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Benthic diatoms in the Salinas river: characteristics, relationships to habitat

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**BENTHIC DIATOMS IN THE SALINAS RIVER: CHARACTERISTICS,
RELATIONSHIPS TO HABITAT**

A Thesis

Presented to

The Faculty of Moss Landing Marine Laboratories

and the Department of Marine Sciences

San Jose State University

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

By

Zoe B. Knesl

August 2002

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ABSTRACT

BENTHIC DIATOMS IN THE SALINAS RIVER: CHARACTERISTICS, RELATIONSHIPS TO HABITAT

The species composition and abundance of benthic diatoms in the Salinas River are poorly known. Diatoms were quantitatively sampled in April 2000. The most abundant species were usually found in eutrophic or sedimentary areas and tolerant of a wide range of ecological conditions. Shannon-Weiner diversity, evenness (J), number of species per sample, and percent motile diatoms were analyzed using a two factor ANOVA, revealing a complex pattern influenced by both site and substrate. A cluster analysis resulted in five clusters, with site and substrate as the grouping factors. A Principal Components Analysis using chemical data revealed that (PC1) was most influenced by turbidity, nitrate, phosphate, and suspended solids, and (PC2) by conductivity, chloride, dissolved solids, and nitrate. A BIOENV analysis revealed no single chemical factor was strongly responsible for the assemblage characteristics. The Salinas River diatoms may be more influenced by the disturbance regime and seasonality than by nutrient or sediment concentrations.

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Introduction:

Diatoms have been studied by scientists since the early 1800's. Originally, these glass walled unicellular algae were merely a beautiful fascination for early microscopists and biologists, including Darwin, who noted "infusoria with silicious shields" in dust collected from the sails of the HMS Beagle (Darwin, 1860). Diatoms have since been described in detail (van Heurck, 1896, and many others) and have been used to ascertain environmental conditions for decades beginning with Kolckwitz and Marsson (1908). They are now used by the EPA as indicators of water quality in streams in several states (Barbour et. al., 1997), and recently became part of the USGS National Water Quality Assessment Program (NAWQA) program in all fresh-water bodies (Porter et. al. 1993). In contrast to stream studies, however, diatom surveys in the rivers in the continental US are rare and for the most part unpublished so descriptive studies are needed prior to water quality assessment. There have been very few diatom surveys conducted in the freshwater rivers of coastal central California, and none have been published. My objective is to describe the diatom assemblages of portions of the Salinas River and, by comparing the results to physical and chemical data collected by the Central Coast Regional Water Quality Control Board (CCRWQCB) and using known habitat preferences of the species found, suggest local environmental factors that may be influencing the flora. Moreover, knowing the conditions in which the existing flora reside will facilitate the future use of these plants as water and habitat quality indicators in the region.

The presence, absence, dominance, and rarity of certain species and types of species can be used to assess water and habitat quality in what is known as rapid bio-assessment (Porter et. al. 1993, Barbour et. al., 1997). Bio-assessment surveys may use fish, benthic macro-invertebrates, or diatoms. Biological, chemical, and physical data are gathered in surveys of rivers and streams and are then used to calculate indices which rank the level of "health" of an ecosystem, or sites along a continuum, and provide management with a hierarchy of areas needing assistance (Karr and Chu, 1999). Several indices based on diatom species and group abundances have been used for assessment in the past. These include the saprobien system, developed to indicate the level of organic pollution in a stream (Lowe, 1974; Kolkwitz and Marsson, 1908), the halobion system designed to indicate salt ion levels (Lowe, 1974; Kolbe, 1927), and the nutrient system which shows the level of enrichment (Lowe, 1974, Smith 1966). All of these indices use the known preferred habitats of diatom species based on decades of research on the upper and lower survival limits of individual species, and hundreds of surveys conducted globally, to produce a numerical rating for the species, then use this number to qualitatively describe the habitat. These systems, however, do not consider the adaptability of most diatom species and the difficulties in identifying some species with extreme certainty. These indices also require a large amount of taxonomic knowledge, as the species in one genus - often similar in form - do not always live in similar conditions. The EPA and the USGS presently recommend using several indices calculated using the assemblage characteristics rather than a species-wise numerical ranking system. These indices include the Shannon-Wiener diversity index, the total number of species found,

the percent motile diatoms and the percent *Achnanthes minutissima* (Barbour et al., 1997). These indices are less dependent on the accuracy of identification, but may be less sensitive to changes in the assemblages due to environmental perturbation, especially by humans (Patrick, 1977. Stevenson, 1984). They also do not recognize the possible importance of a single rare or dominant species whose presence may signal a very specific habitat condition. The Salinas River undergoes many chemical, physical, and biological changes along its length which may be undetectable using these recommended calculations.

The Salinas River

I surveyed the benthic diatoms of the Salinas River in coastal central California. The Salinas River is located in Monterey and San Luis Obispo counties, California, and is fed by 23 smaller watersheds in the Salinas River basin (Figure 1). The river drains an area of 2,099,440.67 acres and is connected to 4,872.39 miles of naturally occurring waterways (<http://endeavor.des.ucdavis.edu>). There are 17 dams in the Salinas basin, and two large reservoirs. Lake Nacimiento and Lake San Antonio, discharge into the Salinas river just south of Bradley, California. These reservoirs discharge approximately 160,000 acre-feet every two to three weeks from May to September in order to prevent winter flooding and ameliorate salt water intrusion (Joe Madruga, Monterey County Water Resources Agency, pers. com.).

The Salinas is a 150-mile long, slowly flowing, sedimentary river whose water characteristics and other environmental variables change considerably along its course. These variables presumably influence the structure of the diatom assemblages found in

the river. Some of these environmental variables were measured by the Central Coast Regional Water Quality Control Board (CCRWQCB) in 1999-2000. Factors that may influence the diatom assemblage include substrate type (Aloi, 1990; Barbour et al., 1997; Blinn et al., 1980; Patrick, 1977; Porter et al., 1977; Sabater et al., 1998; Tuchman and Stevenson, 1980), irradiance (Lowe, 1996; McLeod and Barton, 1998; Patrick, 1977), temperature, hardness, grazing (Patrick, 1977), turbidity, current velocity (Patrick, 1977, Patrick, 1963; Power and Stewart, 1987; Stevenson, 1984; Stevenson, 1990; McLeod and Barton, 1998; Biggs et al., 1998), pH, salinity (Patrick, 1977, Patrick, 1963, Barbour et al., 1997), nutrients (Barbour et al., 1997; Karr and Chu, 1999; Lowe, 1974; Marcus, 1980; McCormick and Stevenson, 1998; Palmer, 1969; Patrick, 1963; Patrick, 1977; Porter et al., 1993; Stevenson, 1984), carbon-dioxide or bicarbonate, oxygen, trace elements and the available species pool (Patrick, 1977). With the exception of substrate types, trace elements, CO₂, grazing and the available species pool, these variables were measured by the CCRWQCB. Several environmental characteristics showed trends downstream along the river (Appendices A and B). The grain size tends to become smaller (Appendix B), the nutrients, temperature, pH, hardness, salinity, and turbidity tend to increase, and current velocity, oxygen content, and light intensity tend to decrease (Appendix A). These factors, in combination with direct human and animal disturbances such as range use, recreational use, and water releases, and internal biological and physical factors such as grazing by insect larvae and disturbance-recovery regimes no doubt have important effects on the structure of the diatom assemblages in the Salinas River.

In its upper reaches the Salinas River is a narrow (2 to 3 m), shallow (<0.5m) slowly flowing stream (Sites H41 and PAR, Figure 1). The substrate available for algal colonization consists of small rocks, cobbles, and pebbles with interspersed patches of large grained sand. Large plants and trees grow to almost the river banks, although there is minimal shading. The water is cooler, less turbid, contains less inorganic nutrients, is of medium hardness, and has a higher oxygen content in this area than in the lower sections of the river (Appendix A). The diatom flora in this area should consist of benthic stream, river, and lake species. Due to the abundant plant substrate, epiphytic genera that live in slow moving (limnophilous) waters such as *Gomphonema*, *Cocconeis*, and *Synedra* should be present in high numbers (Patrick, 1977). The rock substrate would provide habitat for epilithic genera that live in slow moving water such as *Fragilaria*, *Synedra*, *Melosira*, *Cymbella*, *Diatoma*, and *Meridion* (Patrick, 1977). Due to the salt content of the water, the species found here should be oligohalobous (freshwater with salt concentrations of less than 500mg/L) (Lowe, 1974), halophilous (optimum in weakly brackish waters) (Hofmann, 1997) or indifferent (optimum in freshwater but tolerant of slightly brackish water) (Hofmann, 1997) and include species that prefer high light conditions due to the lack of turbidity such as *F. capucina* and *Cyclotella meneghiniana* (Patrick, 1977). In a non-impacted or disturbed environment, no single species or suite of species should have an advantage over any other, each species should be equally able to survive and reproduce, species abundances should be evenly distributed with no single species having a very large density (Patrick, 1977). In

this part of the Salinas River, species richness should be high, with small populations of many different species.

The middle reaches of the river are wider, slower flowing, and deeper, with fewer large plants near the banks, and there are often beaches along the shore (Sites USA, GRN and CHU, Figure 1). The substrates are mostly unstable habitats including large and small grained sands, with small areas of rocks and woody debris and minimal silty habitats (Appendix B). The water in these stretches is slightly warmer, is of increasing turbidity, contains more inorganic nutrients, is softer, has a lower chloride concentration, and has lower oxygen (Appendix A). This area of the Salinas is influenced the most directly by the releases from the reservoirs which cause periodic washouts of the entire habitat; the water released is probably nutrient-rich and hypolimnetic. The diatom flora of this region should be consistent with nutrient enriched and frequently disturbed assemblages. The assemblage should be dominated by raphid species which are able to regain the surface after burial such as members of the *Navicula*, *Nitzschia*, and *Surirella* (Barbour et al., 1999), it should also contain the largest numbers of planktonic and tychoplanktonic (normally associated with periphytic or terrestrial habitats but often suspended in the water column) (Lowe, 1974) genera such as *Synedra*, *Melosira*, and *Cyclotella* due to the proximity of the reservoir assemblages (Patrick, 1977). Members of the genera *Fragilaria*, *Caloneis*, *Neidium*, and *Diploneis* could also be present in relatively high numbers as these genera tend to live in slow flowing water of high sediment content (Patrick, 1977). The assemblage as a whole should consist of oligohalobous and halophobous (exclusively freshwater) species (Hofman, 1997) and

would probably have a smaller number of species with larger individual populations than the upper reaches (Patrick, 1977).

The lower stretches of the Salinas River are very wide, deep, and very slow flowing, with almost no plants on the banks which often are large mud flats (Site DAV, Figure 1). The substrate is almost all mud, often with a layer of fine sand several millimeters below (Appendix B). The water here is high in conductivity, although there is little influence from the ocean. The Salinas River lagoon is approximately 5 miles downstream and is not often open to Monterey Bay. The outflow from the lagoon is regulated using slide gates during the summer, which are opened when the water in the lagoon reaches a depth of 5 feet or higher. Occasionally in the winter, when a large storm or very high tides occur, the dune separating the lagoon from the ocean is breached, either naturally or by bulldozer, to prevent flooding of the fields nearby (Dennis Lebow, Monterey Country Water Resources Agency, pers. com.). The water here is high in inorganic nutrients, of increasing hardness, and very high in turbidity, dissolved solids, and conductivity (Appendix A). This stretch should be dominated by estuarine, β -mesohalobous (brackish water of 0.2-1.00 ‰) (Hofman, 1997) or extremely salt- and sediment-tolerant species from the genera *Amphora*, *Nitzschia*, *Fragilaria*, *Navicula*, *Surirella*, and *Diploneis* (Patrick, 1977). This assemblage should be even more dominated by a few very abundant species than those at the other sites on the river (Patrick, 1977).

The Salinas River as a whole is slow-flowing (in comparison to mountain streams), of slightly alkaline pH, and the water is relatively hard, high in calcium, and

variable but warm temperature (5.8-26°C) (Appendix A). Species preferring low pH (acidobiontic), soft water, cold temperatures or fast currents (such as *Achnanthes minutissima*) should be rare or absent from the system (Pentecost, 1997). Species preferring slow moving or standing water (limnophilous and limnobiontic respectively), warm temperatures (mesothermal - occurring between 15° and 30° C, or eurythermal - occurring over a range of 15° C or more) (Lowe, 1974) or which are indifferent to temperature, and those found in pH close to 7 or 8 (indifferent, alkaliphilous – pH 7 or higher, or alkalibiontic – pH over 7) (Lowe, 1974) should dominate the assemblage at all sites.

Site Descriptions:

Six sites along the Salinas River were chosen to represent low human impact, medium human impact, and high human impact environments using chemical data from CCRWQCB (CCRWQCB, 2000). The data available for site selection included pH, conductivity, turbidity, dissolved oxygen, salinity, water temperature, air temperature, coliform concentrations, chloride, nitrate-NO₃, nitrate-N, ammonia, ortho-phosphate, total phosphate, total dissolved solids, and others (Appendix A). As the data represented only a small portion of the annual cycle at the time when the sites were selected, their designations as low, medium, and high, are considered semi-quantitative. The major human impacts in the Salinas River are agricultural run-off, sedimentation, and water reclamation (Karen Worcester, CCRWQCB, pers. com.). There are no significant point sources of pollutants along most of the river (Karen Worcester, CCRWQCB, pers. com.). For this reason sites were chosen mainly according to nutrient and sediment loading. The

cities closest to the six sites, from low to high impact, are: Atascadero, Paso Robles, San Ardo, Greenfield, Chualar and Salinas, California. These sites are spread out along the length of the entire river (Figure 1) and fall into three categories: 1) Low (low turbidity, low nutrients, and upstream of the major reservoir influx); 2) Medium (Medium turbidity, low to mid-range nutrients, and downstream of the reservoir influx; 3) High (high turbidity, high nutrients and severe sedimentation).

Site H41 is located just upstream of the bridge over the Salinas River on Highway 41 in Atascadero, San Luis Obispo county, California. Atascadero Creek joins the river approximately one mile downstream. The river here is a small, shallow, and slowly flowing, with high vegetative cover on both banks. The substrate available for diatom colonization is gravel and large grained sand with small rocks, and filamentous algae and aquatic plants along the edges of the water (Appendix B). In April 2000, there was considerable diatom growth on all available surfaces, often at 100% cover (Appendix C). There was evidence of off road vehicle traffic in the river bed and along the banks as this site is easily accessible from several local roads. The water quality was relatively good at this site as it is surrounded by vineyards and livestock pastures and only a small proportion of its drainage area is used as impervious urban neighborhoods or intensive row-agriculture.

Site PAR is approximately 30 miles downstream, just upstream from the bridge at 12th Street in Paso Robles, San Luis Obispo County, California. Several seasonal streams join the river before Paso Robles, and a small reservoir is located on Santa Ysabel Creek, 2 miles upstream from the site. The Salinas at this site is slightly wider than at H41, but

still small, shallow, and slow flowing. The substrates available for diatoms are similar to H41, with a slightly larger proportion of sand and slightly less vegetation along the river banks (Appendix B). When the site was sampled, diatom cover was high on all substrates (Appendix C), and there were several types of grazers encountered with a very patchy distribution (pers. obs.). There was no evidence of vehicular disturbance, but there was evidence of hikers and dog walkers on the river banks. The water chemistry at this site was almost identical to that of H41 and there are similar land-uses in the surrounding area.

Site USA is 35 miles downstream of PAR, just downstream of Bradley, Monterey County, California. The Salinas River at this point is much wider, but is still wadable in most places in late spring when it was sampled. This is the first site below the introduction of the water from the Nacimiento and San Antonio Reservoirs. There is little vegetation on the banks and there are large sandy beaches along the edges of the river and several sandy islands in the center. The substrate here is almost entirely sand and small gravel, with a few rocks here and there and very little filamentous growth (Appendix C) or available aquatic plants (Appendix B). The diatom cover was low and not very thick, even in areas of very slow flow. The water chemistry at this site was only slightly different from the first two sites, probably due to its proximity to the reservoirs and the similarity of land use in the surrounding area. There was evidence of cattle crossing the river and wandering the banks but none were seen during sampling.

Site GRN is located at the Elm Street bridge in Greenfield, Monterey County, California, about 40 miles downstream from USA. This site is less wide than USA, but

considerably deeper, although crossings could still be made with caution in late spring. There are a few trees on the western bank and also high cover of annual and perennial vegetation, mostly *Orundo donax*. There are also several stretches of aquatic plants in the river's edges. This site has the most diverse substrate availability, including large sand stretches, gravel, small rocky areas, mud, and several patches of woody debris (Appendix B). The diatom assemblage covered almost 100% of the substrate, with a few filamentous species which were also covered with diatoms (Appendix C). Although the reservoir input is many miles upstream, the water was murky and sediment laden, probably due to the water's tendency to pick up sediment as it flows downstream. In contrast to the three upstream sites, the water quality here was more turbid, had slightly higher nutrient levels, and the pH was higher than any other site (Appendix A). There is extensive irrigated and fertilized row agriculture on the west side of the river and cattle grazing lands on the east side.

Site CHU is at Chualar River Road in Chualar, Monterey County, California, below the confluence of Arroyo Seco Creek, the only non-dammed creek originating in the Santa Lucia Range (Fred Watson, Watershed Institute, CSUMB pers. com.). Arroyo Seco discharges large amounts of water to the Salinas River seasonally at Soledad, California. This site has the least visually obvious riparian habitat. The Salinas River here is very wide, with extensive sand beaches. There are trees on the west bank, but they are far from the water's edge. There is almost no vegetation near the river, the bottom is almost exclusively sand, and there were almost no algal filaments (Appendix C). Diatom cover was very low and thin, and almost non-existent on the few available

rocks (Appendices B and C). Water characteristics were similar to USA, but turbidity was increased, possibly from Arroyo Seco, or due to a naturally increased sediment load (Appendix A). This site is surrounded on both sides by intensive row agriculture for many kilometers in all directions.

Site DAV is located at the Davis Road bridge near Salinas, Monterey County, California. There are a few small streams and creeks flowing into the Salinas upstream of this site and agricultural run-off is heavy due to several drainage ditches and sloughs in the area. There are large trees on one bank and fields on the other; the river is not very wide, but rather deep with considerable mud flats on both sides. The substrate is muddy, often with sand underneath, and there was a small cover of plants on one bank near the trees (Appendix B). The water quality was different from all the other sites; it had high conductivity, Ca and chloride (possibly due to tidal influence), and dissolved solids and nutrient levels were very high (Appendix A). This site is surrounded by the most intensive agriculture and has only a minor outlet to the ocean.

Methods and Materials

At each site, 50 10X10 cm quadrats were randomly placed along a haphazardly located 250m transect to estimate percent cover of filamentous algae, diatom film, and to note the underlying substrate type. These data were used to ascertain differences in the relative amounts of the algal forms and substrate types amongst sites. Data were plotted graphically and qualitatively analyzed (Appendices B and C).

The diatom sampling was done in the spring 2000, one month after the last rain and before the beginning of the water release season. The rain usually washes and scours

the algal assemblage causing high degrees of variability and initiating a successional process (Sabater, 1998, Stevenson, 1990, Power and Stewart, 1987, Blinn, 1980). Blinn (D. Blinn, pers.com.) recommends sampling 3 to 5 weeks following the last major rain. The water releases from the two reservoirs along the Salinas River have the same effect as a large rainstorm so sampling had to be accomplished before the first release. The releases are not definitively scheduled, but begin roughly in mid May (Joe Madruga, Monterey County Water Resources Agency, pers com.).

The EPA periphyton sampling protocols were used as a sampling design (Barbour et al., 1997). The Salinas River, however, is not a small stream containing pools and riffles, but a large slow-flowing river. The protocols were modified to accommodate both the water body and the scope of the study. At each site, samples of the naturally occurring periphyton assemblage were collected randomly along a haphazardly located 250m transect, preferably upstream from the bridge, using a large (18.85 cm²) sampler. An earlier pilot study using only algal filaments revealed that this size reduced effects of patchy algal distributions. The samplers used were PVC couplings with rubber o-rings to create a seal with the rock substrate. Three samples of each available substrate were collected: rock, sand, mud, and gravel. To do this, a random number was selected, and the substrate noted. Numbers were chosen until three samples of each substrate were obtained. All samples were in full sunlight and water depth was held constant at between 5 to 10 cm, as depth has been shown to affect assemblage parameters (Lowe, 1996). Rocks were collected whole and scraped clean of periphyton in the lab using a sampler, tweezers, small brush, and a suction device; the o-rings provided a seal with the rocks to

ensure that the entire sample came from only within the sampler. Mud and sand assemblages were collected by pushing a PVC sampler into the substrate and removing the top layer of sediment with a suction device. Gravel samples were collected by pushing the sampler into the substrate and using a spatula to scoop the sample into a jar.

All samples were preserved in 3% formalin to prevent changes in relative abundance due to reproduction and deterioration of filamentous algae (P. Kociolek, pers.com.). The rock and sand samples were the only substrate types represented adequately at all sites and only these were further analyzed. They were split in half to retain filaments for possible future use. The diatom frustules were cleaned using the hydrogen peroxide and potassium dichromate method described in Barbour et al., (1997). The resulting sample was diluted to 5mL and a sub-sample of 100 μ L was mounted permanently on a slide using Naphrax, a mountant made for diatoms. Diatom samples and slides have been deposited at the Diatom Collection, California Academy of Sciences.

As the diatoms did not dry evenly on the cover slip, the left and right sides of each slide were blocked off with tape 4 mm from the edges of the cover slip to ensure a representative sweep across the slide. The diatom counting method used was a series of three randomly located sweeps across each slide. This resulted in unequal numbers of diatoms counted per slide, but these data can be used to relate algal abundances to the surface area sampled by multiplying by 1326, resulting in an abundance/cm². There were 75 possible rows across the slide, of which 3 were counted, consisting of one one-thousandth of the original 18.85cm² sample ($1326 = (75/3 \times 1000)/18.85$). Diatoms were

identified to the lowest taxon using Patrick and Reimer, (1966, 1975), van Heurck, (1896), Krammer and Lange-Bertalot (1986-1991), with the assistance of Dr. Patrick Kociolek, California Academy of Sciences.

Data Analyses:

A descriptive table was constructed of the 53 most abundant diatom species, where they were found in highest abundance, general site characteristics and their known habitat preferences according to the literature (Table 1).

For each sample the applicable indices recommended by the EPA were calculated (Barbour et al., 1997). These included the Shannon-Wiener diversity, number of species per sample, and percent motile species; evenness (j) was calculated as suggested by Patrick (1977). Percent motile species, used to ascertain sedimentation intensity, is calculated using the total percentage per sample of three mobile genera: *Navicula*, *Nitzschia* and *Surirella* (Barbour et al., 1997). As no *Achnanthes minutissima* were found in the samples, this index was not calculated. Percent sensitive diatoms was also not calculated as very few species considered sensitive in the literature were found. The indices were averaged by site and substrate category and a standard deviation was calculated. The indices were tested for normality using the Kolmogorov-Smirnov test and homogeneity using Cochran's C (Zar, 1996). A two way fixed factor ANOVA was used to test for differences amongst sites and substrates, alpha level 0.05. When interactions were significant, means were graphed to determine which sites or substrates were responsible. A post-hoc Bonferroni was done when the interaction was

insignificant. Power was calculated using Cohen, 1988. in order to estimate the sample size that would have detected a difference at 80% power.

A cluster analysis was done using Primer 5.0 (Clarke and Gorley, 1994, Clarke and Warwick, 1994), with a Bray-Curtis similarity matrix on fourth root transformed relative abundance data. Clusters were considered to consist of at least three samples, were designated at 60% or more similar, and named according to their general characteristics. The results of this analysis were further analyzed with SIMPER (Primer 5.0) to calculate the percent of the total similarity value contributed by each species within and between clusters.

A Principal Components Analysis was performed using the chemical data from the CCRWQCB collected at the closest date to the algal sampling (CCRWQCB, unpublished data, 2001). For all sites except DAV, this was the April water sampling data; DAV's water data were from February as April sampling did not take place at this site. Data were log-transformed when a draftsman's plot revealed skewness (Clarke and Gorley, 1994, Clarke and Warwick, 1994). The PCA information was then plotted and compared with a multi-dimensional (MDS) scaling plot of the species abundances. The MDS is a two-dimensional unit-less plot which is constructed using the species abundance data from each sample and the Bray Curtis similarity matrix described above. A BIOENV (Primer 5.0) procedure was done excluding the chemical variables which were correlated at more than 90%. The variables used were: conductivity, turbidity, pH, NH_3 , NO_3 , and orthophosphate. The BIOENV procedure uses the Bray-Curtis similarity matrix of the diatom data and the uncorrelated chemical variables to match the chemical

data with the biological data. The procedure then lists the most influential environmental factors in combinations that produce the highest degree of correlation with the diatom assemblages.

Results

The 53 most abundant species, their total relative abundance, their habitat preferences along the Salinas River, and their known habitat preferences from the available literature are listed in Table 1. Of the 53 species, the habitat characteristics of 25 species had been reported in the literature. Thirteen of these 25 species are usually found in eutrophic waters, defined as having high nutrient concentrations. All of them are either alkaliphilous (occurring at pH around 7 but having best development at pH of more than 7), or indifferent to pH (Lowe, 1974). Using the Halobion system, based on affinity to salinity, all 25 species were either indifferent to small amounts of salt or halophilous (presumably stimulated by small amounts of salt), with one species being mesohalobous (brackish water) (Lowe, 1974, Patrick and Reimer, 1966). The Saprobien system (Kolkwitz and Marston, 1908) classified 11 out of the 24 species as usually found in areas where the oxidation of organic matter is complete and the water is high in inorganic nutrients (Lowe, 1974). Only 5 species out of the 25 were usually found in areas where decomposition is in progress (Lowe, 1974). All known species were eurythermal (existing over a wide range of temperatures), and 18 were cosmopolitan (having a very wide geographical distribution) (Lowe, 1974). Two are indicators of pollution, and one is resistant to pollution. Most of the species are periphytic (growing on a solid substrate), epiphytic (growing on a plant or other alga), tychoplanktonic (living

in both the water column and on the substrate), or epilithic (living on rocks). No decisively euplanktonic species were found.

Shannon-Wiener diversity for the site and substrate categories ranged from 3.1052 (CHU sand), to 4.2874 (USA sand) with standard deviations ranging from 0.1362 to 0.9849 (Table 2). Evenness (J) ranged from 0.5341 (CHU sand) to 0.8747 (CHU rock), with standard deviations ranging from 0.0126 to 0.1367 (Table 2). Mean number of species ranged from 20 (CHU rock), to 64.7 (GRN rock), with standard deviations ranging from 4.58 to 25.42 (Table 2). Mean percent motile species ranged from 38.37%, H41 sand, to 85.52%, GRN rock, with standard deviations ranging from 3.66% to 22.55% (Table 2).

The two factor ANOVAs on the indices produced variable results. Shannon-Wiener diversity was not significantly different among sites or between substrates and the interaction factor was also not significant (with relatively low power, Table 3). More than 1000 samples would have had to be analyzed to have a significant result between substrates at 80% power, and 15 for site, given the range of values (d) (Cohen, 1988, Table 3). Evenness was significantly different among sites, the interaction factor was significant, but substrate was not (also with relatively low power, Table 3). Forty-four samples would be necessary to have a significant result at 80 % power for substrates, and 7 samples would be necessary for site (Table 3). The graphed means showed that the sites are variable with respect to substrates, with three sites more even on sand and three on rock. CHU had the largest difference between sites (Figure 2). Mean number of species was significantly different among sites and between substrates, but the interaction

was not significant (with an acceptable power rating, Table 3). Twenty-five samples would have been necessary to allow 80% certainty for the substrate factor (Table 3). A post-hoc Bonferroni revealed that CHU was different from all other sites and that the substrates were different from each other (Table 3). Percent motile species was significantly different among sites, the interaction was significant, but substrate was not (Table 3). The graph of means per site and substrate showed a similar distribution as the evenness index: three sites had more motile species on sand, and three on rock (Figure 3). The sample size needed to guarantee 80% certainty of the substrates' insignificance was 180 (Table 3).

The cluster analysis based on species composition and relative abundance (%) resulted in 5 clusters of more than three samples at 60% or more similarity and five that samples did not cluster (Figure 4). The clusters are named for their location on the Salinas River and the substrate type, USA rock, USA sand, Upstream, Downstream sand, and Downstream rock (Figure 4). The SIMPER analysis revealed that the most abundant species (see Table 1) were responsible for the clustering patterns (Table 4). The downstream rock cluster was similar due to the abundances of five *Nitzschia* species, *N. palea*, *N. microcephala*, *N. frustulum*, *N. acicularis*, and *N. linearis* (Table 4). The species most responsible for the similarity within the downstream sand cluster included *N. palea* – at a higher abundance, and *N. linearis*, but also *F. construens* var. *venter*. The USA rock cluster was highly delineated by *N. frustulum*, *A. perpusilla*, *N. pelliculosa* and *A. deflexa*, responsible for a total of 46.14% of the similarity. All the other clusters had a more evenly distributed similarity spread. The USA sand cluster had a high abundance

of *N. microcephala* and *N. frustulum* but *N. gregaria*, *A. perpusilla* and *N. cincta* also accounted for a total of 18.71 % similarity. The Upstream cluster had a high abundance of *N. gregaria*, *N. cincta*, and *M. varians*, which were responsible for a large portion of the similarity (See Table 4 for details).

The differences between the downstream rock and downstream sand clusters (Figure 4) were related to the dominants *N. cf. subminuscula*, *Nitzschia sp. 3* and *N. pelliculosa*, which were responsible for a total of 11.25% of the dissimilarity (Table 5). The differences in the Downstream rock and USA rock clusters were mostly due to the abundances of *N. palea* and *N. acicularis*. Differences between the downstream sand and USA rock clusters were mostly due to the high abundance of *N. palea* in the downstream sand cluster and the high abundances of *A. deflexa* and *N. pelliculosa* in the USA rock cluster. The species responsible for the dissimilarities between the downstream rock and USA sand clusters were more evenly distributed but were related to domination by *N. acicularis*. The differences between the downstream sand and USA sand clusters were mostly due to the high abundance of *N. palea* in the downstream sand cluster and the high abundance of *N. frustulum* in the USA sand cluster. USA rock and USA sand clusters were different due to the high abundance of *A. deflexa* in the USA rock cluster and the absence of *A. delicatula*, *Amphora sp. 1* and *Fragilaria sp. 3* in the USA sand cluster. The downstream rock and upstream cluster were different mostly due to the high abundance of *M. varians* in the upstream cluster. The differences between the downstream sand and upstream clusters were due to *M. varians* in the upstream cluster and *N. palea* in the downstream sand cluster. The USA rock and upstream clusters were

different due to the varying abundances of *A. delicatula*, *M. varians* and *C. meneghiniana* (Table 5).

The chemical data from the CCWRQCB were used in a Principal Components Analysis to qualitatively connect the diatom assemblage patterns revealed in the cluster analysis to the water quality characteristics at each site. The PCA results were interpreted as follows: the most influential chemical variables in principal component #1 were turbidity, ammonia, nitrite, phosphate, total nitrogen, and suspended solids (Table 6). Principal component #2 was influenced by conductivity, chloride, dissolved solids, and nitrate (Table 6). Principal components #3 (pH and phosphorous species), #4 (pH and nitrogen species), and #5 (pH and phosphorous species) show high eigenvalues for some of the chemical variables, indicating that the system is more complex than any simple two dimensional combination of variables (Table 6). The resultant diagram shows that DAV was very different chemically from the other sites, with two other groups forming, GRN and CHU together, and PAR, H41, and USA together (Figure 5).

The BIOENV analysis, used to match the chemical data with the species abundance data, resulted in relatively low correlation coefficients (Table 7). The chemical variables most highly correlated with the structure of the diatom distributions were nitrate, turbidity, chloride concentration and, to a lesser degree, pH and orthophosphate (Table 7). Comparison with the multi-dimensional scaling (MDS) graphic, produced using the same similarity matrix as the cluster diagram, showed that the sites do tend to exhibit similar differences both chemically and biologically (Figure 4). The MDS diagram (Figure 6) places the samples in a unit-less 2 dimensional space

using the species abundance similarity data. This diagram was compared to the PCA plot (Figure 5) revealing similar placement of site (due to their chemistry) and samples (due to their diatom assemblages). Chemically, DAV is very dissimilar to the other sites (Figure 5), whereas biologically, it is interspersed with the other downstream sites (Figure 4). H41, PAR, and USA group near each other in both diagrams, as do GRN and CHU sand (Figures 5 and 6). The MDS further shows that CHU rock samples were highly anomalous in all the analyses done. These did not cluster, and may have been partially responsible for the significant interaction factors in the ANOVAs. See discussion below.

Discussion:

The diatom assemblage in the Salinas River appears to be characteristic of water bodies that are considered human-impacted in other regions (Table 1). Most of the species with known habitat associations are from eutrophic or sedimentary environments. All were alkaliphilous to some degree, indicating that the water in the Salinas is rarely, if ever, at a pH of less than 7 (Appendix A). Most species were indifferent to conductivity and current, which was not measured directly in this survey. Several species have seasonal peaks in density e.g., *Melosira varians*, which becomes highly abundant in summer eutrophic lakes (Patrick, 1977). *Nitzschia palea* is well known as an indicator of pollution and human impact as it is found in high numbers in eutrophic, sedimented, and otherwise disturbed areas globally (Lowe, 1974, Patrick, 1977, Pentecost et al., 1997). The assemblage was generally made up of tolerant, weedy species that are able to rapidly reach high numbers in non-optimum waters. There was, however, no obvious trend from upstream to downstream; several species, such as *N. frustulum*, *N. microcephala*, *F.*

construens var. *venter*, and *G. olivaceum* were found in highest abundance at opposite ends of the river, in markedly different habitat conditions (Table 1 and Appendix A). Regardless, the most abundant species in the river are tolerant of a broad range of ecological conditions, found over large geographic areas, and are known to exist in high numbers in relatively "poor" water quality. Although "poor" water quality is not well defined, it is considered here as any situation where a large number of species are no longer present due to direct human-induced changes in the environment. As no previous information on the diatom flora and little chemical data for the Salinas River are available, it is difficult to label this water "poor" quality, as no changes in the diatom species and their abundances have been recorded.

Surprisingly, many species were found in the highest abundance at sites that were not within their published habitat range. For example, the periphytic *F. construens* var. *venter*, *N. linearis* and *A. lanceolata* (amongst others) were relatively more abundant on sand, not rocks, as indicated by Lowe, 1974 (Table 1). *Amphora perpallida*, which is usually found in somewhat brackish water (Patrick and Reimer, 1975), was found abundant at USA, a site which has no salt water influence and low conductivity in general. In the slow-flowing Salinas River, one would not expect halophilous species in high numbers, but four were present. Possibly these species are highly adaptable and acclimatization has taken place. The Salinas River water characteristics change drastically seasonally (note the size of the Appendix A error bars), which probably selects for species able to adapt to a new set of conditions without changing their genetic

makeup. These would presumably be the same species that are able to compete and thrive in "poor" waters elsewhere.

The EPA indices by site and substrate did not show any obvious trends in either the upstream versus downstream direction, or between the different substrates sampled (Table 2). The absence of *Achnanthes minutissima* indicates that either a later successional stage was sampled (Barbour et. al., 1997) or true absence of this species, but, regardless, this index could not be calculated. The ranges in the indices were quite high, indicating some differences amongst categories, but exhibited no identifiable trend. Most intriguing was the presence of only 20 species on the CHU rocks, indicating a possible scouring event or a high number of grazers at this site, as chemically, the site is not unique. The number of diatoms on these rocks was exceptionally low in comparison to the other sites. The highest number of species was found at GRN which is effectively the middle site with the most diverse substrates and a mix of chemical levels (Appendices A and B). Contrary to expectations, none of the indices declined noticeably towards the more impacted end of the river (DAV), and there was not a higher number of species on the more stable rock substrate. These indices may not be sensitive enough to discriminate between these similarly impacted sites, they may only be able to reveal differences between extremely different environments. The distribution of percent motile species was also unexpected as the percentage should have increased dramatically downstream as the river becomes more sediment-laden. This index should also have been able to differentiate sand from rock substrates (Table 2).

The ineffectiveness of the assemblage-wide indices to distinguish among sites in the Salinas River is even more apparent from the two way ANOVAs (Table 3). This lack of obvious pattern may be due to the highly variable seasonal flow in the river; chemical and physical variables may change so often and rapidly, that samples taken at any one time may not be indicative of the general environment. Monthly water sampling may not be adequate to reveal the temporal variation in habitat conditions in this river. The diatom assemblage could be in constant state of adjustment to the new environment and may never reach an appropriate successional stage for rapid bio-assessment sampling. The indices recommended by the EPA are generally applied in stable habitats, such as spring fed streams and rivers with a natural year-round flow (Barbour et al., 1997). The flora in these water bodies have time to stabilize (at least within a season) with respect to the more general assemblage indices. In any case, the indices were confounded by unknown factors.

Shannon-Wiener diversity was insignificant for both factors. The use of the diversity index for water quality has long been questioned by freshwater diatomists as it is relatively sensitive to dominance and insensitive to changes in the species themselves or the numbers of species, which are important variables in most water quality analyses (Patrick, 1977, Stevenson, 1984). This lack of sensitivity could also be due to the relatively high diversity values that all diatom assemblages seem to possess (Stevenson, 1984). Shannon-Weiner rarely exceeds 5.0, suggesting that most diatom assemblages are already in the 'above normal' diversity range (Krebs, 1989). Therefore, a low value within a diatom study is not necessarily low enough to indicate ecological "disturbance"

or stress in comparison to low diversity values found in other types of assemblages. The highest value found in this study was 4.46, the lowest was 2.45. Stevenson (1984) suggested that a low diversity value itself does not indicate a "depressed" assemblage, but rather it is the differences in diversity that is important, differences such as a drop in diversity along a gradient, or a low value at a specific site with an extreme environment. Neither of these situations appeared to exist on the Salinas. CHU rocks, with low number of species, still had high diversity in comparison with the other sites (Table 3).

As diversity indices are more sensitive to dominance than changes in the number of species or the species themselves, Patrick (1977) suggested that perhaps a percent dominance value would be more appropriate for indicating a stressed diatom assemblage. Impacted areas should have a few very abundant species and several relatively rare species and lesser impacted areas should have more evenly distributed dominance, with several abundant species and several mid-range species. The evenness (J) values in the Salinas River diatom data showed a higher sensitivity to possible changes in the assemblage due to habitat differences. This index discriminated amongst sites but not substrates, with a significant interaction (Table 3, Figure 2). This interaction makes interpretation difficult if not impossible as half the rocks were more even than the sand samples (Table 2, Figure 2). The graphed means (Figure 2) showed no identifiable pattern from upstream to downstream, e.g., rocks were not more even upstream and sand downstream. Some sites had already been identified as somewhat anomalous, such as CHU rocks and USA rocks, both with low densities of diatoms. CHU rock samples were much more even than CHU sand, probably due to their low densities in general, but USA

rock samples were no more even than sand (Figure 3). Regardless, there is no discernible pattern that can be identified to explain the interaction, perhaps a larger sample size ($n > 3$) would have been more revealing. There is a general trend towards lower evenness values downstream at the more impacted sites, but due to the significant interaction factor, this observation is not statistically founded. Perhaps as the environment becomes more stressful chemically, the additional stress of a shifting substrate becomes intolerable, and a suite of diatom species that prefers rocks becomes more prominent. The CHU rock samples displayed a very high evenness. However, it was calculated with an average of only 87 individuals per sample, and this may be artificially inflated due to the small sample size. Although it has a complex distribution, my results suggest this index should be included in the suggested indices of the periphyton protocols of the EPA.

The number of species per sample was the most statistically useful index in this study, perhaps due to the quantitative sampling method. Both factors were significant with high power, but the interaction was not (Table 3). Only one site proved different from the others (Table 3), most likely due to the low numbers of diatoms on rocks at CHU (Table 2). In general, there were fewer species on rocks than sand (Table 3), however, there was no upstream-downstream trend (Table 2). One might expect a higher number of species on rocks due to the stability of the substrate in comparison to sand. Rocks support a larger three dimensional habitat (microscopically) than sand, allowing species to grow on or very close to other species. Although the sand substrate has a larger surface area the crevices in between grains are very small and may not be appropriately sized for diatoms. The interstitial spaces would provide good habitat for

some species, but the lack of light penetration and water circulation would become limiting to the number of individuals able to occupy this area. In addition, rocks support green filamentous growth, which allows additional attachment surfaces for epiphytic and motile species. Some species such as *Gomphonema* sp. only immigrate when horizontal space is limited. In this case, they secrete stalks and live vertically (Patrick, 1977). This vertical growth form allows for more individuals and species to colonize a given area. Sand is relatively unstable, subjected to a high level of disturbance both temporally and spatially. Fewer species should be present, weedy species should dominate, and diversity, evenness, and the number of species present should be lower.

In this study, however, the sand proved more able to support more species. Sand does have a highly irregular microscopic three dimensional structure (Yamamoto and Lopez, 1985), perhaps irregular enough to provide space for many individuals. The diatoms found there were very abundant, sometimes several millimeters thick, obviously growing on top of one another. In contrast to the tumbling disturbances experienced by species on sand, there must be a certain amount of scour experienced by the species living on rocks due to the high turbidity of the river and the fact that the rocks are usually surrounded by sand. Although it was not measured directly, flow in the Salinas is similar overall during this season, so scour should be experienced at a similar rate at all sites. This could explain the rocks having a lower or statistically the same number of species as sand at all sites. The rocks also form a good habitat for grazing insects and larvae that may not move easily on sand. Some diatom species may also be able to survive on the sandy substrate after having been knocked off the adjacent rock if the water flow is slow

enough so they are not swept away or buried. Perhaps the sand is disturbed at some intermediate level, allowing the highest possible number of species by keeping a constant area open for colonization, effectively holding the assemblage at several levels of succession within the sampling area (Sousa, 1979).

Regardless of substrate, the number of species does not decline from upstream to downstream, which would be the case if fewer species were able to live in the more stressful conditions. This suggests that either the species themselves are different, or there is little effect of nutrient and sediment loading on the number of species present, or that there are other complex factors operating in this river that have not yet been identified. Drift may have brought more species downstream and therefore there would be higher numbers available for colonization in the species pool (Patrick, 1977). Perhaps the water quality downstream, although unpleasant for human use, has little effect on diatoms.

The ANOVA on percent motile diatoms per sample was significant for site and the interaction factor, but not substrate (Table 3). The graphed means did not show any interaction trend from upstream to downstream, or any pattern in general (Figure 3). Again, perhaps more samples would have clarified the interaction, or rendered it insignificant. The expected result was that there would be more motile diatoms on sandy substrates as they would be able to regain the surface after burial or migrate, etc., whereas non-motile diatoms would remain buried if buried. Non-motile species should have had a much higher abundance on rocks, where they can attach and remain in place. Although the significant interaction makes interpretation of the main effects impossible, there were,

on average, more motile species on rocks than on sand (Table 2). The different sites somehow reacted differently in this index, perhaps due to the relative availability of the substrates, or the diatom species pool. It may be that an unmeasured disturbance occurs at the sites where there are more motile diatoms on rocks, making it more difficult to remain on the sand surface. The interaction factor complicated this analysis, but this motility-specific index should be able to discern amongst substrates (Figure 3). Site-wise there was a slight trend towards a higher percentage of motile diatoms downstream, where the river is siltier (Appendices A and B), but this result was not consistent (Table 2, Figure 3). The EPA calculates this index using only three motile genera, *Nitzschia*, *Navicula* and *Surirella* (Barbour et al., 1997), whereas in reality there are many more motile species than those in these three genera. Any diatom with a raphe is motile and has the potential to unbury itself after a disturbance. However, even with a raphe, some species are very small or shaped inappropriately for movement through sand. Most of the abundant species found in this study were motile, for example *Achnanthes* sp., *Gomphonema* sp., *Amphora* sp, and *Sellaphora* sp. (Table 3 and Appendix D). Only a few genera found in the Salinas river are non-motile; these include *Fragilaria* sp., *Melosira varians*, *Cyclotella* sp., and *Diatoma* sp. (Table 3 and Appendix D). These araphid genera made up only a small fraction of those found in this study (Table 1 and Appendix D). Perhaps if all the motile genera are included in the calculation, this index would detect a difference between substrates. The fact that the three genera used by the EPA to calculate this index are the most abundant at all sites and on both substrates may have confounded the calculation.

Perhaps a more revealing approach for assemblage analysis is the cluster diagram made using the Bray-Curtis similarity measures (Figure 4). When the cut off point is arbitrarily set at 60% similar, only three samples remain completely isolated from any others, with two clustering with each other (not considered a cluster here). Three of the five samples that did not cluster are the CHU rock samples, which have already been noted as anomalous, having very low diatom density and a low number of species (Table 2). At 60 % similarity, the clusters group mostly according to site, or upstream versus downstream. At similarities higher than 60 %, however, the differences between the substrates become apparent, with smaller, substrate driven clusters appearing within the larger ones (Figure 4). It would appear that on a broader scale, site is more important in structuring the assemblage, whereas within site, substrate becomes definitive. For example, the downstream rock and downstream sand samples are similar to each other at approximately 70%, however, the downstream sand cluster groups separately from the from the rock cluster at almost 80% similarity (Figure 4). All five of the non-clustering samples are rock samples. Rocks may be patchy on a larger scale than sand, producing higher variability within the same sampling area. USA sand and rock samples separated out from each other, but these samples were also dissimilar to any other sites. This site is the nearest to the influx of the water released from the two large reservoirs each summer. The higher level of physical disturbance here could be structuring the assemblage, as it does not appear to be particularly different from the other sites chemically (Appendix A) or substrate-wise (Appendix B). There may be some unknown and unmeasured variable at USA that is responsible for the differences in its diatom assemblage structure. These

samples were also highly variable, shown by the high standard deviation for 'mean number of species' on USA rock in Table 2. However, the sand samples from this site have very high diversity and evenness values (Table 2), suggesting that the optimum disturbance regime responsible for healthy diatom assemblage characteristics is different on sand and rock. Rocks may produce a more diverse and even assemblage at lower levels of disturbance than sand.

The SIMPER analysis revealed that the five clusters were considered similar due to similar abundances of the most abundant species (Tables 1 and 4). *Nitzschia palea* was present as a major contributor to similarity in four out of the five clusters. This species is known for its ability to survive and thrive in conditions considered adverse for other species and is a known indicator of pollution (Table 1; Lowe, 1974). It is the most abundant species along the Salinas River and is found globally. It has, for example, been reported in a river on Mount Kilemonjaro, Kenya (Pentecost et al., 1997). It was much more abundant (relatively) downstream than upstream, perhaps indicating a less than optimal environment for other species. It was abundant, however, at all sites, showing that for *N. palea*, the habitats along the Salinas River are not unsuitable. Several other species contributed to the similarities within clusters, many of which belong to the genera *Nitzschia* and *Navicula* (Table 4). Some species have obvious habitat-oriented divisions. *Melosira varians*, for example, was much more abundant upstream than downstream (Tables 4 and 5). This species is non-motile and reaches high abundances in still or slowly flowing water in warm eutrophic areas (Patrick, 1977, Lowe, 1974). It is a large, barrel-shaped, centric diatom, unlikely to be able to tolerate the repeated burial or scour

experienced downstream of the reservoirs. It was much less abundant at USA than upstream and was absent downstream. Other species were more ubiquitous, with differences in abundance dictating the cluster groupings instead of presence or absence. For example, *N. frustulum* 's average abundance within USA rock cluster was 31.9%, but it was only 11.78% in the downstream rock cluster (Table 4). This indicates that, like *N. palea*, *N. frustulum* is able to live and reproduce at all sites, but may have some advantage at a few sites. This is a very small species that was highly abundant in the Salinas River in areas (like USA) where not many other species were found. It is tolerant of many ecological conditions (Table 1). In addition, its small size may enable it to escape grazing or simply fit into smaller spaces on surfaces that other species would be swept out of during a disturbance. All of the species listed in Table 4 as the most important to structuring the clusters are also listed in Table 1 as the most abundant species in the Salinas River. The assemblages in the clusters are, not surprisingly, driven by the most abundant species, most of which are known for their tolerance of a wide range of ecological conditions. Rather than presence or absence of species, the similarities are driven most often by differing abundances, suggesting that all the abundant species can survive at all sites, but that they can be at a disadvantage in some areas, depending on chemistry, disturbance, and the presence of other species that could out-compete them for space.

The results of the SIMPER analysis depicting the species responsible for the dissimilarities between cluster-pairs contains most of the same species as the similarity table (Tables 4 and 5). *Nitzschia palea* again predominated, present in 8 of the 10 lists of

the top ten species operating on the cluster pairs (Table 5). Only three of the species listed are not in the top 53 in Table 1. Again, the differences in the clusters are due to the most abundant species and their relative abundances within each cluster. The most decisive differences occur between the downstream and upstream clusters, with several species having high abundances in one cluster and none in the other, such as *Melosira varians*, *Nitzschia lacuum*, and *Fragilaria capucina* in the downstream sand versus upstream comparison (Table 5). This is not due to the complete absence of the species, but a result of the species having an average abundance of less than 0.005% within the cluster. The USA samples differed from upstream as a result of two *Achnanthes* species and *Melosira varians*. No data other than the alkaliphilous nature of *A. deflexa* were available for the *Achnanthes* species, but *M. varians* displays similar chemical preferences to *N. palea* (Table 1). The three genera, *Nitzschia*, *Navicula*, and *Achnanthes* are heavily represented again, all have motile species with broad ecological ranges and a tolerance if not a preference for high nutrient levels. Although the diatoms along the Salinas River are found in differing abundances in different areas and, at least downstream and at USA, on different substrates, none of the more abundant species (other than *M. varians*) is absent from any one site. This indicates that at least the most abundant species can survive in the conditions at all sites. The differing abundances and relative abundances must be due to a factor that interacts with the water chemistry to produce the observed habitats, such as disturbance, grazing, ease of reproduction and colonization, or competition for space.

The PCA analysis revealed that DAV is chemically very different from the other sites, at least at the time of its sampling, which was in February of 2000 instead of April 2000 as for the other sites (Figure 5). GRN and CHU had similar chemical components as did PAR and USA, H41 was slightly different from PAR and USA, but not very much (Figure 5). The first principal component, responsible for 65.8% of the total variation, was found to be related to turbidity, ammonia, nitrite, phosphate, total nitrogen, and suspended solids, all factors influencing diatom growth and survival; it follows that the diatom assemblages should be very different at the sites progressing from the origin to infinity (Table 6). The diatoms found at DAV, however, do not differ in the cluster analysis or the SIMPER analysis from those of the other downstream samples, possibly due to the time of the water sampling. The second principal component, responsible for 25.9% of the total variation, was related to conductivity, chloride, dissolved solids, and nitrate, also factors important for diatom life; DAV again does not biologically match the chemical situation (Table 6). There could be a chemical threshold beyond which the species settle into a predictable pattern. If this were reached at GRN, all sites downstream would be similar, regardless of minor chemical differences beyond the hypothetical threshold. Perhaps there is a more important factor in the Salinas River influencing diatom assemblages and populations than the chemical constituents measured in 1999-2000. More likely, there are several factors operating at once, which are not identifiable without explicit and complex field experiments manipulating the chemistry and the rate of flow and scour along the river.

The results of the BIOENV procedure showed again that there is not a single chemical factor determining the structure of diatom assemblages along the Salinas River (Table 7, Figure 6). At best, at a correlation value of less than 0.34, five chemical factors can be identified that have an influence on the diatom assemblage structure: nitrate, turbidity, chloride, pH, and orthophosphate (Table 7). Although these are highly influential constituents in any freshwater body, the coefficients of correlation for all factors and combinations of factors was very low. In most other freshwater studies of this kind, phosphorous plays a much more important role in structuring the assemblage than nitrogen (Lund, 1969). The diatoms in the Salinas appear to be more influenced by nitrogen, possibly due to the high amounts applied to the agricultural fields along its length. When comparing the MDS (Figure 6), essentially a cluster diagram in two dimensions, to the PCA diagram (Figure 5), it can be seen that the biological DAV samples again do not group separately from the GRN and CHU samples. However, the PAR and H4I samples do group together, as they did in the cluster and the PCA. The USA samples are scattered amongst the PAR and H4I samples in the MDS, supporting to some extent the cluster analysis and the PCA.

Further investigation in the Salinas River into factors such as disturbance regimes (scour and sediment turnover), preferential grazing by insects and larvae, colonization and reproductive rates of the species dominating the assemblages, and field experiments manipulating different chemical constituents would prove informative in disentangling the reasons for the displayed diatom assemblage structure (Powers et al., 1972). The low abundances of diatoms on rocks at CHU and USA is intriguing, as such a difference

between substrates cannot be chemically caused; this is likely to be due to scour or grazing. The similarity in diatom assemblages from DAV to GRN and CHU (with very different chemistry) could also be due to some physical or biological variable; this particular discrepancy could easily be explained by the timing of the water sampling, however. Introducing small point sources of nutrients at GRN and CHU and monitoring changes in species abundance could resolve this question (Underwood, 1998). There are many factors that influence diatom growth and survival that were not measured in this survey, most of which should be experimentally manipulated in order to determine their relative importance. For instance, small point sources of nutrients or sediment could be added at several sites while monitoring changes in the assemblage over time and space, providing direct evidence for human impacts. Other recommendations include quantitatively surveying more rivers in coastal Central California, in all seasons, to determine the effects that periodic flooding and drying may have on both diatom assemblages and water chemistry. The water chemistry could change on the order of days, not months – as was sampled here. The diatoms may change distributions very rapidly in response to these large scale and abrupt disturbances. These data would provide a sound frame of reference, not only for comparison to rivers in other regions, but also to establish whether or not these waters can truly be considered impaired, or whether these seasonally flowing rivers merely have very different ecological characteristics.

Conclusions:

The diatom assemblages in the Salinas river were structured by a few highly dominant species. These species are cosmopolitan, tolerant taxa which are present in many types of environments globally. The assemblages cluster together due to differing densities of these species; they were all, however, present at all sites, and thus available in the species pool for colonization. Some factors in the environment were governing which species dominated in which habitats. These factors could be chemical, physical, and biological.

The species inhabiting specific sites are somewhat correlated to the water quality and substrate characteristics of the area. Several chemical factors probably operate together to produce these correlations. No single factor such as nutrient concentration or turbidity could be identified as the most influential assemblage-structuring variable. Field experiments with small point sources at several sites could untangle the effects of the many variables.

The indices recommended for habitat assessment by the EPA and USGS should be modified for rivers that have a seasonal flow regime, are highly sediment-laden, and do not have point sources of pollution. Sediment load and disturbance levels may be more important than nutrient and mineral levels in these types of rivers in structuring the diatom assemblage. The number of species per sample and the evenness indices were the best at revealing possible trends associated with abiotic environmental change in the Salinas River. The motile species index should be modified to include all motile species because, although the *Nitzschia-Navicula-Surirella* calculations are based on these

genera's tolerance to sedimentation and pollution in general, in some regions they may be so ubiquitous that other genera become more important to the index. In addition, more species-specific indices, such as the *Achnanthes minutissima* index, should be established as individual species' sensitivities can be much more informative than assemblage-wide characteristics. Also, the protocols should be adjusted to include an ability to calculate actual abundance per surface area, so that species abundances can be compared individually, in an index format or otherwise, without the complicating factor of all the other species present in the sample. This method would also allow comparisons to be made amongst water body types and across regions.

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Table 1: The 53 most abundant species in the Salinas River, their relative abundance (%), sites where found in the highest abundance, and habitat characteristics from this study and available literature (from Lowe, 1974, and Patrick and Reimer, 1966 and 1975). Definitions to limnological terms are listed at the end of the table.

Site Characteristics are noted by number: 1 – (H41) medium conductivity, chloride, dissolved solids, pH, hardness, low suspended solids, nutrients, turbidity. 2 – (PAR) medium conductivity, chloride, dissolved solids, pH, hardness, low suspended solids, nutrients, turbidity. 3 – (USA) medium pH, low conductivity, chloride, dissolved, and suspended solids, nutrients, hardness and turbidity. 4 – (GRN) high suspended solids, pH, medium conductivity, dissolved solids, hardness, nutrients, turbidity, low chloride. 5 – (CHU) high suspended solids, turbidity, medium conductivity, dissolved solids, pH, hardness, nutrients, low chloride. 6 – (DAV) high conductivity, chloride, dissolved solids, nutrients and hardness, medium suspended solids, pH, turbidity.

TAXON	Percentage of total	Occurrence in this study	General habitat characteristics	Known habitat associations
<i>Nitzschia palea</i>	22.9%	DAV sand CHU sand	6, 5	Eutrophic conditions, indifferent to pH, conductivity, current, mesosaprobic to polysaprobic, eurythermal, periphytic, tychoplanktonic, euplanktonic, lakes and ponds, cosmopolitan, indicator of pollution, extremely tolerant
<i>Nitzschia frustulum</i>	8.2%	GRN rock H41 rock	4, 1	Eutrophic conditions, alkaliphilous, euryhalobous, limnobiontic, periphytic, tychoplanktonic, euplanktonic, cosmopolitan
<i>Nitzschia microcephala</i>	7.7%	USA sand DAV rock	3, 6	alkaliphilous, halophilous, indifferent to current, eurythermal, cosmopolitan
<i>Nitzschia acicularis</i>	6.1%	GRN sand DAV rock	4, 6	Eutrophic conditions, alkaliphilous, indifferent to conductivity, mesosaprobic, limnophilous, euplanktonic, cosmopolitan
<i>Fragilaria construens</i> v. <i>venter</i>	5.8%	H41 sand DAV sand	1, 6	Oligotrophic to mesotrophic conditions, indifferent to conductivity, mesosaprobic to saprophobic, limnobiontic, periphytic, tychoplanktonic, cosmopolitan
<i>Nitzschia linearis</i>	4.8%	GRN sand DAV sand	4, 6	Eutrophic conditions, alkaliphilous, indifferent to conductivity, oligosaprobic, rheobiontic, eurythermal, periphytic, springs and streams, cosmopolitan, autotrophic

Table 1 continued

TAXON	Percentage of total	Occurrence in this study	General habitat characteristics	Known habitat associations
<i>Navicula gregaria</i>	4.0%	PAR sand H41 rock	2, 1	No data available
<i>Navicula cincta</i>	3.5%	H41 rock H41 sand	1	No data available
<i>Amphora perpusilla</i>	3.0%	USA rock DAV rock	3, 6	Alkaliphilous, mesohalobous, epilithic, tends to occur in large numbers on fixed solid substances
<i>Melosira varians</i>	2.9%	PAR sand H41 rock	2, 1	Eutrophic conditions, alkaliphilous, indifferent to conductivity, current, mesosaprobic, eurythermal, periphytic, lakes, ponds, streams, springs and ditches, cosmopolitan, forms large blooms in summer in eutrophic waters
<i>Navicula cf. subminuscula</i>	2.0%	DAV rock GRN rock	6, 4	No data available
<i>Navicula cari</i>	2.0%	PAR sand CHU sand	2, 5	No data available
<i>Navicula pelliculosa</i>	2.0%	PAR rock USA rock	2, 3	No data available
<i>Nitzschia constricta</i>	1.5%	DAV sand GRN sand	6, 4	No data available
<i>Fragilaria pinnata</i>	1.5%	H41 sand H41 rock	1	Eutrophic conditions, alkaliphilous, indifferent to conductivity, current, mesosaprobic to oligosaprobic, periphytic, lakes and ponds, cosmopolitan
<i>Surirella angusta</i>	1.4%	DAV sand CHU sand	6, 5	Alkaliphilous, indifferent to conductivity, rheobiontic, eurythermal, periphytic, tychoplanktonic, cosmopolitan
<i>Nitzschia fruticosa</i>	1.4%	CHU rock GRN sand	5, 4	No data available

Table 1 continued

TAXON	Percentage of total	Occurrence in this study	General habitat characteristics	Known habitat associations
<i>Fragilaria</i> sp. 3	1.1%	H41 sand CHU sand	1, 5	Not identified to species
<i>Cyclotella meneghiniana</i>	1.0%	PAR rock GRN rock	2, 4	Alkaliphilous, halophilous, mesosaprobic, indifferent to current, periphytic, tychoplanktonic, euplanktonic, cosmopolitan
<i>Nitzschia dissipata</i>	0.9%	USA rock H41 sand	3, 1	Eutrophic conditions, alkalibiontic to alkaliphilous, indifferent to conductivity, rheophilous, eurythermal, periphytic, lakes and ponds, cosmopolitan
<i>Fragilaria chain</i>	0.9%	GRN sand USA sand	4, 3	Not identified to species
<i>Cocconeis pediculus</i>	0.9%	GRN rock USA rock	4, 3	Alkaliphilous, indifferent to conductivity, current, saproxenous, epiphytic, cosmopolitan, resistant to moderate organic pollution
<i>Achnanthes lanceolata</i>	0.8%	USA sand H41 sand	3, 1	Alkaliphilous, indifferent to conductivity, oligosaprobic, rheophilous to rheobiontic, periphytic, springs and streams, cosmopolitan, does not appear to live in waters with high organic enrichment
<i>Sellaphora pupula</i>	0.8%	CHU sand GRN rock	5, 4	No data available
<i>Amphora</i> sp 1	0.8%	USA sand GRN rock	3, 4	Not identified to species – new species?
<i>Fragilaria construens</i>	0.8%	H41 sand H41 rock	1	Eutrophic, alkaliphilous, indifferent to conductivity and current, oligosaprobic, eurythermal, periphytic and tychoplanktonic, lakes, ponds, springs and streams, cosmopolitan

Table 1 continued

TAXON	Percentage of total	Occurrence in this study	General habitat characteristics	Known habitat associations
<i>Achnanthes deflexa</i>	0.7%	USA rock CHU rock	3, 5	alkaliphilous
<i>Nitzschia</i> sp. 3	0.7%	CHU rock GRN rock	5, 4	Not identified to species
<i>Navicula decussis</i> var. <i>decussis</i>	0.7%	USA sand USA rock	3	Alkaliphilous, indifferent to conductivity, oligosaprobic, rheophilous,
<i>Nitzschia</i> sp. 4	0.7%	USA sand DAV sand	3, 6	Not identified to species
<i>Nitzschia lacuum</i>	0.6%	CHU rock GRN sand	5, 4	No data available
<i>Navicula viridula</i> v. <i>rostillata</i>	0.6%	PAR sand H41 rock	2, 1	No data available
<i>Gomphonema olivaceum</i>	0.6%	DAV rock H41 rock	6, 1	Eutrophic conditions, alkalibiontic, indifferent to conductivity, current, mesosaprobic to oligosaprobic, eurythermal, periphytic, lakes and ponds,
<i>Diatoma vulgare</i>	0.6%	H41 rock H41 sand	1	Eutrophic conditions, alkalibiontic to alkaliphilous, indifferent to conductivity, mesosaprobic to oligosaprobic, rheophilous, eurythermal, periphytic and epiphytic, cosmopolitan, seldom in tropics, summer dominant in eutrophic lakes,
<i>Surirella brebissonia</i>	0.6%	GRN sand GRN rock	4	No data available
<i>Surirella minuta</i>	0.5%	GRN sand GRN rock	4	No data available
<i>Nitzschia sublinearis</i>	0.5%	DAV sand CHU sand	6, 4	No data available

Table 1 continued

TAXON	Percentage of total	Occurrence in this study	General habitat characteristics	Known habitat associations
<i>Nitzschia amphibia</i>	0.4%	USA sand DAV rock	3, 6	Eutrophic conditions, alkalibiontic to alkaliphilous, indifferent to conductivity, current, eurythermal, periphytic, lakes, streams, and ponds, cosmopolitan
<i>Navicula protracta</i>	0.4%	CHU sand USA sand	5, 3	No data available
<i>Tryblionella</i> sp.	0.3%	DAV sand GRN sand	6, 4	Not identified to species
<i>Achnanthes delicatula</i>	0.3%	USA sand	3	No data available
<i>Gomphonema parvulum</i>	0.3%	PAR sand PAR rock	2	indifferent to pH, conductivity, mesosaprobic, rheophilous, mesothermal, periphytic, cosmopolitan, pollution indicator
<i>Navicula capitoradiata</i>	0.3%	CHU rock PAR sand	5, 2	No data available
<i>Cocconeis placentula</i> v. <i>euglypta</i>	0.3%	USA sand CHU rock	3, 5	Alkaliphilous, indifferent to conductivity, current, oligosaprobic, eurythermal, periphytic and epiphytic, cosmopolitan, resistant to moderate organic pollution
<i>Opephora</i> sp. 1	0.3%	CHU rock H41 sand	5, 1	Not identified to species
<i>Cocconeis placentula</i> v. <i>lineata</i>	0.2%	GRN rock CHU rock	4, 5	Alkaliphilous, indifferent to conductivity, current, oligosaprobic, periphytic, cosmopolitan resistant to moderate organic pollution
<i>Navicula variostraga</i>	0.2%	CHU rock H41 sand	5, 1	No data available
<i>Cocconeis</i> sp. 1	0.2%	GRN rock	4	Not identified to species

Table 1 continued

TAXON	Percentage of total	Occurrence in this study	General habitat characteristics	Known habitat associations
<i>Fragilaria capucina</i>	0.2%	PAR rock PAR sand	2	Eutrophic conditions, alkaliphilous, indifferent to conductivity, current, oligosaprobic, eurythermal, periphytic, tychoplanktonic, lakes and ponds, cosmopolitan
<i>Nitzschia sp. 1</i>	0.2%	GRN sand DAV sand	4, 6	Not identified to species
<i>Diatoma sp. 1</i>	0.1%	H41 rock PAR sand	1, 2	Not identified to species
<i>Nitzschia sp. 2</i>	0.1%	GRN sand CHU sand	4, 5	Not identified to species
<i>Reimeria sinuata</i>	0.1%	USA rock USA sand	3	indifferent to pH, conductivity, limnobiontic, eurythermal, periphytic, cosmopolitan

Definitions: (from Lowe, 1974)

Nutrients:

Eutrophic: water with high nutrient concentrations
 Oligotrophic: water with low nutrient concentrations
 Mesotrophic: water with moderate nutrient concentrations

PH:

Indifferent: best development around pH 7
 Alkaliphilous: Occurring at a pH of 7, with best development at pH over 7
 Alkalibiontic: occurring only in pH greater than 7

Conductivity:

Indifferent: tolerates small amounts of salt
 Euryhalobous: occurring over broad ranges of salt concentration
 Halophilous: stimulated by small amounts of salt

Current:

Mesohalobous: brackish water forms occurring in salt concentrations of 500-30,000 mg/L.
 Indifferent: common in both flowing and standing water
 Limnobiontic: characteristic of only standing water
 Limnophilous: characteristic of standing water, but may be found in running water

Rheobiontic: characteristic of only running water
 Rheophilous: characteristic of running water, but may be found in standing water
 Saprobie index: Mesosaprobic: where oxidation of organic matter is proceeding
 Polysaprobic: zone of degradation and putrification, low oxygen levels
 Oligosaprobic: zone where oxidation is complete, inorganic nutrient concentration is high
 Saprophobic: characteristic of waters that have not been exposed to pollutants
 Saproxenus: occurring in clean and polluted water
 Temperature: Eurythermal: occurring over a temperature range of 15 °C or more
 Growth habit: Periphytic: occurring on, but not penetrating the substrate
 Tychoplanktonic: normally periphytic of terrestrial, but often found suspended in the water
 Euplanktonic: normally suspended in water, distribution is current dependent
 Epilithic: occurring on rock
 Cosmopolitan: found over a wide geographical range

Table 2: Shannon-Weiner diversity (H'), mean number of species per sample, and the mean percent motile species plus evenness (J) for each site and each substrate category ($n = 3$).

Site and Substrate	Mean Diversity (H')	Stan. Dev.	Mean Evenness (J)	Stan. Dev.	Mean Number of Species	Stan. Dev.	Mean Percent Motile	Stan. Dev.
DAV Rock	3.7731	0.1556	0.6315	0.0240	63	4.58	83.79%	7.09
DAV Sand	3.4885	0.3698	0.5869	0.0719	62	4.58	76.37%	4.58
CHU Rock	3.7418	0.2515	0.8747	0.0280	20	5.57	61.33%	22.55
CHU Sand	3.1052	0.5690	0.5341	0.0895	56	5.57	81.08%	4.43
GRN Rock	3.5254	0.5290	0.5929	0.0265	64.7	25.42	85.52%	4.21
GRN Sand	3.7644	0.5136	0.6303	0.0941	63.3	6.66	84.08%	3.66
USA Rock	3.5596	0.5352	0.6616	0.0126	45	23.52	60.24%	9.60
USA Sand	4.2874	0.2172	0.7224	0.0536	62	8.89	67.17%	9.06
PAR Rock	3.9980	0.2354	0.7222	0.0269	47	9.17	78.67%	6.35
PAR Sand	4.0628	0.1362	0.6962	0.0713	59.7	15.31	70.82%	9.71
H41 Rock	3.6487	0.9849	0.6437	0.1367	50.7	15.57	68.70%	16.20
H41 Sand	4.1210	0.2339	0.7188	0.0378	54	10.15	38.37%	7.14

Table 3: The results of the two factor ANOVAs done on the Shannon-Wiener diversity, evenness, number of species per sample and percent motile diatoms indices. Asterisks indicate factors that were significant at the $p < 0.05$ level. All indices were tested for normality and equal variances. Power and estimated sample size for 80% was calculated using Cohen, 1988.

Index	Factor	P-value	Power	Sample size
Diversity	Site	0.23	45%	15
	Substrate	0.53	8%	> 1000
	Substrate x Site	0.16	42%	6
Evenness	Site	0.04*	60%	7
	Substrate	0.09	42%	44
	Substrate x Site	0.0001*	>53%	3
Number of Species	Site	0.03*	80%	6
Post-hoc Bonferroni	2 < 1, 3, 4, 5, 6			
	Substrate	0.02*	68%	25
Post-hoc Bonferroni	Rock < Sand			
	Substrate x Site	0.16	>53%	5
Percent Motile	Site	0.0004*	>94%	6
	Substrate	0.44	~14%	180
	Substrate x Site	0.007*	>53%	5

Table 4: Top ten species dominating the similarities within each cluster and the top ten species dominating the dissimilarities between each pair of two clusters. Results of the SIMPER analysis including the average similarity of the cluster, the average abundance of the species in the cluster, the overall abundance of the species, the percentage and the cumulative percentage of the similarity due to the particular species.

Cluster: Downstream rock		Average similarity: 78.70			
Species	Overall Abundance	Average Abundance within cluster	Average Similarity within cluster	Species Contributing %	Cumulative Contributing %
<i>Nitzschia palea</i>	22.9	22.79	3.94	5.01	5.01
<i>Nitzschia microcephala</i>	7.7	11.78	3.34	4.25	9.26
<i>Nitzschia frustulum</i>	8.2	11.03	3.27	4.15	13.41
<i>Nitzschia acicularis</i>	6.1	10.80	3.21	4.07	17.49
<i>Nitzschia linearis</i>	4.5	4.03	2.76	3.50	20.99
<i>Fragilaria construens v. venter</i>	5.8	3.50	2.51	3.18	24.17
<i>Nitzschia sp. 3</i>	0.7	2.00	2.05	2.60	26.77
<i>Surirella angusta</i>	1.4	1.08	1.94	2.47	29.24
<i>Navicula cf. subminuscula</i>	2.0	5.59	1.90	2.41	31.65
<i>Nitzschia constricta</i>	1.5	1.21	1.90	2.41	34.06
Cluster: Downstream sand		Average similarity: 80.20			
<i>Nitzschia palea</i>	22.9	39.57	5.09	6.35	6.35
<i>Nitzschia linearis</i>	4.5	8.41	3.52	4.40	10.75
<i>Fragilaria construens v. venter</i>	5.8	9.61	3.45	4.30	15.05
<i>Nitzschia acicularis</i>	6.1	7.07	2.88	3.59	18.64
<i>Surirella angusta</i>	1.4	3.66	2.70	3.37	22.01
<i>Nitzschia constricta</i>	1.5	3.44	2.69	3.36	25.36
<i>Navicula cari</i>	2.0	2.33	2.34	2.91	31.27
<i>Nitzschia microcephala</i>	7.7	2.62	2.40	2.99	28.35
<i>Fragilaria sp. 3</i>	1.1	1.65	2.22	2.77	34.04
<i>Sellaphora pupula</i>	0.8	1.25	2.09	2.61	36.65

Table 4 continued:

Cluster: USA rock		Average similarity: 67.22			
Species		Average Abundance within cluster	Average Similarity within cluster	Species Contributing %	Cumulative Contributing %
<i>Nitzschia frustulum</i>	8.2	31.90	7.04	10.47	10.47
<i>Amphora perpusilla</i>	3.0	16.78	5.56	8.27	18.74
<i>Navicula pelliculosa</i>	2.0	15.07	5.56	8.27	27.01
<i>Achnanthes deflexa</i>	0.7	12.40	5.21	7.74	34.75
<i>Navicula cincta</i>	3.5	6.45	3.85	5.73	40.48
<i>Achnanthes lanceolata</i>	0.8	2.26	3.81	5.66	46.14
<i>Nitzschia microcephala</i>	7.7	2.73	3.58	5.32	51.47
<i>Navicula gregaria</i>	4.0	1.17	2.96	4.40	55.87
<i>Nitzschia fruticosa</i>	1.4	0.42	2.47	3.68	59.55
<i>Fragilaria construens</i> v. <i>venter</i>	5.8	0.37	2.22	3.31	62.86
Cluster: USA sand		Average similarity: 77.50			
<i>Nitzschia microcephala</i>	7.7	18.72	3.45	4.45	4.45
<i>Nitzschia frustulum</i>	8.2	15.58	3.29	4.24	8.69
<i>Navicula gregaria</i>	4.0	5.60	2.70	3.48	12.17
<i>Amphora perpusilla</i>	3.0	4.57	2.54	3.28	15.45
<i>Navicula cincta</i>	3.5	4.05	2.53	3.26	18.71
<i>Fragilaria construens</i> v. <i>venter</i>	5.8	4.50	2.41	3.11	21.82
<i>Navicula decussis</i> var. <i>decussis</i>	0.7	5.63	2.39	3.08	24.91
<i>Achnanthes lanceolata</i>	0.8	4.67	2.25	2.90	27.81
<i>Nitzschia palea</i>	22.0	2.41	2.05	2.65	30.46
<i>Fragilaria 'chain'</i>	0.9	2.51	1.97	2.54	33.00

Table 4 continued:

Cluster: Upstream				Average similarity: 73.45	
<i>Navicula gregaria</i>	4.0	12.11	3.40	4.63	4.63
<i>Navicula cincta</i>	3.5	10.30	3.36	4.57	9.20
<i>Melosira varians</i>	3.0	10.66	3.01	4.10	13.30
<i>Nitzschia frustulum</i>	8.2	5.85	2.88	3.92	17.22
<i>Nitzschia palea</i>	22.9	6.96	2.76	3.76	20.98
<i>Nitzschia microcephala</i>	7.7	5.26	2.67	3.64	24.62
<i>Cyclotella meneghiniana</i>	0.9	3.27	2.45	3.34	27.96
<i>Fragilaria construens</i> v. <i>venter</i>	5.8	5.92	2.27	3.09	31.05
<i>Navicula viridula</i> v. <i>rostellata</i>	0.6	2.03	2.12	2.88	33.93
<i>Fragilaria pinnata</i>	1.5	5.35	2.11	2.87	36.81

Table 5: Top ten species dominating the dissimilarities between each pair of two clusters. Results of the SIMPER analysis including the average dissimilarity between the cluster-pair, the overall abundance of the species, the average abundance of the species in each cluster, the percentage of the dissimilarity that the species is responsible for and the cumulative percentage responsibility.

Clusters: Downstream rock & Downstream sand				Average dissimilarity = 30.96		
Species	Overall abundance	Average Abundance within first cluster	Average Abundance within second cluster	Average Dis-similarity between clusters	Species Contributing %	Cumulative Contributing %
<i>Navicula cf. subminuscula</i>	2.0	5.59	0.02	1.29	4.17	4.17
<i>Nitzschia sp. 3</i>	0.7	2.00	0.00	1.18	3.82	7.98
<i>Navicula pelliculosa</i>	2.0	3.24	0.04	1.01	3.27	11.25
<i>Nitzschia lacuum</i>	0.6	0.77	0.00	0.89	2.88	14.13
<i>Nitzschia frustulum</i>	8.2	11.03	1.41	0.80	2.58	16.71
<i>Achnanthes deflexa</i>	0.7	0.35	0.00	0.75	2.42	19.13
<i>Cocconeis pediculus</i>	0.9	1.59	0.06	0.65	2.11	21.24
<i>Amphora perpusilla</i>	3.0	5.27	0.45	0.63	2.03	23.27
<i>Nitzschia microcephala</i>	7.7	11.78	2.62	0.62	2.00	25.27
<i>Navicula paucivittata</i>	0.0007	0.20	0.00	0.61	1.97	27.23

Table 5 continued:

Clusters: Downstream rock & USA rock				Average dissimilarity = 47.01		
Species	Overall abundance	Average Abundance within first cluster	Average Abundance within second cluster	Average Dis-similarity between clusters	Species Contributing %	Cumulative Contributing %
<i>Nitzschia palea</i>	22.9	22.79	0.11	2.16	4.59	4.59
<i>Nitzschia acicularis</i>	6.1	10.80	0.22	1.58	3.35	7.95
<i>Achnanthes deflexa</i>	0.7	0.35	12.40	1.36	2.89	10.84
<i>Nitzschia constricta</i>	1.5	1.21	0.00	1.27	2.70	13.54
<i>Amphora sp. 1</i>	0.8	1.10	0.00	1.24	2.64	16.18
<i>Nitzschia sp. 3</i>	0.7	2.00	0.11	1.10	2.33	18.51
<i>Fragilaria sp. 3</i>	1.1	0.61	0.00	1.01	2.15	20.66
<i>Nitzschia sublinearis</i>	0.5	0.43	0.00	0.99	2.10	22.76
<i>Surirella minuta</i>	0.5	0.61	0.00	0.98	2.08	24.85
<i>Surirella angusta</i>	1.4	1.08	0.09	0.97	2.06	26.91
Clusters: Downstream sand & USA rock				Average dissimilarity = 57.14		
<i>Nitzschia palea</i>	22.9	39.57	0.11	2.72	4.76	4.76
<i>Achnanthes deflexa</i>	0.7	0.00	12.40	2.36	4.12	8.88
<i>Navicula pelliculosa</i>	2.0	0.04	15.07	2.19	3.83	12.71
<i>Nitzschia constricta</i>	1.5	3.44	0.00	1.73	3.03	15.74
<i>Nitzschia frustulum</i>	8.2	1.41	31.90	1.72	3.01	18.75
<i>Amphora perpusilla</i>	3.0	0.45	16.78	1.57	2.75	21.50
<i>Surirella angusta</i>	1.4	3.66	0.09	1.46	2.55	24.05
<i>Fragilaria sp. 3</i>	1.1	1.65	0.00	1.44	2.52	26.57
<i>Nitzschia acicularis</i>	6.1	7.07	0.22	1.32	2.31	28.89
<i>Nitzschia sublinearis</i>	0.5	1.06	0.00	1.27	2.23	31.11

Table 5 continued:

Clusters: Downstream rock & USA sand				Average dissimilarity = 31.97		
Species	Overall abundance	Average Abundance within first cluster	Average Abundance within second cluster	Average Dis-similarity between clusters	Species Contributing %	Cumulative Contributing %
<i>Nitzschia acicularis</i>	6.1	10.80	0.18	1.10	3.45	3.45
<i>Navicula decussis</i> <i>var. decussis</i>	0.7	0.07	5.63	0.93	2.92	6.38
<i>Nitzschia sp. 3</i>	0.7	2.00	0.09	0.92	2.88	9.25
<i>Nitzschia palea</i>	22.9	22.79	2.41	0.89	2.78	12.04
<i>Achnanthes lanceolata</i>	0.8	0.07	4.67	0.86	2.71	14.74
<i>Achnanthes delicatula</i>	0.3	0.03	3.04	0.81	2.54	17.29
<i>Achnanthes deflexa</i>	0.7	0.35	1.66	0.73	2.29	19.57
<i>Cocconeis pediculus</i>	0.9	1.59	0.36	0.62	1.95	21.52
<i>Achnanthes lanceolata v. dubia</i>	0.0006	0.00	0.62	0.62	1.95	23.47
<i>Navicula cf. subminuscula</i>	2.0	5.59	0.51	0.59	1.85	25.32
Clusters: Downstream sand & USA sand				Average dissimilarity = 35.40		
<i>Nitzschia palea</i>	22.9	39.57	2.41	1.27	3.59	3.59
<i>Nitzschia frustulum</i>	8.2	1.41	15.58	0.91	2.58	6.17
<i>Nitzschia acicularis</i>	6.1	7.07	0.18	0.90	2.54	8.71
<i>Navicula pelliculosa</i>	2.0	0.04	1.84	0.84	2.36	11.08
<i>Navicula decussis</i> <i>var. decussis</i>	0.7	0.22	5.63	0.82	2.32	13.40
<i>Achnanthes delicatula</i>	0.3	0.06	3.04	0.81	2.28	15.68
<i>Nitzschia microcephala</i>	7.7	2.62	18.72	0.81	2.28	17.96
<i>Achnanthes lanceolata</i>	0.8	0.16	4.67	0.80	2.25	20.21
<i>Navicula cf. subminuscula</i>	2.0	0.02	0.51	0.75	2.11	22.32
<i>Amphora perpusilla</i>	3.0	0.45	4.57	0.71	2.00	24.32

Table 5 continued:

Clusters: USA rock & USA sand				Average dissimilarity = 43.97		
Species	Overall abundance	Average Abundance within first cluster	Average Abundance within second cluster	Average Dis-similarity between clusters	Species Contributing %	Cumulative Contributing %
<i>Achnanthes deflexa</i>	0.7	12.40	1.66	1.71	3.90	3.90
<i>Achnanthes delicatula</i>	0.3	0.00	3.04	1.41	3.20	7.10
<i>Amphora sp. 1</i>	0.8	0.00	1.46	1.25	2.85	9.94
<i>Fragilaria sp. 3</i>	1.1	0.00	1.10	1.20	2.72	12.67
<i>Navicula pelliculosa</i>	2.0	15.07	1.84	1.00	2.28	14.95
<i>Navicula protracta</i>	0.4	0.00	0.68	0.97	2.21	17.16
<i>Nitzschia palea</i>	22.9	0.11	2.41	0.97	2.20	19.36
<i>Nitzschia microcephala</i>	7.7	2.73	18.72	0.94	2.14	21.49
<i>Nitzschia constricta</i>	1.5	0.00	0.41	0.92	2.09	23.58
<i>Navicula decussis</i> var. <i>decussis</i>	0.7	0.42	5.63	0.86	1.95	25.53
Clusters: Downstream rock & Upstream				Average dissimilarity = 38.31		
<i>Melosira varians</i>	3.0	0.01	10.66	1.56	4.08	4.08
<i>Nitzschia sp. 3</i>	0.7	2.00	0.00	1.11	2.91	6.99
<i>Navicula cf. subminuscula</i>	2.0	5.59	0.73	1.07	2.79	9.78
<i>Navicula gregaria</i>	4.0	0.83	12.11	0.88	2.31	12.09
<i>Nitzschia acicularis</i>	6.1	10.80	1.92	0.81	2.12	14.21
<i>Fragilaria capucina</i> v. 2	0.002	0.00	0.60	0.79	2.06	16.27
<i>Diatoma vulgare</i>	0.6	0.02	1.94	0.74	1.93	18.20
<i>Fragilaria pinnata</i>	1.5	0.16	5.35	0.71	1.85	20.05
<i>Cyclotella meneghiniana</i>	0.9	0.17	3.27	0.69	1.79	21.84
<i>Navicula cincta</i>	3.5	2.11	10.30	0.66	1.72	23.56

Table 5 continued:

Clusters: Downstream sand & Upstream					Average dissimilarity = 39.82	
Species	Overall abundance	Average Abundance within first cluster	Average Abundance within second cluster	Average Dis-similarity between clusters	Species Contri-buting %	Cumulative Contributing %
<i>Melosira varians</i>	3.0	0.00	10.66	1.73	4.35	4.35
<i>Nitzschia palea</i>	22.9	39.57	6.96	1.01	2.54	6.89
<i>Nitzschia lacuum</i>	0.6	0.00	0.85	0.98	2.46	9.35
<i>Nitzschia constricta</i>	1.5	3.44	0.12	0.96	2.41	11.76
<i>Surirella angusta</i>	1.4	3.66	0.09	0.92	2.30	14.06
<i>Navicula pelliculosa</i>	2.0	0.04	4.10	0.90	2.26	16.32
<i>Fragilaria capucina</i> v. 2	0.002	0.00	0.60	0.88	2.21	18.53
<i>Navicula cincta</i>	3.5	0.89	10.30	0.86	2.16	20.69
<i>Tryblionella</i> sp.	0.3	0.72	0.01	0.83	2.07	22.76
<i>Navicula gregaria</i>	4.0	1.43	12.11	0.79	1.99	24.75
Clusters: USA rock & Upstream					Average dissimilarity = 48.19	
<i>Achnanthes deflexa</i>	0.7	12.40	0.34	1.98	4.10	4.10
<i>Melosira varians</i>	2.9	0.05	10.66	1.87	3.87	7.98
<i>Amphora perpusilla</i>	3.0	16.78	0.64	1.47	3.05	11.03
<i>Nitzschia palea</i>	22.9	0.11	6.96	1.44	2.99	14.02
<i>Cyclotella meneghiniana</i>	0.9	0.03	3.27	1.41	2.92	16.94
<i>Navicula viridula</i> v. <i>rostillata</i>	0.6	0.05	2.03	1.18	2.46	19.39
<i>Navicula pelliculosa</i>	2.0	15.07	4.10	1.16	2.40	21.79
<i>Amphora</i> sp. 1	0.8	0.00	0.71	1.12	2.32	24.12
<i>Fragilaria</i> sp. 3	1.1	0.00	1.00	1.06	2.19	26.31
<i>Nitzschia frustulum</i>	8.2	31.90	5.85	1.03	2.14	28.45

Table 5 continued:

Clusters: USA sand & Upstream				Average dissimilarity = 33.47		
Species	Overall abundance	Average Abundance within first cluster	Average Abundance within second cluster	Average Dis-similarity between clusters	Species Contributing %	Cumulative Contributing %
<i>Achnanthes delicatula</i>	0.3	3.04	0.00	1.13	3.38	3.38
<i>Melosira varians</i>	2.9	0.11	10.66	1.12	3.35	6.72
<i>Cyclotella meneghiniana</i>	0.9	0.18	3.27	0.76	2.28	9.00
<i>Navicula decussis</i> var. <i>decussis</i>	0.7	5.63	0.48	0.71	2.12	11.12
<i>Achnanthes lanceolata</i> v. <i>dubia</i>	0.0006	0.62	0.00	0.68	2.03	13.15
<i>Navicula</i> cf. <i>subminuscule</i>	2.0	0.51	0.73	0.65	1.95	15.09
<i>Gomphonema parvulum</i>	0.3	0.07	1.10	0.65	1.94	17.03
<i>Amphora perpusilla</i>	3.0	4.57	0.64	0.64	1.92	18.95
<i>Diatoma vulgare</i>	0.6	0.13	1.94	0.62	1.85	20.80
<i>Cocconeis placentula</i> v. <i>euglypta</i>	0.3	1.15	0.08	0.62	1.85	22.65

Table 6: Eigenvectors from Principal Components Analysis results using chemical data taken closest to the algal sampling date. FTDS denotes fixed total dissolved solids, FTSS denotes fixed total suspended solids, OP-P denotes orthophosphate as phosphate, OP-PO₄ denotes orthophosphate as phosphate, PHOS-P denotes total phosphate, TKN-N denotes total Kjeldahl nitrogen, TSS denotes total suspended solids, VTDS denotes volatile total dissolved solids, and VTSS denotes volatile total suspended solids.

Variable	PC1	PC2	PC3	PC4	PC5
log Conductivity	-0.219	-0.346	0.036	-0.009	0.145
log turbidity	0.290	-0.137	-0.208	-0.056	-0.006
log pH	-0.243	-0.100	0.491	0.508	-0.494
log chloride	0.232	-0.313	0.152	-0.053	0.221
log FTDS	-0.210	-0.361	0.022	0.012	0.058
log FTSS	0.235	-0.311	0.103	-0.192	-0.041
log NH ₃	0.288	0.156	-0.062	0.203	-0.187
NO ₂	0.294	-0.102	0.045	0.348	0.059
log NO ₃	0.033	-0.471	-0.149	0.358	0.247
log OP-P	0.269	0.129	0.447	0.059	0.151
log OP-PO ₄	0.267	0.117	0.480	0.057	0.164
log PHOS-P	0.277	-0.038	-0.390	0.220	-0.530
log TKN_N	0.293	-0.063	-0.181	0.342	0.251
log TSS	0.262	-0.230	0.143	-0.294	-0.232
log VTDS	-0.169	-0.408	0.074	-0.105	-0.242
log VTSS	0.287	-0.121	0.104	-0.374	-0.275
Percent variation explained by principal component	65.8	25.9	4.8	2.5	1.1

Table 7: Results of the BIOENV procedure, matching the species data to the chemical data used in the PCA. Variables were excluded if correlated more than 90%. Variables included were: NO₃-N (nitrate), conductivity, turbidity, pH, NH₃-N (ammonia), chloride, and OP-PO₄ (orthophosphate).

Best results

Number of Variables	Correlation Coefficient	Variables Used
2	0.355	log NO ₃ -N, log turbidity
2	0.352	log NO ₃ -N, log chloride
3	0.339	log NO ₃ -N, log turbidity, log chloride
1	0.334	log NO ₃ -N
3	0.331	log NO ₃ -N, log pH, log chloride
1	0.331	log chloride
2	0.302	log NO ₃ -N, log pH
2	0.295	log turbidity, log chloride
4	0.285	log NO ₃ -N, log turbidity, log pH, log chloride
3	0.278	log NO ₃ -N, log chloride, log OP-PO ₄

Figure 1: The Salinas River Basin, with the watershed delineated by the dashed line. The study sites are circles, the nearby cities are squares. Base map provided by Central Coast Watershed Studies (CCoWS), The Watershed Institute, California State University, Monterey Bay.

Figure 2: Mean Evenness (J) for each site x substrate combination. Error bars are one standard deviation.

Figure 3: Mean Percent Motile Diatoms for every site x substrate combination. Errors bars are one standard deviation.

Figure 5: Principal Components Plot of the chemical data from the six sites along the Salinas River. Chemical data were log transformed when appropriate in order to maximize linearity. Chemical components constituting the first two principal components, and the eigenvectors in Table 6.

Diatom relative abundance

Figure 6: Multi-dimensional scaling plot of the fourth root transformed diatom relative abundance data. This is a non-metric procedure; the axes are hypothetical and non-numeric. The samples are only related to each other and no other factor, essentially a two dimensional cluster diagram. The stress value totals the scatter around the regression line, a value of 0.12 indicates that higher dimensional solutions would not be more reliable and that the placement of the samples is repeatable over several iterations.

Appendix A: Chemical characteristics of the six chosen sites on the Salinas River. These data were used to choose sites and to designate the sites as 'high, medium and low'. Bars are the mean value for the entire sampling season. Points and lines indicate the data value closest to the algal collection time, used in the PCA and BIOENV analyses. Error bars are one standard deviation. DAV denotes the Davis Road site, CHU denotes the Chualar site, GRN denotes the Greenfield site, USA denotes the Bradley site, PAR denotes the Paso Robles site, and H41 denotes the Atascadero site.

Hardness as Calcium Carbonate

Ammonia (NH₃) Concentrations

Nitrite (NO₂) Concentrations

Nitrate (NO₃) Concentrations

Orthophosphate Concentrations

Appendix B: Relative abundance of available substrates in summer 1999 and in April 2000, at the same time as the algal collections. Data from 50 10X10 cm random quadrats along on a haphazardly placed 250m transect. These data were used to differentiate sites according to the available substrate. Site definitions as in Appendix A.

Appendix C: Percent cover of algal filaments, film and total percent cover at all sites in April 2000. Data from 50 10X10 cm random quadrats along 250m of the river's edge. Error bars are one standard deviation. (n = 50)

Appendix D: Species List

Achnanthes deflexa
Achnanthes delicatula
Achnanthes exigua
Achnanthes haukiana
Achnanthes hustedtii
Achnanthes lanceolata
Achnanthes lanceolata v. *dubia*
Achnanthes linearis
Achnanthes linearis v. *pusilla*
Achnanthes saxonica
Achnanthes wellsiae
Amphora acutiuscula
Amphora ovalis
Amphora perpusilla
Amphora sp. 1
Amphora sp. 2
Caloneis amphisbaena
Caloneis bacillum
Caloneis sp. 1
Caloneis sp. 2
Caloneis ventricosa
Caloneis ventricosa v. *alpina*
Caloneis ventricosa v. *minuta*
Caloneis ventricosa v. *subundulata*
Caloneis ventricosa v. *truncatula*
Cocconeis disculus
Cocconeis pediculus
Cocconeis placentula v. *euglypta*
Cocconeis placentula v. *lineata*
Cocconeis sp. 1
Craticula cuspidata
Cyclotella meneghiniana
Cymatopleura solea
Cymbella affinis
Cymbella mexicana
Cymbella minuta
Cymbella sp. 1
Cymbella sp. 2
Cymbella sp. 3
Diatoma moniliformis

Diatoma sp. 1
Diatoma vulgare
Diploneis elliptica
Diploneis puella
Epithemia sorex
Epithemia sp. 1
Fallacia pygmaea
Fragilaria capucina
Fragilaria capucina v. 1
Fragilaria capucina v. 2
Fragilaria construens
Fragilaria construens v. venter
Fragilaria pinnata
Fragilaria pinnata v. lancettula
Fragilaria sp. 1
Fragilaria sp. 2
Fragilaria sp. 3
Gomphonema affine
Gomphonema augustatum v. intermedia
Gomphonema dichototum?
Gomphonema mexicanum
Gomphonema olivaceum
Gomphonema parvulum
Gomphonema sp. 1
Gomphonema sp. 2
Gomphonema subclavatum v. commutatum
Gomphonema subclavatum v. mexicanum
Gomphonema tenellum
Gomphonema truncatum
Hippodonta capitata
large fragilaria chain
Melosira varians
Meridion circulare
Navicula capitoradiata
Navicula cari
Navicula cascadiensis
Navicula cf. subminuscule
Navicula cincta
Navicula cocconeiformis
Navicula decussis var. decussis
Navicula festiva
Navicula graciloides

Navicula gregaria
Navicula halophila
Navicula laterpuncta
Navicula mutica v. *undulata*
Navicula paucivittata
Navicula pelliculosa
Navicula protracta
Navicula pupula
Navicula radiosa
Navicula sp. 1
Navicula tenelloides
Navicula tripunctata
Navicula variostraga
Navicula viridula v. *rostillata*
Neidium dubium v. *constrictum*
Nitzschia acicularis
Nitzschia amphibia
Nitzschia constricta
Nitzschia dissipata
Nitzschia filiformis
Nitzschia frustulum
Nitzschia fruticosa
Nitzschia lacuum
Nitzschia linearis
Nitzschia microcephala
Nitzschia palea
Nitzschia sp. 1
Nitzschia sp. 2
Nitzschia sp. 3
Nitzschia sp. 4
Nitzschia sublinearis
Opephora martyi
Opephora sp. 1
Paralia sp.
Pinnularia brebisonii
Pinnularia brebisonii v. *diminuta*
Pleurosigma australe
Reimeria sinuata
Rhoicosephnia curvata
Rhopalalodia gibba
Rhopalalodia musculus
Rhopalalodia sp. 1

Sellaphora bacillum
Sellaphora pupula
Surirella angusta
Surirella brebissonia
Surirella minuta
Surirella sp. 1
Synedra rumpens
Synedra sp. 1
Synedra ulna
Tryblionella sp.