resources decline associated with changes due to global warming. Many aridland rivers are naturally nutrient-limited in a landscape that is volcanic in origin. In the middle Rio Grande in central New Mexico, nutrient sources, especially nitrogen, are related primarily to wastewater effluent and irrigation practices.

With several years of data, we have been able to begin assessment of the temporal abiotic patterns, as well as spatial components, that affect the periphyton biomass and community composition in the middle Rio Grande. These repeated surveys of the river enable the examination of seasonal trends in water quality and biological communities in a highly variable system.

Spatial trends in water quality and nutrient concentration

There is a gradient from upstream to downstream in algal parameters and significant abiotic factors (e.g. NO3, turbidity). This gradient can be attributed to anthropogenic and natural origins: nitrate contributions from wastewater treatment plants in the Albuquerque region and suspended solids from tributary inputs during runoff events.

Spatial trends in diatoms

Diatom taxa differed among the five survey sites. At Angostura, the diatom community is dominated by taxa that are tolerant of low nutrient concentrations and high alkalinity. Alameda is a ‘low nutrient’ site, but has a very different substrate to that at Angostura so the diatom is characterized by epipsammic taxa. The three southern sites (Los Lunas, Sevilleta and Bosque del Apache) have similar diatom communities, dominated by epipelic taxa, taxa that are tolerant of brackish or high conductivity water and taxa that are associated with higher nutrient concentrations.

Temporal trends in water quality and nutrient concentration

Turbidity, temperature, specific conductivity, and nitrate concentrations in the water column all differed significantly among sites and among all seasons sampled. It is likely that several of these parameters are interrelated and associated with flow. Because of the need to sample low flow habitats, our direct flow measurements do not reflect the status of the main channel flow. However, the effects of high flow can be seen in higher levels of turbidity and specific conductivity, for example. Nutrient levels become elevated in higher flow because of the longer distance it takes for biota and sediment to adsorbed nutrient particles (termed a longer “spiraling length”) (Newbold et al. 1982). Therefore nutrients remain in the water column for a longer distance while moving downstream before being removed by biota (i.e., microbes, algae). When the flows are lower, the nutrients are more readily absorbed by biota, which is described as a shorter spiraling length. There is also an effect of inputs from tributaries that may be related to high spring tributary flows and monsoonal flows in late summer. These tributary inputs contribute to turbidity levels and substrate type.

Temporal trends in diatoms

We continue to work through the literature to describe these unknown taxa. There is a paucity of taxonomic literature and studies for the southwestern US; it is likely that a number of the taxa reported in the research are new to science. Notable, this work represents some of the first work to record algal communities in larger rivers in the southwestern USA. Species richness and overall diversity at the five locations was consistently high and there was strong seasonal variation in the diatom assemblages at each location. The temporal patterns in diatoms reflect the change in turbidity associated with changes in flow and with tributary inputs, especially in the northern sampling sites. At Angostura, diatom communities were dominated by *Cocconeis pediculus* and *Epithemia sorex* in the summer months when turbidity was relatively high compared to cooler times of the year. During winter months, *Diatoma vulgare*, *Diatoma moniliformis* and *Rhopalodia gibberula* were dominant. In all sampling periods, *Cocconeis placentula* var. *lineata* was common, reflecting its cosmopolitan autecology. *Epithemia sorex*, *Rhopalodia gibberula*, *R. gibba*, and *Reimeria sinuata* were common during all sampling periods, associated with low nitrogen levels and influence of alkaline soils. Substrate, flow, and relatively high nutrient levels shaped algal communities at the downstream sites compared to the upstream sites. In reaches downstream from wastewater effluents with a wide, sand-silt riverbed, epipelic diatoms (e.g., *Surirella minuta*, *S. angusta*, *Nitzschia dissipata*, *Navicula* cf *radiosa*, and *Navicula symmetrica*) were abundant during summer months when turbidity levels were relatively high. Months with lower turbidity levels were dominated by *Nitzschia palea* and several *Surirella* taxa.

Associations between the diatom assemblages and water quality parameters

Patterns in the diatom communities indicate that the assemblages were shaped by a number of abiotic factors, particularly nitrate and turbidity. However, low correlation values from the BEST analysis demonstrated that there was additional variability in the diatom communities that was not explained by the parameters that were measured. We predict that discharge (and flow) and substrate may account for some of this additional community variation but we did not effectively measure discharge and substrate. Flow was measured at our low flow sampling locations but may not have reflected flows in the main channel. Secondly, we did not link samples and substrate type, but collected general sediment samples from each subsite; therefore we cannot link a diatom community type in a sample to a substrate type.

Analyses on sediment composition showed pronounced differences among locations and seasons. The northern sites appeared to be affected by stable flows in the non-irrigation months. The southern reaches of the middle Rio Grande are affected by tributary inputs from the Rio Puerco and Rio Salado which contribute high sediment loads composed of silts and clays into the Rio Grande when they flow. Finally, the Bosque del Apache site was sampled in the floodplain, rather than the main channel, during most of 2009. The high silt and clay content of that floodplain sediment is reflected in the data and potentially will influence the structure of the diatom assemblage. The substrate structure may be reflected in shifts between epipsammic and epipelic diatoms.

NDS experiment and the biological response

The NDS experiment reflected some of the patterns that were observed in the river. Past experimental NDS runs (see annual report 2007-2008) seemed to indicate that high turbidity played an important role in limiting algal biomass. The final experimental design remedied low light issues, as evidenced by high levels of algal biomass and moderate numbers of invertebrates found on the treatments. In part, the high algal biomass through time may be a consequence of the hard surfaces that the substrates provide, compared to the shifting sand and silt substrate of the natural river bed. Algal taxa often vary by substrate (Stevenson 1996); epipsammic diatoms are limited in their abundances because the sand is unstable and may crush the cells. Biomass tends to be lower in epipsammic communities compared to epipelic, epilithic and epiphytic communities (Stevenson and Hashim 1989, Burkholder 1996, Potapova and Charles 2005).

We have concluded that the NDS experiment did not work as effectively as we expected. It is not clear if our data supports the hypothesis of nitrate limitation in the middle Rio Grande. Increases in algal biomass, in general, show colonization patterns through time but do not convincingly support nitrogen limitation for primary producers. With nutrient limitation, we expected there to be significant increases of algal biomass (as chlorophyll *a*) in the treatments that provided the additional nutrients through the NDS (Tank and Dodds 2003, Tank et al. 2006). No significant differences in biomass response among nutrient treatments imply that there were sufficient levels of nitrate and phosphorus for algal production. The lack of a response by algal biomass was surprising given the N:P ratios in the middle Rio Grande which indicate strong nitrogen limitation (Passell et al. 2005, D. Van Horn, pers. comm.). Again initial surveys of the periphyton community showed no differences among treatments.

Furthermore, it was difficult to determine the extent of grazing effects on periphyton communities. Invertebrate densities were relatively low, and did not differ among the treatments. Outcomes of the experiment were limited by the complexities of working in this natural system (e.g. variability in flow velocity, turbidity, light penetration).

Transverse surveys

Seasonally, shifts in turbidity (related to tributary flows) played a major role in shaping algal communities. In the summer months, high turbidity associated with tributary inputs created a light-limited environment where primary production was limited to a littoral zone “bathtub ring.” In 2010 (spring, summer and fall), we tested the idea of a “bathtub ring” by conducting detailed transverse surveys at Los Lunas to document the relationships among depth, algal parameters and invertebrate parameters. Depth and flow velocity are critical in the biological communities: high densities of diatoms and invertebrate fauna were only collected at shallow depths and low flow velocities. The bathtub ring is somewhat seasonal. In spring and fall, there was clear evidence that primary production and was limited to the shallow littoral zone in a compressed habitat. Similarly, the invertebrate fauna was also limited to this compressed habitat. In contrast, there was no evidence of a bathtub ring or concentration of invertebrate fauna in the littoral zone in summer when flows were low and the water was very shallow.

Conclusions

Our results indicate that periphyton and water quality (including nutrient loads) vary widely, both spatially and temporally, in aridland rivers. Three parameters that we have tested directly or indirectly (turbidity, substrate, and nitrate) appear to be the main drivers for algal biomass and species assemblages in this ecosystem; in fact, the relationships among these variables may directly or indirectly co-vary. For example, tributary inputs affected main channel flows and increased the amount of suspended solids, which influences turbidity and substrate composition, which subsequently influences biological components of the river, such as algae. Sampling difficulties have prevented us from testing the direct effects of discharge, although this parameter is likely to play a primary role in shaping algal communities as well. These abiotic factors that affect algal communities cause a bottom-up effect on the higher trophic levels, including invertebrates and fishes like the RGSM. Overall, understanding the spatial and temporal patterns of algae, driven, in part by abiotic factors, adds to our knowledge of the effects of stochasticity and seasonality on foodwebs in aridland riverine ecosystems.

Literature Cited

Alexander, R. B., R. A. Smith, and G. E. Schwartz. 2000. Effect of stream channel size on the delivery of nitrogen to the Gulf of Mexico. Nature **403**:758-761.

Bernhardt, E. S. and G. E. Likens. 2004. Controls on periphyton biomass in heterotrophic streams. Freshwater Biology **49**:14-27.

Biggs, B. J. F. and C. W. Hickey. 1994. Periphyton responses to a hydraulic gradient in a regulated river in New Zealand. Freshwater Biology **32**:49-59.

Bixby, R. J., M. B. Edlund, and E. F. Stoermer. 2005a. *Hannaea superiorensis* sp. nov., an endemic diatom from the Laurentian Great Lakes. Diatom Research **20**:227-240.

Bixby, R. J., U. Wydrzycka, and C. M. Pringle. 2005b. Diatom assemblages as indicators of solute levels in lowland Neotropical streams. Bulletin of the North American Benthological Society **22**:425-426.

Bunn, S. A., P. M. Davies, and M. Winning. 2003. Sources of organic carbon supporting the food web of an arid floodplain river. Freshwater Biology **48**:619-635.

Burkholder, J. M. 1996. Interactions of benthic algae with their substrata. Pages 253-289 *in* R. J. Stevenson, M. L. Bothwell, and R. L. Lowe, editors. Algal Ecology: Freshwater Benthic Ecosystems. Academic Press, New York.

Clarke, K. R. 1993. Non-parametric multivariate analyses of changes in community structure. Australian Journal of Ecology **18**:117-143.

Clarke, K. R. and R. N. Gorley. 2006. PRIMER v6: User Manual/Tutorial. PRIMER-E. Plymouth.

Coleman, R. L. and C. N. Dahm. 1990. Stream geomorphology: effects on periphyton standing crop and primary production. Journal of the North American Benthological Society **9**:293-302.

Connelly, S., C. M. Pringle, R. J. Bixby, R. Brenes, M. R. Whiles, K. R. Lips, S. Kilham, and A. D. Huryn. 2008. Changes in stream primary producer communities resulting from large-scale catastrophic amphibian declines: Can small-scale experiments predict effects of tadpole loss? . Ecosystems **11**:1262-1276.

Cowley, D. E., P. D. Shirey, and M. D. Hatch. 2006. Ecology of the Rio Grande silvery minnow (Cyprinidae:*Hybognathus amarus*) inferred from specimens collected in 1874. Reviews in Fisheries Science **14**:111-125.

Czarnecki, D. B. and D. W. Blinn. 1978. Diatoms of the Colorado River. Bibliotheca Phycologica **38**:1-181.

Czarnecki, D. B., D. W. Blinn, and M. Penton. 1981. Diatoms of Chevelon Creek, Navajo Co., Arizona. Southwestern Naturalist **26**:311-317.

Day, P. R. 1965. Particle fractionation and particle-size analysis. Pages 545-567 *in* C. A. Black, editor. Methods in Soil Analysis. Part I. Soil Science Society of America.

Eichhorst, K. D., D. C. Shaw, J. F. Schuetz, A. D. Gebauer, and C. S. Crawford. 2006. Bosque Ecosystem Monitoring Program (BEMP) (2004-2005), Albuquerque, NM.

Elsner, J. J., M. M. Elsner, N. A. MacKay, and S. R. Carpenter. 1988. Zooplankton-mediated transitions between N- and P-limited algal growth. Limnology and Oceanography **33**:1-14.

Feminella, J. W. and C. P. Hawkins. 1995. Interactions between stream herbivores and periphyton: a quantitative analysis of past experiments. Journal of the North American Benthological Society **14**:465-509.

Filemaker. 2009. Filemaker, Inc., Santa Clara, CA.

Gelwick, F. P. and W. J. Matthews. 2006. Trophic relations of stream fish. Pages 611-635 *in* R. M. Hauer and G. A. Lamberti, editors. Methods in Stream Ecology. Elsevier, New York.

Greenwood, J. L. and A. D. Rosemond. 2005. Periphyton response to long-term nutrient enrichment in a shaded headwater stream. Canadian Journal of Fisheries and Aquatic Sciences **62**:2033-2045.

Grimm, N. B., S. G. Fisher, and W. L. Minckley. 1981. Nitrogen and phosphorus dynamics in hot desert streams in the southwestern U.S.A. Hydrobiologia **83**:303-312.

Hanson, M. A., K. D. Zimmer, M. G. Butler, B. A. Tangen, B. R. Herwig, and N. H. J. Euliss. 2005. Biotic interactions as determinants of ecosystem structure in prairie wetlands: an example using fish. Wetlands **25**:764-775.

Hauer, F. R. and W. R. Hill. 2006. Temperature, light, and oxygen. Pages 103-117 *in* F. R. Hauer and G. A. Lamberti, editors. Methods in Stream Ecology, 2nd edition. Academic Press, New York.

Hlohowskyj, C. P., M. M. Coburn, and T. M. Cavender. 1989. Comparison of a pharyngeal filtering apparatus in seven species of the herbivorous cyprinid genus *Hybognathus* (Pisces: Cyprinidae). Copeia **1989**:172-183.

Kociolek, J. P. and S. A. Spaulding. 2000. Freshwater diatom biogeography. Nova Hedwigia **71**:223-241.

Lowe, R. L. 1974. Environmental requirements and pollution tolerance of freshwater diatoms. Environmental Monitoring Series. U.S. Environmental Protection Agency, Cincinnati, Ohio.

Lowe, R. L., S. W. Golladay, and J. R. Webster. 1986. Periphyton response to nutrient manipulation in streams draining clearcut and forested watersheds. Journal of the North American Benthological Society **5**:221-229.

Magaña, H. A. 2007. A case for classifying the Rio Grande silver minnow (*Hybognathus amarus*) as an omnivore. University of New Mexico, Albuquerque.

Merritt, R. W., K. W. Cummins, and M. B. Berg. 2008. An introduction to the aquatic insects of North America, 4th edition. Kendall Hunt, Dubuque, IA.

Mosisch, T. D., S. E. Bunn, and P. M. Davies. 2001. The relative importance of shading and nutrients on algal production in subtropical streams. Freshwater Biology **46**:1269-1278.

Moulton, T. P., M. L. DeSouza, R. M. L. Silveira, and F. A. M. Krsulovis. 2004. Effects of ephemeropterans and shrimps on periphyton and sediments in a coastal stream (Atlantic Forest, Rio de Janeiro, Brazil). Journal of the North American Benthological Society **23**:868-881.

Newbold, J. D., R. V. O'Neill, J. W. Elwood, and W. VanWinkle. 1982. Nutrient spiralling in streams: implications for nutrient limitation and invertebrate activity The American Naturalist **120**:628-652.

Oelsner, G. P., P. D. Brooks, and J. F. Hogan. 2007. Nitrogen sources and sinks within the Middle Rio Grande. Journal of the American Water Resources Association **43**:850-863.

Pan, Y., A. Herlihy, P. Kaufmann, J. Wigington, J. van Sickle, and T. Moser. 2004. Linkages among land-use, water quality, physical habitat conditions and lotic diatom assemblages: A multi-spatial scale assessment. Hydrobiologia **515**:59-73.

Passell, H. D., C. N. Dahm, and E. J. Bedrick. 2005. Nutrient and organic carbon trends and patterns in the upper Rio Grande, 1975-1999. Science in the Total Environment **345**:239-260.

Pease, A. A., J. J. Davis, M. S. Edwards, and T. F. Turner. 2006. Habitat and resource use by larval and juvenile fishes in an arid-land river (Rio Grande, New Mexico). Freshwater Biology **51**:475-486.

Potapova, M. and D. F. Charles. 2005. Choice of substrate in algae-based water-quality assessment. Journal of the North American Benthological Society **24**:415-427.

Potapova, M. G. and D. F. Charles. 2002. Benthic diatoms in USA rivers: Distributions along spatial and environmental gradients. Journal of Biogeography **29**:167-187.

Pringle, C. M. and G. A. Blake. 1994. Quantitative effects of atyid shrimp (Decapoda: Atyidae) on the depositional environment in a tropical stream: use of electricity for experimental exclusion. Canadian Journal of Fisheries and Aquatic Sciences **51**:1443-1450.

Pringle, C. M., R. J. Naiman, G. Bretschko, J. R. Karr, M. W. Oswood, J. R. Webster, R. L. Welcomme, and M. J. Winterbourn. 1988. Patch dynamics in lotic systems: the stream as a mosaic. Journal of the North American Benthological Society **7**:503-524.

Pringle, C. M. and F. J. Triska. 2006. Effects of nutrient enrichment on periphyton. Pages 743-759 *in* R. M. Hauer and G. A. Lamberti, editors. Methods in stream methods, 2nd edition. Elsevier.

Ranvestel, A. W., K. R. Lips, C. M. Pringle, M. R. Whiles, and R. J. Bixby. 2004. Neotropical tadpoles influence stream benthos: evidence for ecological consequences of amphibian declines. Freshwater Biology **49**:274-285.

Richards, C. R., D. H. Franklin, M. L. Cabrera, R. J. Bixby, M. A. Risse, and D. E. Radcliffe. in review. Periphyton response to two nutrient diffusing substrate methods in small southeastern U.S.A. piedmont streams Journal of Environmental Quality.

Rosemond, A. D., P. J. Mulholland, and J. W. Elwood. 1993. Top-down and bottom-up control of stream periphyton: effects of nutrients and herbivores. Ecology **74**:1264-1280.

Sartory, D. P. and J. U. Grobbelaar. 1984. Extraction of chlorophyll-*a* from freshwater phytoplankton for spectophotometric analysis. Hydrobiologia **114**:177-187.

Shirey, P. D. 2004. Foraging habits and habitat utilization of the Rio Grande silvery minnow as inferred by diatom frustules. New Mexico State University, Las Cruces.

Sicko-Goad, L. and N. A. Andresen. 1991. Effect of growth and light/dark cycles on diatom lipid content and composition. Journal of Phycology **27**:710-718.

Smith, D. G. 2001. Pennak's freshwater invertebrates of the United States: porifera to crustacea, 4th edition. John Wiley and Sons, New York.

Spaulding, S. A., J. P. Kociolek, and D. R. Davis. 2002. A new diatom (Bacilliariophyceae) genus with two new species from New Mexico, USA. European Journal of Phycology **37**:135-143.

Stanley, E. H., G. G. Fisher, and N. B. Grimm. 1987. Ecosystem expansion and contraction in streams: desert streams cary in both space and time and fluctuate dramatically in size. Bioscience **47**:427-435.

Steinman, A. D. 1996. Effects of grazers on freshwater benthic algae. Pages 341-373 *in* R. J. Stevenson, M. L. Bothwell, and R. L. Lowe, editors. Algal Ecology: Freshwater Benthic Ecosystems. Academic Press, New York.

Steinman, A. D., C. D. McIntire, S. V. Gregory, G. A. Lamberti, and L. R. Ashkenas. 1987. Effects of herbivore type and density on taxonomic structure and physiognomy of algal assemblages in laboratory streams. Journal of the North American Benthological Society **6**:175-188.

Steinman, A. D., P. J. Mullholland, and D. B. Kirschtel. 1991. Interactive effects of nutrient reduction and herbivory on biomass, taxonomic structure, and P uptake in lotic periphyton communities. Canadian Journal of Fisheries and Aquatic Sciences **48**:1951-1959.

Stelzer, R. S. and G. A. Lamberti. 2001. Effects of N:P ratio and total nutrient concentration on stream periphyton community structure, biomass, and elemental composition. Limnol. Oceanog. **46**:356-367.

Stevenson, R. J. 1996. An introduction to algal ecology in freshwater benthic habitats. Pages 3-26 *in* R. J. Stevenson, M. L. Bothwell, and R. L. Lowe, editors. Algal Ecology: Freshwater Benthic Ecosystems. Academic Press, New York.

Stevenson, R. J. and L. L. Bahls. 1999. Periphyton protocols. Pages 6-1 to 6-22 *in* M. T. Barbour, J. Gerritsen, and B. D. Snyder, editors. Rapid Bioassessment Protocols for Use in Streams and Rivers: Periphyton, Benthic Macroinvertebrates, and Fish, Second Edition EPA 841-B-99-002., U.S. Environmental Protection Agency, Washington D.C.

Stevenson, R. J. and S. Hashim. 1989. Variation in diatom community structure among habitats in sandy streams. Journal of Phycology **25**:678-686.

Stevenson, R. J. and Y. Pan. 1999. Assessing environmental conditions in rivers and streams using diatoms. Pages 11-13 *in* E. F. Stoermer and J. P. Smol, editors. The Diatoms: Applications for the Environmental and Earth Sciences University of Cambridge Press, Cambridge.

Stewart, A. J. 1987. Responses of stream algae to grazing minnows and nutrients: a field test for interactions. Oecologia **72**:1-7.

Stoermer, E. F., M. B. Edlund, C. Pilskaln, and C. L. Schelske. 1995. Siliceous microfossil distribution in the surficial sediments of Lake Baikal. Journal of Paleolimnology **14**:69-82.

Tank, J. L., M. J. Bernot, and E. J. Rosi-Marshall. 2006. Nitrogen limitation and uptake. Pages 213-238 *in* R. M. Hauer and G. A. Lamberti, editors. Methods in Stream Ecology, Second Edition. Elsevier, New York.

Tank, J. L. and W. K. Dodds. 2003. Nutrient limitation of epilithic and epixylic biofilms in ten North American streams. Freshwater Biology **48**:1031-1049.

Triska, F. J., C. M. Pringle, J. H. Duff, R. J. Avanzino, A. Ramirez, M. Ardon, and A. P. Jackman. 2006. Soluble, reactive phosphorus transport and retention in tropical, rainforest streams draining a volcanic and geothermally active landscape in Costa Rica: Long-term concentration patterns, pore water environment and response to ENSO events. Biogeochemistry **81**:131-143.

Vadeboncoeur, Y., J. Kalff, K. Christoffersen, and E. Jeppesen. 2006. Substratum as a driver of variation in periphyton chlorophyll and productivity in lakes. Journal of the North American Benthological Society **25**:379-392.

Van Dam, H., A. Mertens, and J. Sinkeldam. 1994. A coded checklist and ecological indicator values of freshwater diatoms from the Netherlands. Netherlands Journal of Aquatic Ecology **28**:117-133.

Van Horn, D., L. H. Zeglin, and C. N. Dahm. 2006. Seasonal and longitudinal trends in the Middle Rio Grande water quality. Bulletin of the North American Benthological Society **23**:351.

Zeglin, L. H., D. J. Van Horn, C. N. Dahm, O. G.P., and P. D. Brooks. 2006. Controls on water quality in a heavily managed aridland river. Bulletin of the North American Benthological Society **23**:290-291.

Appendix 1 – Water quality data by season

Table A1-1. Mean (± SE) data for water quality variables at each of the five sampling locations on twelve sampling periods. *Samples not collected because of high water or equipment failure.

location	year	season	n	turbidity (NTU)	water temperature (°C)	flow velocity (m/s)	DO (mg/L)	pH	salinity (ppt)	specific conductivity (μS/cm)
Angostura	2007	fall	3	13.3 ± 3.1	11.9 ± 0.8	0.00 ± 0.00	3.2 ± 0.0	8.40 ± 0.24	0.07 ± 0.03	219.4 ± 103.1
	2008	winter	3	6.2 ± 0.3	6.0 ± 0.3	0.17 ± 0.12	4.4 ± 0.3	8.94 ± 0.11	0.10 ± 0.00	304.9 ± 1.1
		spring	3	37.8 ± 14.6	17.4 ± 2.1	0.06 ± 0.04	7.3 ± 3.2	6.94 ± 0.08	0.10 ± 0.00	250.7 ± 22.6
		summer	3	24.1 ± 4.4	23.5 ± 0.6	0.07 ± 0.04	4.2 ± 0.3	8.12 ± 0.15	0.10 ± 0.00	292.4 ± 2.3
		fall	3	13.7 ± 4.4	12.5 ± 0.1	0.00 ± 0.00	5.0 ± 1.2	7.13 ± 0.25	0.20 ± 0.00	351.3 ± 19.4
	2009	winter	3	9.6 ± 3.4	3.8 ± 0.5	0.03 ± 0.03	4.2 ± 0.6	6.75 ± 0.09	0.10 ± 0.00	313.8 ± 2.9
		spring	1	12.5	15.1	0.26		8.11	0.20	315.7
		summer	3	51.5 ± 3.2	21.9 ± 0.1	0.41 ± 0.22	8.5 ± 0.1	8.22 ± 0.01	0.15 ± 0.00	262.7 ± 30.3
		fall	3	30.7 ± 19.3	11.9 ± 0.6	0.07 ± 0.04	10.8 ± 0.6	8.33 ± 0.01	0.16 ± 0.00	334.3 ± 4.9
	2010	winter	3	3.9 ± 0.2	4.0 ± 0.4	0.08 ± 0.08	15.5 ± 0.2	8.26 ± 0.13	0.15 ± 0.00	310.0 ± 1.0
		spring	1	*	18.4	0.02	10.3	8.32	0.12	212.0
		summer	3	190.7 ± 26.3	22.1 ± 0.1	0.00 ± 0.00	6.9 ± 0.3	8.30 ± 0.02	0.14 ± 0.00	275.3 ± 0.3
Alameda	2007	fall	3	25.8 ± 2.0	14.3 ± 0.1	0.11 ± 0.06	3.2 ± 0.1	7.81 ± 0.24	0.20 ± 0.00	359.4 ± 18.6
	2008	winter	3	29.2 ± 0.8	7.9 ± 1.0	0.10 ± 0.04	3.5 ± 0.2	9.15 ± 0.27	0.17 ± 0.03	307.9 ± 35.7
		spring	1	48.2	12.7	0.61	14.3	7.03	0.10	239.0
		summer	3	92.8 ± 7.3	24.6 ± 0.7	0.02 ± 0.02	3.4 ± 0.2	8.42 ± 0.09	0.10 ± 0.00	301.7 ± 3.1
		fall	3	21.8 ± 3.1	17.9 ± 1.1	0.08 ± 0.08	6.6 ± 0.7	7.71 ± 0.36	0.20 ± 0.00	240.6 ± 103.3
	2009	winter	3	40.4 ± 17.5	14.7 ± 1.6	0.00 ± 0.00	4.6 ± 0.4	6.44 ± 0.13	0.17 ± 0.02	331.8 ± 10.2
		spring	1	18.2	15.4	0.20		7.90	0.20	315.9
		summer	3	351.3 ± 130.8	29.5 ± 1.2	0.14 ± 0.14	7.2 ± 0.4	8.41 ± 0.10	0.16 ± 0.00	372.3 ± 9.6
		fall	3	24.6 ± 1.5	14.7 ± 0.6	0.06 ± 0.03	10.8 ± 0.1	8.41 ± 0.32	0.17 ± 0.00	276.0 ± 2.6
	2010	winter	3	13.0 ± 4.7	12.3 ± 2.2	0.04 ± 0.04	8.4 ± 1.8	8.07 ± 0.21	0.20 ± 0.02	307.7 ± 43.2
		spring	1	*	18.0	0.17	10.3	8.26	0.10	182.0
		summer	3	1075.7 ± 149.5	32.0 ± 1.8	0.00 ± 0.00	4.1 ± 0.3	8.67 ± 0.06	0.14 ± 0.00	340.3 ± 11.3

Table A1-1 continued.

location	year	season	n	turbidity (NTU)	water temperature (°C)	flow velocity (m/s)	DO (mg/L)	pH	salinity (ppt)	specific conductivity (µS/cm)
Los Lunas	2007	fall	3	191.6 ± 85.7	12.5 ± 0.4	0.15 ± 0.09	4.2 ± 0.3	8.10 ± 0.37	0.20 ± 0.00	425.3 ± 27.4
	2008	winter	3	34.7 ± 6.8	12.6 ± 1.1	0.01 ± 0.01	3.6 ± 0.2	8.07 ± 0.59	0.17 ± 0.03	346.0 ± 75.2
		spring	2	253.8 ± 162.3	16.0 ± 1.3	0.39 ± 0.39	14.0 ± 6.0	6.66 ± 0.81	0.10 ± 0.00	265.1 ± 12.0
		summer	3	107.5 ± 29.8	31.6 ± 0.2	0.00 ± 0.00	3.7 ± 0.3	8.72 ± 0.17	0.20 ± 0.00	358.4 ± 3.6
		fall	3	41.9 ± 14.3	17.9 ± 1.1	0.00 ± 0.00	6.4 ± 0.8	7.99 ± 0.23	0.23 ± 0.03	510.4 ± 79.2
	2009	winter	1	33.9	9.1	0.11	3.3	8.05	0.20	421.4
		spring	1	31.5	18.7	0.00		7.80	0.20	344.6
		summer	3	89.6 ± 36.6	26.7 ± 0.7	0.02 ± 0.02	5.7 ± 2.5	8.44 ± 0.22	0.19 ± 0.03	391.7 ± 53.3
		fall	3	117.0 ± 52.0	15.0 ± 0.4	0.03 ± 0.01	11.7 ± 0.5	8.39 ± 0.15	0.16 ± 0.04	340.7 ± 85.2
	2010	winter	3	38.4 ± 3.0	9.3 ± 0.5	0.00 ± 0.00	11.9 ± 0.1	8.39 ± 0.02	0.21 ± 0.00	300.3 ± 3.7
		spring	1	*	19.6	0.14	9.3	8.17	0.13	244.0
		summer	3	61.2 ± 29.2	34.5 ± 0.8	0.00 ± 0.00	5.8 ± 0.4	9.25 ± 0.09	0.19 ± 0.00	482.3 ± 8.1
Sevilleta	2007	fall	3	169.7 ± 39.2	14.5 ± 1.6	0.03 ± 0.03	3.4 ± 0.3	8.65 ± 0.11	0.23 ± 0.07	471.1 ± 98.0
	2008	winter	3	53.2 ± 9.7	9.4 ± 1.4	0.16 ± 0.09	4.3 ± 0.1	9.00 ± 0.45	0.17 ± 0.03	385.9 ± 94.9
		spring	3	89.4 ± 4.1	14.2 ± 0.8	0.16 ± 0.11	4.3 ± 0.8	7.22 ± 0.10	0.10 ± 0.00	294.3 ± 7.1
		summer	3	4000.0 ± 0.0	28.5 ± 0.5	0.13 ± 0.03	3.7 ± 0.1	7.89 ± 0.17	0.30 ± 0.06	615.3 ± 96.0
		fall	3	89.2 ± 24.2	12.5 ± 2.2	0.00 ± 0.00	7.7 ± 0.6	6.83 ± 0.15	0.27 ± 0.03	537.1 ± 61.7
	2009	winter	3	22.2 ± 9.3	8.5 ± 1.6	0.09 ± 0.09	6.2 ± 0.5	7.09 ± 0.16	0.23 ± 0.03	502.4 ± 18.3
		spring	1	48.3	21.8	0.33	8.1	7.96	0.20	331.3
		summer	3	36.1 ± 17.0	30.6 ± 0.5	0.04 ± 0.03	10.1 ± 0.8	8.85 ± 0.02	0.27 ± 0.00	514.0 ± 77.9
		fall	3	173.7 ± 31.8	13.3 ± 0.8	0.00 ± 0.00	10.7 ± 0.2	8.45 ± 0.02	0.25 ± 0.00	401.7 ± 7.2
	2010	winter	3	41.8 ± 5.7	10.2 ± 0.9	0.04 ± 0.01	14.1 ± 0.7	8.85 ± 0.12	0.24 ± 0.00	335.3 ± 10.4
		spring	1	94.0	22.8	0.35	7.7	8.26	0.14	292.0
		summer	1	4000.0	25.4		6.1	8.53	1.45	913.0

Table A1-1 continued.

location	year	season	n	turbidity (NTU)	water temperature (°C)	flow velocity (m/s)	DO (mg/L)	pH	salinity (ppt)	specific conductiv (μS/cm)
Bosque del Apache	2007	fall	3	1433.9 ± 1378.6	16.7 ± 2.0	0.02 ± 0.02	3.6 ± 0.5	8.40 ± 0.06	0.30 ± 0.00	582.3 ±
	2008	winter	3	198.0 ± 88.0	4.3 ± 0.1	0.12 ± 0.10	3.6 ± 0.1	8.19 ± 0.68	0.23 ± 0.03	470.7 ±
		spring	3	87.5 ± 7.4	13.3 ± 0.3	0.23 ± 0.13	10.2 ± 4.9	7.05 ± 0.07	0.20 ± 0.00	320.6 ±
		summer	3	3585.0 ± 1216.2	23.2 ± 0.6	0.03 ± 0.02	4.5 ± 0.1	7.58 ± 0.18	0.40 ± 0.00	790.0 ±
		fall	3	48.3 ± 19.6	8.7 ± 0.8	0.00 ± 0.00	7.7 ± 0.2	7.82 ± 0.23	0.20 ± 0.06	390.2 ±
	2009	winter	3	377.2 ± 348.4	10.8 ± 0.6	0.00 ± 0.00	2.2 ± 0.3	6.99 ± 0.35	0.30 ± 0.00	537.0 ±
		spring	3	145.8 ± 43.8	24.8 ± 4.4	0.00 ± 0.00		7.54 ± 0.09	0.20 ± 0.00	413.0 ±
		summer	3	380.7 ± 269.2	25.8 ± 1.6	0.00 ± 0.00	7.5 ± 1.7	8.03 ± 0.20	0.21 ± 0.06	463.0 ±
		fall	3	130.6 ± 58.8	14.1 ± 2.3	0.00 ± 0.00	11.1 ± 0.6	8.51 ± 0.13	0.22 ± 0.03	447.7 ±
	2010	winter	3	108.2 ± 34.8	10.8 ± 1.7	0.10 ± 0.07	12.0 ± 0.6	8.35 ± 0.08	0.38 ± 0.12	581.0 ±
		spring	1	185.0	22.5	0.69	8.5	8.36	0.15	300.0
		summer	1	4000.0	24.9	0.82	5.1	8.21	0.38	782.0

Appendix 2 – Diatom taxa list

Table A2-1. Summary of all diatom taxa collected during longitudinal surveys, 2007-2010.

Taxa	Angostura	Alameda	Los Lunas	Sevilleta	Bosque del Apache
Achnanthes cf. *levanderi*	+		+		
Achnanthes linearis	+		+		
Achnanthes sp GV			+		
Achnanthes sp. 7	+				
Achnanthes sp. 1	+		+		
Achnanthes sp. 2		+			
Achnanthes sp. 3		+			
Achnanthes sp. 5	+				
Achnanthes sp. 6	+				
Achnanthes subsalsa	+		+		
Achnanthidium cf. *linearis (or biasolittiana)*	+		+		+
Achnanthidium exiguum	+			+	
Achnanthidium minutissimum	+		+	+	+
Amphipleura pellucida	+	+		+	
Amphora holsatica	+				
Amphora inariensis	+				
Amphora libya	+				
Amphora ovalis	+			+	+
Amphora pediculus	+	+	+		
Amphora perpusilla	+	+		+	+
Amphora sp. 1		+		+	
Amphora veneta	+				
Anomoeoneis sphaerophora			+	+	+
Asterionella formosa	+	+	+	+	
Aulacoseira granulata	+		+	+	+
Aulacoseira italica	+	+	+	+	+
Bacillaria paxillifera				+	
Caloneis amphisbaena			+	+	+
Caloneis bacillum	+	+	+	+	+
Caloneis schumanniana			+		
Caloneis silicula				+	
Cocconeis pediculus	+	+	+	+	+
Cocconeis placentula var. *lineata*	+	+	+	+	+
Craticula ambigua	+		+	+	+
Craticula sp.			+		
Cyclotella antiqua?	+				
Cyclotella bodanica	+				
Cyclotella cf. meneghiniana			+		

Table A2-1 continued.

Taxa	Angostura	Alameda	Los Lunas	Sevilleta	Bosque del Apache
Cyclotella meneghiniana	+	+	+	+	+
Cyclotella sp.	+				
Cymatopleura elliptica				+	
Cymatopleura solea			+	+	
Cymatopleura solea var. *apiculata*	+	+	+	+	+
Cymbella affinis	+	+			+
Cymbella caepitosa	+				
Cymbella cf. *affinis*	+	+			+
Cymbella sp. 1 *(Amphora?)*	+				
Cymbella tumida	+	+	+		+
Denticula elegans	+				
Diadesmis confervacea		+	+	+	+
Diatoma (round)		+		+	
Diatoma moniliformis	+	+	+	+	+
Diatoma vulgare	+	+	+	+	+
Diatoma-oval	+	+	+	+	+
Diploneis ovalis		+			
Encyonema minutum	+	+	+	+	+
Encyonema silesiacum	+	+			+
Eolimna minima	+	+			+
Epithemia adnata					
Epithemia cf. *sorex*	+	+			
Epithemia sorex	+	+	+	+	+
Eunotia incisa	+	+			
Eunotia intermedia	+				
Fallacia insocialibilis	+				
Fragilaria capucina var. *mesolepta*	+	+		+	+
Fragilaria crotonensis	+				
Fragilaria sp. (GV)			+		
Fragilaria sp. 1			+		
Fragilaria vaucheriae	+			+	+
Geissleria decussis	+	+	+		
Geissleria sp.			+		
Gomphoneis cf. *herculeana*	+	+			
Gomphonema acuminatum					+
Gomphonema angustatum?		+			
Gomphonema cf. *lagenula*		+			
Gomphonema cf. *pumilum*			+	+	
Gomphonema cf. olivaceum		+			

Table A2-1 continued.

Taxa	Angostura	Alameda	Los Lunas	Sevilleta	Bosque del Apache
Gomphonema clavatum					+
Gomphonema gracile	+	+	+		
Gomphonema insigne	+				
Gomphonema lagenula	+			+	+
Gomphonema minutum	+		+		
Gomphonema olivaceum	+	+	+	+	+
Gomphonema parvulum	+	+	+	+	+
Gomphonema parvulum?		+			
Gomphonema pumilum	+	+		+	+
Gomphonema rhombicum	+		+		
Gomphonema sp.					+
Gomphonema sp.	+				
Gomphonema sp.	+				
Gomphonema sp. 1	+				
Gomphonema sp. 2		+			
Gomphonema subclavatum	+				
Gomphonema truncatum	+		+		
Gomphonema sp.		+			
Gomphonema/Gomphoneis sp.	+				
Gyrosigma acuminatum	+				
Gyrosigma cf. sciotoense					+
Gyrosigma scalproides	+	+	+		+
Gyrosigma sciotoense	+	+	+		+
Gyrosigma sp.			+		
Hanzschia amphioxys	+	+	+	+	+
Hanzschia amphioxys sp. 2			+		
Hippodonta capitata	+		+	+	
Hippodonta capitata var. *hungarica*	+				
Hippodonta cf. *capitata*	+	+	+	+	
Hippodonta sp. 1	+				
Karayevia clevei	+				+
Luticola goeppertiana					+
Luticola mutica	+		+		+
Luticola mutica var. ventricosa					+
Luticola muticoides	+		+	+	+
Luticola sp.					+
Mastogloia elliptica		+		+	
Mastogloia sp.					+
Melosira varians	+	+	+	+	+

Table A2-1 continued.

Taxa	Angostura	Alameda	Los Lunas	Sevilleta	Bosque del Apache
Navicula angusta	+			+	
Navicula capitatoradiata	+	+	+	+	+
Navicula cf accomoda	+	+	+	+	
Navicula cf angusta	+				
Navicula cf buderi	+				
Navicula cf cinta			+		
Navicula cf cocconeiformis	+		+		
Navicula cf constans			+		+
Navicula cf constans var. symmetrica	+				
Navicula cf cryptocephala	+				
Navicula cf cryptotenella			+		
Navicula cf elginensis	+				
Navicula cf *halophiloides*	+	+	+		+
Navicula cf radiosa				+	+
Navicula cf rhynchocephala	+				
Navicula cf rostellata	+				
Navicula cf symmetrica	+	+	+	+	+
Navicula cf tripartita				+	
Navicula cf upsaliensis		+			
Navicula cf viridula					+
Navicula clementis	+				
Navicula cryptocephala	+	+	+	+	+
Navicula cryptotenella	+	+	+	+	+
Navicula elginensis	+				
Navicula germainii	+		+	+	+
Navicula gregaria		+		+	
Navicula lanceolata	+		+		+
Navicula libonensis	+		+		
Navicula meniculus	+				
Navicula pseudoanglica	+	+	+		
Navicula radiosa	+	+		+	+
Navicula recens	+	+			
Navicula rostellata	+	+	+	+	+
Navicula seminulum	+	+	+	+	+
Navicula sp. 1	+				
Navicula sp. 10	+				
Navicula sp. 11	+				
Navicula sp. 13					+
Navicula sp. 15	+	+			

Table A2-1 continued.

Taxa	Angostura	Alameda	Los Lunas	Sevilleta	Bosque del Apache
Navicula sp. 16	+		+		+
Navicula sp. 17	+			+	
Navicula sp. 2	+				+
Navicula sp. 3	+				
Navicula sp. 4	+				
Navicula sp. 5			+		
Navicula sp. 6	+	+			
Navicula sp. 7	+			+	
Navicula sp. 8				+	
Navicula sp. 9				+	
Navicula sp.18				+	
Navicula sp. 19			+		
Navicula sp. 20			+		
Navicula subminuscula		+	+	+	
Navicula symmetrica	+	+	+	+	+
Navicula tripunctata	+	+	+	+	+
Navicula trivialis	+	+	+	+	
Navicula veneta	+	+	+	+	+
Navicula viridula		+	+	+	+
Navicula viridula var. linearis					+
Navicula/Fallacia sp.			+	+	
Neidium dubium		+			
Nitzschia acicularis	+			+	
Nitzschia amphibia	+	+	+	+	+
Nitzschia angustata					+
Nitzschia capitellata	+	+	+	+	+
Nitzschia cf filiformis				+	
Nitzschia cf frustulum			+		
Nitzschia cf perminuta	+		+		
Nitzschia clausii	+	+	+	+	+
Nitzschia communis	+				
Nitzschia dissipata	+	+	+	+	+
Nitzschia filiformis	+	+	+		+
Nitzschia flexoides	+				
Nitzschia fonticola	+				
Nitzschia frustulum	+	+	+	+	+
Nitzschia gracilis	+		+	+	+
Nitzschia heufleriana	+	+	+	+	+
Nitzschia heufleriana	+				

Table A2-1 continued.

Taxa	Angostura	Alameda	Los Lunas	Sevilleta	Bosque del Apache
Nitzschia inconspicua	+	+	+	+	+
Nitzschia linearis	+	+	+	+	+
Nitzschia microcephala	+				
Nitzschia palaeaformis	+	+	+		+
Nitzschia palea	+	+	+	+	+
Nitzschia palea-large	+				
Nitzschia palea-sigmoid				+	
Nitzschia perminuta	+	+	+	+	+
Nitzschia recta	+	+	+	+	+
Nitzschia sigmoidea	+				
Nitzschia sinuata var. *delognei*				+	
Nitzschia sp. 1	+				
Nitzschia sp. 10	+			+	
Nitzschia sp. 11				+	
Nitzschia sp. 12				+	
Nitzschia sp. 13				+	
Nitzschia sp. 14				+	
Nitzschia sp. 2		+			
Nitzschia sp. 3	+	+		+	+
Nitzschia sp. 4		+		+	+
Nitzschia sp. 5		+			
Nitzschia sp. 6			+	+	
Nitzschia sp. 7					+
Nitzschia sp. 8	+				
Nitzschia sp. 9					+
Nitschia sp. 15	+				
Nitzschia subacicularis				+	
Nitzschia supralitorea	+		+		
Nitzschia terrestris	+	+	+		+
Nitzschia vermicularis		+			
Nitzschia wuellerstorfii	+		+		
Pinnularia borealis	+	+	+		
Pinnularia divergens				+	
Pinnularia GV	+				
Pinnularia sp. 1			+		
Pinnularia sp. 2		+			
Placoneis gastrum	+				
Planothidium delicatulum	+	+	+		+
Planothidium lanceolatum	+	+	+	+	+

Table A2-1 continued.

Taxa	Angostura	Alameda	Los Lunas	Sevilleta	Bosque del Apache
Planothidium lanceolatum var. *dubium*	+	+	+		
Planothidium lanceolatum var. *frequentissimum*	+	+	+	+	
Planothidium lanceolatum var. *rostratum*	+	+	+	+	+
Planothidium sp. 1	+				
Planothidium sp. 2	+				
Pleuroseira laevis	+				+
Psammothidium subatomoides	+	+			
Pseudostaurosira brevistriata	+	+	+	+	+
Pseudostaurosira brevistriata var. *inflata*		+			+
Raphid GV	+				
Raphid GV	+				
Reimeria cf sinuata (punctate striae)	+	+	+		+
Reimeria sinuata	+	+		+	+
Rhoicosphenia abbreviata	+	+	+	+	+
Rhoicosphenia sp.	+				
Rhopalodia brebissoni			+		+
Rhopalodia gibba	+	+	+	+	+
Rhopalodia gibberula	+	+	+		+
Sellaphora bacillum	+				
Sellaphora cf pupula)				+	
Sellaphora cf pupula				+	
Sellaphora cf. bacillum		+			
Sellaphora pupula	+	+	+	+	+
Stauroneis anceps	+				
Staurosira cf. construens		+			
Staurosira construens	+	+	+	+	+
Staurosira construens var. *binodis*	+	+	+		+
Staurosira construens var. *subsalina*			+		
Staurosira construens var. *venter*	+	+	+	+	+
Staurosira elliptica		+	+		
Staurosira sp.	+	+		+	
Staurosirella cf. *pinnata*	+				
Staurosirella leptostauron	+	+		+	+
Staurosirella pinnata	+	+	+	+	+
Staurosirella pinnata var. intercedens		+			
Staurosirella sp.	+	+			
Stephanodiscus hantzschiana	+			+	+
Stephanodiscus niagarae		+		+	+
Stephanodiscus sp.		+			

Table A2-1 continued.

Taxa	Angostura	Alameda	Los Lunas	Sevilleta	Bosque del Apache
Surirella angusta	+		+	+	+
Surirella linearis	+				
Surirella linearis v. constricta					+
Surirella minuta	+	+	+	+	+
Surirella minuta 1	+		+	+	
Surirella minuta 2				+	
Surirella ovalis	+		+	+	+
Surirella sp.					+
Surirella sp. 1	+				
Surirella sp. 2			+		
Synedra cf. acus					+
Synedra cf. ulna		+		+	+
Synedra delicatissima	+				
Synedra goulardii			+	+	+
Synedra parasitica			+		
Synedra rumpens var. *familaris*	+				
Synedra rumpens var. fragilarioides	+				
Synedra sp.	+				
Synedra ulna	+	+	+	+	+
Tryblionella angustata	+		+		+
Tryblionella calida			+		
Tryblionella constricta	+	+	+	+	+
Tryblionella hungarica 1	+	+		+	+
Tryblionella hungarica 2	+	+	+	+	+
Tryblionella lacunarum				+	+

Appendix 3 - Tumbleweed as an algal substrate

During the initial review process for this contract, reviewers hypothesized about the role of submerged tumbleweed as an algal substrate in the middle Rio Grande. Given that submerged tumbleweed often provide good habitat for fishes, tumbleweed may be an important component for evaluating algal food resources in the middle Rio Grande.

An experiment to determine colonization rates and diversity of algae on tumbleweed was performed in the middle Rio Grande in November 2007. Tumbleweed segments were placed in the river at the Angostura, Alameda, and Sevilleta locations used in our longitudinal survey. These sites were chosen based on differences in nutrient inputs above and below the Albuquerque reach. Segments of tumbleweeds were attached to posts with nylon fishing line and placed in the river to be colonized by algae in the water column. Segments for tumbleweed were removed and sampled weekly for four weeks.

Diatoms and soft algae were enumerated and identified to genus (soft algae by colleague Dr. R. Verb) from tumbleweeds and compared within and among sites. Data were analyzed by site and week using Multi-response Permutation Procedure and Non-metric Multidimensional Scaling ordinations.

From the three sites, 75 algal genera were recorded from the tumbleweeds . Results showed that tumbleweeds at the Angostura site were colonized by the dominant diatoms *Epithemia* and *Cocconeis* and filamentous cyanobacteria *Phormidium*, *Oscillatoria* and a unicellular cyanobacterium *Synechocystis*. The Alameda site was dominated by a different assemblage of diatom genera (i.e., *Cocconeis, Cymbella, Diatoma, Epithemia, Navicula,* and *Nitzschia*) and the unicellular cyanobacterium *Synechocystis.* Finally, the Sevilleta site had only a few dominant taxa which supported the community (diatoms *Navicula* and *Nitzschia*, and *Synechocystis).*

There was considerable variation among algal colonizers among sample weeks, and there were no significant differences detected (MRPP, $P = 0.4664$). Constant water flow and moving tumbleweed substrate may be continually resetting the system. Comparatively, there were clear differences among algal communities by site with significant differences among sites ($P < 0.0001$). The MDS visually supports these results with a clear pattern of increasing nutrients and conductivity from upstream to downstream sites (Figure A3-1A). Interestingly, the patterns are driven by the diatoms (Figure A3-1B), rather than the entire algal community (Figure A3-1C).

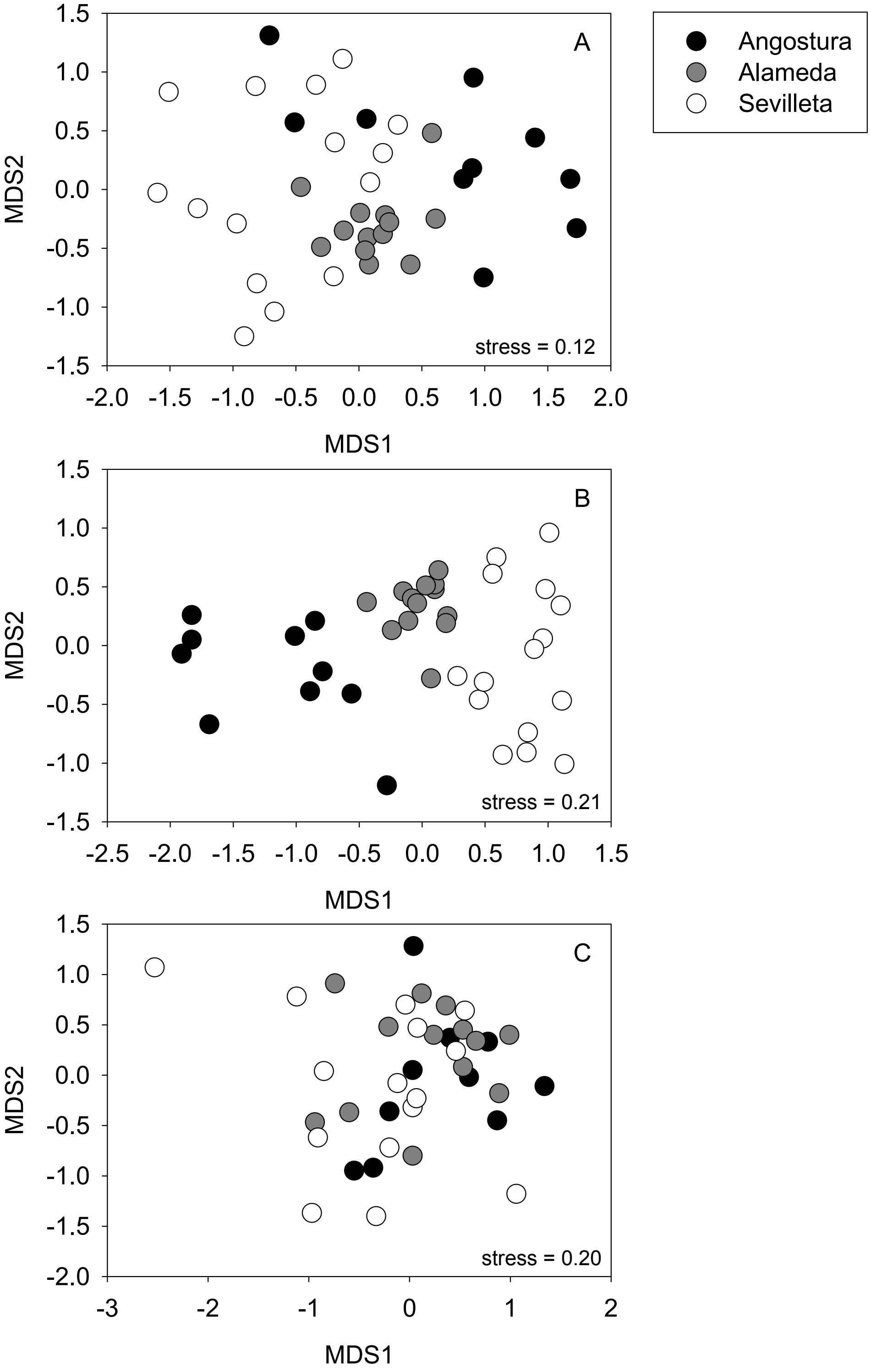


Figure A3-1. Multidimensional Scaling ordination of (a) all taxa, (B) diatom taxa only and (C) algal taxa only, indicating assemblage structure at three sites. The MDS plots were calculated separately and are plotted on different axes.

Table A3-1. Summary of algal and diatom taxa collected on tumbleweed over the experimental period at Angostura, Alameda and Sevilleta. Note that now samples were collected at Angostura in week 4 due to vandalism.

location		**Angostura**			**Alameda**				**Sevilleta**			
week		**week 1**	**week 2**	**week 3**	**week 1**	**week 2**	**week 3**	**week 4**	**week 1**	**week 2**	**week 3**	**week 4**
n		*4*	*4*	*2*	*4*	*4*	*2*	*3*	*4*	*4*	*4*	*3*
green algae	*Anabaena*	+		+	+	+	+					
	Ankistrodesmus		+									
	Auxospores					+						
	Bulbochaete		+		+							
	Characium	+	+					+		+		
	cf *Cladophora*									+		
	Chlamydomonas	+	+	+	+	+	+		+	+		+
	Chlorella	+	+	+	+	+	+		+	+	+	+
	Chroococcus	+	+	+	+	+		+	+		+	+
	Closterium							+	+			
	Coleochaete		+		+	+						
	Cosmarium	+						+	+			
	cf *Dinobryon*						+					
	Dinoflagellate	+		+	+	+		+		+		
	Euglena				+		+		+		+	
	Gleocapsa						+					
	Gymnodinium	+										
	Lepocinclis					+		+	+	+	+	+
	Malomonas		+			+						
	Mougeotia	+	+		+			+	+	+		
	cf *Nostochopsis*		+									
	Oedogonium		+		+	+			+	+		
	Oocystis				+							
	Oscillatoria	+	+	+	+		+	+	+	+	+	
	Peridinium											
	Phormidium			+								
	Plectonema	+	+					+		+		
	Pseudoanabaena					+			+		+	

Table A3-1 continued

location		**Angostura**			**Alameda**				**Sevilleta**			
week		**week 1**	**week 2**	**week 3**	**week 1**	**week 2**	**week 3**	**week 4**	**week 1**	**week 2**	**week 3**	**week 4**
n		*4*	*4*	*2*	*4*	*4*	*2*	*3*	*4*	*4*	*4*	*3*
	Selenastrum						+	+				
	Staurastrum		+									
	Stichococcus				+		+					
	Stigeoclonium		+			+		+				
	Stylosphaeridium	+		+	+						+	
	Synechococcus	+	+	+	+	+			+			
	Synechocystis	+	+	+	+	+	+	+	+	+	+	+
	Trachelomonas				+	+		+				
	Tribonema		+		+	+				+		
	Ulothrix								+			+
diatoms	*Achnanthidium*						+	+	+		+	
	Amphipleura	+	+		+	+						
	Amphora						+	+				
	Aulacoseira	+									+	
	Bacillaria								+	+		
	Cocconeis	+	+	+	+	+	+	+	+	+	+	+
	Cyclotella									+		
	Cymatopleura									+		
	Cymbella	+	+	+	+	+	+	+		+	+	+
	Diatoma	+	+	+	+	+	+	+	+	+	+	+
	Encyonema									+		
	Epithemia	+	+	+	+	+	+	+			+	+
	Fragilaria	+			+		+			+		+
	Geissleria	+	+				+					
	Gomphoneis	+										
	Gomphonema	+		+	+	+	+	+	+	+	+	+
	Gyrosigma	+	+	+				+		+	+	+

Table A3-1 continued

location	**Angostura**			**Alameda**				**Sevilleta**			
week	**week 1**	**week 2**	**week 3**	**week 1**	**week 2**	**week 3**	**week 4**	**week 1**	**week 2**	**week 3**	**week 4**
n	*4*	*4*	*2*	*4*	*4*	*2*	*3*	*4*	*4*	*4*	*3*
Hippodonta	+	+									
Melosira								+	+	+	
Navicula	+		+	+	+	+	+	+	+	+	+
Nitzschia	+		+	+	+	+	+	+	+	+	+
Pinnularia										+	
Planothidium	+	+							+		
Pleurosira		+		+	+			+	+	+	
Pseudostaurosira	+							+	+		
Reimeria	+			+	+						
Rhoicosphenia	+					+				+	+
Rhopalodia	+		+	+		+	+		+		
Sellaphora									+		
Stauroneis							+				
Staurosira	+			+		+		+	+	+	+
Staurosirella					+	+					
Surirella					+			+	+	+	
Synedra	+		+	+	+	+	+	+	±	+	+
Tryblionella							+	+	+	+	+