APPENDIX H

Accuracy, operation and maintenance of Continuous Chlorophyll and Turbidity Sensors (SCUFA)

H.1 Objective

At the conclusion of the 2001 CALFED was anticipated that a more comprehensive watershed monitoring program addressing upstream algal would rely on continuous sensors rather than on weekly or monthly grab samples. In the fall of 2001 Peggy Lehman reported on the availability of a new instrument from Turner Designs Inc. named SCUFA, which provided the capability of continuous measurements of either chlorophyll-a or rhodamine, a built-in datalogger and a submersible battery pack allowing autonomous deployment. Until this innovation from Turner Designs Inc. the company offered a flow-through cell which attached to the 10-AU-005-CE Field Fluorometer. Although the 10AU is a rugged, field-portable instrument it is bulky and expensive – approximately $12,000 with standard options. The SCUFA is less than 50% of the price and offers the same optical sensor as the field instrument. The first objective of this series of experiments was to gain experience with the maintenance and deployment of these instruments and to test their accuracy against standard analytical techniques for chlorophyll-*a* analysis. The sensors were deployed near the inlet and outlet of the San Luis Drain (at stations approximately 26 miles apart). The second objective was to quantify changes in chlorophyll-*a* concentrations between these stations and from these data estimate algae growth rates.

H.2.1 Laboratory Methods

The SCUFA units were calibrated against chlorophyll-*a* concentrations in the laboratory. The method for chlorophyll extraction and quantification was adapted from Standard Methods 10200H by Jeremy Hanlon at LBNL :

2.1.1 Materials needed:

1. Vacuum filtration apparatus to hold 47mm GF/F Whatman glass fiber filters
2. Saturated magnesium carbonate and water solution in squirt bottle
3. Filter forceps (tweezers)
4. Containers (Plastic screw top Falcon vial) for holding and freezing filters
5. Freezer
6. Chilled water bath
7. Tissue grinder (Wheaton pt#358009) 15ml with modified Teflon pestle (see note below)
8. Variable speed hand drill with 3/8" chuck
9. Extraction solution: 90% acetone 10% sat. magnesium carbonate in water solution (in squirt bottle)

10. Graduated 15ml glass centrifuge tubes with caps
11. Benchtop centrifuge
12. Spectrophotometer (Perkin-Elmer UV-Vis) and two 1cm quartz cuvettes
13. 0.1N HCl
14. Pipettes for 1ml and 33 mls

2.1.2 Method: Filtration

1. Dim lights in work area or otherwise keep samples out of light
2. Place GF/F filters on support with the irregularly textured surface up and assemble apparatus
3. Resuspend sample in collection container by shaking before pouring into filter apparatus
4. Quantify volume filtered and try to get at least 500ml through (may take 10-30 minutes) keep filtration apparatus covered from dust and light.
5. Rinse down sides of apparatus with small amount of magnesium carbonate solution (stir before using to resuspend powder)
6. Remove filter from apparatus with forceps and place in labeled container, place container in freezer immediately

2.1.3 Method: Extraction

1. Pre-chill all glassware etc. which will come into contact with sample extract
2. Remove the filter from freezer tear into several pieces (using clean, gloved hands) and place in chilled tissue grinder, add 2 to 3 ml of acetone extraction solution
3. Place the chilled, modified Teflon pestle in the drill chuck , tighten with chuck key
4. Maintain cold temperature while grinding filter in the bottom of tube at top drill speed until no discernable pieces of filter remain. MAKE SURE TUBE DOES NOT BECOME WARM. If grinder starts to become warm; STOP! Place entire apparatus back into chilled water bath to re-cool.
5. Transfer the filter pulp to graduated centrifuge tube and rinse grinder with additional acetone solution into the centrifuge tube. The total volume in centrifuge tube after rinsings should be between 9 and 11 ml with 10 ml being the goal
6. Place capped and labeled centrifuge tube in refrigerator to steep for at least 4 hours but not more than 24 hours
7. After steeping, centrifuge filter extract using bench-top centrifuge at speed setting #4 for 15 to 20 minutes. It is helpful to chill the tube holders before centrifuging to help maintain cold temperature
8. After centrifuging, remove tubes and use foil covers to protect chlorophyll extract from light while at the spectrophotometer.

2.1.4 Method: spectrophotometric determination of chlorophyll content

1. Turn on the Perkin-Elmer spectrophotometer and start UVWinlab software from desktop icon
2. Choose the CHLAPPTN.MWP Method
3. Fill in sample list, first one being "blank" second one being the sample and third being the sample with acid (ie 1.blank 2.MDS 3.MDSacid) repeat sample and sample with acid for each extract to be analyzed
4. Using acetone extraction solution as a blank in both the front and rear cuvettes start the program method and click "OK" when prompted to enter the blank sample
5. Cover the top of rear cuvette with parafilm to avoid evaporation during analysis
6. Rinse front cuvette with sample extract once then pipette 1ml of extract and click "OK"
7. When prompted to place next sample in holder, remove cuvette and pipette in 33mls of 0.1N HCl, cover with parafilm and invert several times to mix
8. Place the cuvette back into spectrophotometer and wait 90 seconds before clicking the "OK" button
9. When prompted, remove the cuvette and rinse with DI water, shake out excess water and repeat steps 6 and 7 for remaining samples
10. Print out results and tape into a notebook
11. Clean both quartz cuvettes and turn off the spectrophotometer.

In the experimental protocol the teflon pestle should be modified to work with glass fiber filters. This requires that the radius of the pestle must be reduced to allow more room between it and the wall of the grinding tube. Sand paper should be used while spinning the pestle in the drill and a small amount of the Teflon material should be gently removed. Grinding was made much easier by adding light spiral grooves in the pestle with a file..

H 2.1 Field and Laboratory Methods

Field samples were collected in 1 liter glass Wheaton media bottles at various sample points in the Grasslands Basin on July 25, 2002 and were immediately put on ice and transported to the Lab. Upon arrival the samples were held at 4 degrees Celsius before starting the analyses. Sample chlorophyll-a and turbidity were measured in the lab using the SCUFA (Turner Designs) instrument using the flow-through cap provided with the unit and a peristaltic pump, using the protocol described in the instrument manual. For the chlorophyll extraction, the Standard Methods 10200H protocol was followed using Whatman GF/F filters and a 90% saturated magnesium carbonate acetone solution. The extracted chlorophyll-a was read in 1 cm quartz cuvettes on a Perkin Elmer UV/Vis spectrophotometer. Total organic carbon (TOC) analysis was performed using an Apollo 9000HS (Tekmar/Dorhman) on 30 ml samples in VOA vials while a stir bar provided constant agitation to the solution.

Calibration of the SCUFA unit was performed in the laboratory using algae grown in a small aquarium in water derived from the San Luis Drain. This was necessary in order to obtain the range of algae concentrations needed to develop a full calibration curve. The algal sample was transferred into a sample cup into which the SCUFA probe was inserted. Care was taken to exclude direct incident light. Serial dilutions were made of the algal sample to create a series of algal concentrations with which to compare the SCUFA readings.

H 2.2 Calibration Results

The data obtained from the calibration experiment is presented in Figure H2(a). The SCUFA units exhibits a linear response with a low error. The R^2 value for the regression of SCUFA fluorescence units and chlorophyll-a is 0.9972. On the basis of this strong correlation an experiment was designed to deploy the SCUFA units in the San Luis Drain.

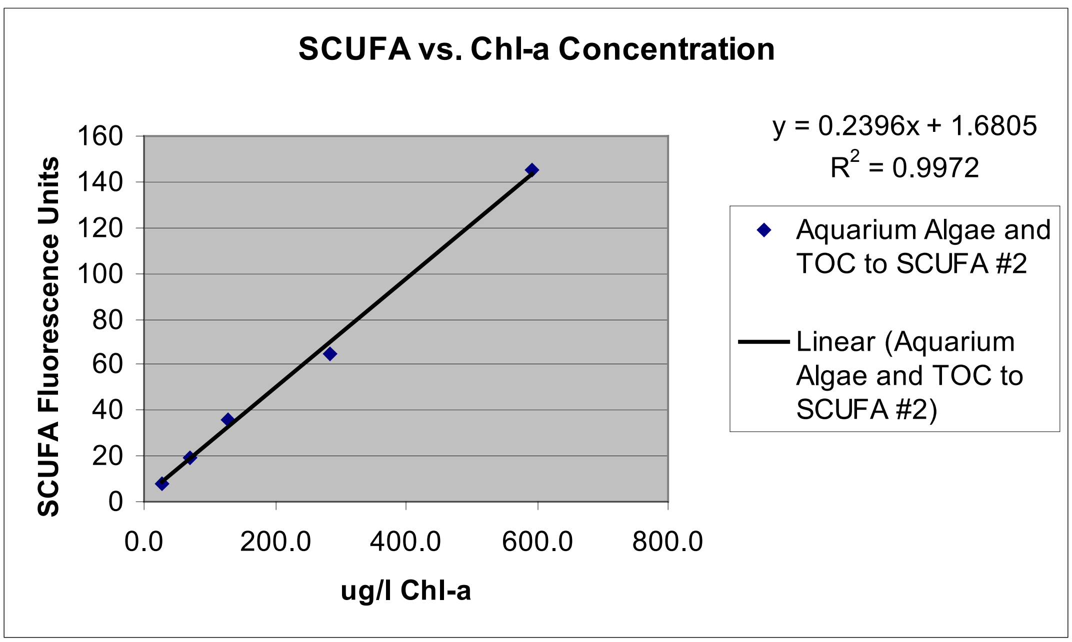


Figure H2(a) : SCUFA reading and chlorophyll-a concentration for a laboratory algae sample.

A second experiment was carried out to develop a relationship between total organic carbon concentration and chlorophyll concentration. Since algae cells have a high concentration of carbon it is expected that the correlation will be high between these parameters. The regression coefficient for the linear relationship between TOC and chlorophyll-a is 0.9935.

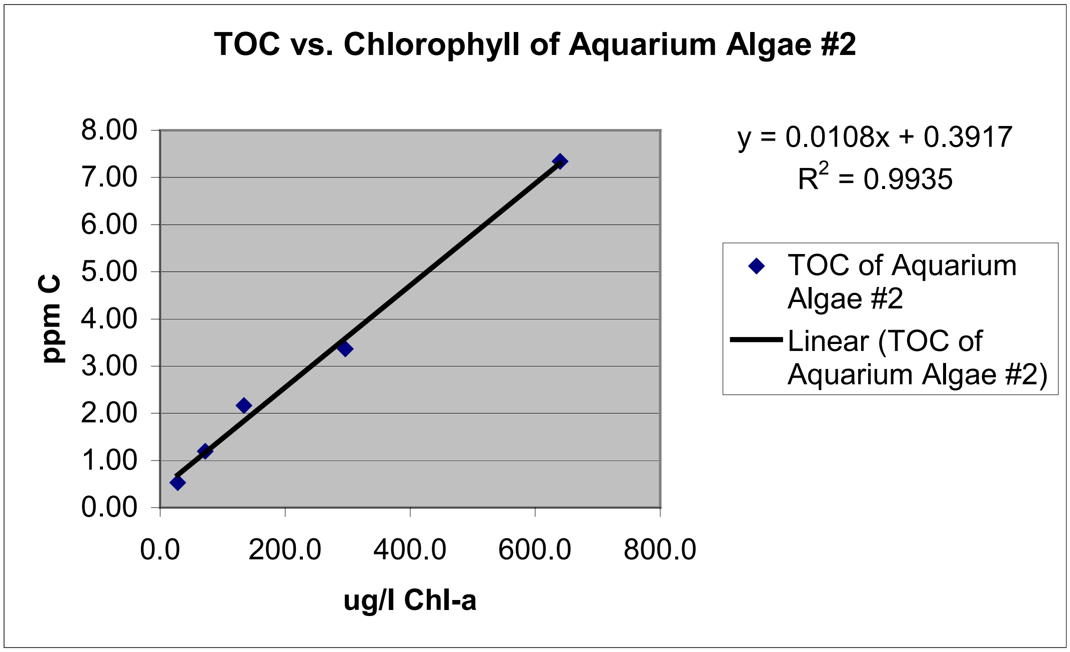


Figure H2(b) : Total organic carbon and chlorophyll-a concentration for a laboratory algae sample.

H 3.1 SCUFA Deployment

The SCUFA sondes were equipped with optional detachable batteries and internal data loggers to allow the instruments to be deployed autonomously. Attention to the connectors used to secure the detachable battery pack to the sonde showed them to be both insecure and prone to vandalism. Since each battery pack costs over $1,000 – loss of the battery pack is a significant cost together with the opportunity cost of the lost data. A secure housing was designed to eliminate this design-flaw and to make the instruments more secure in their deployment. The housing was constructed of ½ inch fiberglass tubing, cut to a length to leave three inches of the sonde body and the probe sensor housing protruding. A brass rod was machined and drilled to form a locking bolt which was inserted though a drilled hole in the fiberglass housing and the top flange on the sonde to secure the sonde in place. The gap between the sonde body and the internal diameter of the housing is less than ¾ inch – allowing no opportunity for a vandal to disconnect the sonde and battery pack.

The sondes were suspended on stout chains from bridges at upstream (Site A) and downstream (Site B) locations (Figure 4.1.b), along the San Luis Drain. The stability and the reliability of the fluorescent measurements recorded on the sondes were evaluated over a three month period. The SCUFA sondes successfully logged chlorophyll data for two weeks between maintenance visits. If the maintenance schedule was extended to longer than two weeks, sensor fouling proceeded rapidly resulting in signal degradation. The sensor maintained calibration against a chlorophyll-*a* standard for the entire three month test period, checked in the field using a solid calibration standard.

(a) SCUFA disassembled

(b) SCUFA in protective housing

Figure H3.1: Self-Contained Underwater Fluorescence Apparatus (SCUFA) shown disassembled (a) and assembled in protective housing (b). SCUFA sondes can be deployed independently or integrated into existing continuous flow monitoring infrastructure.

Data from a typical two-week deployment is presented in Figure H3.2. The data show that chlorophyll-*a* concentrations can vary by a factor of greater than two within a short time (hours) at Site B, but that Site A had less variability. This study illustrates that information collected with SCUFA sondes can help fill data gaps concerning the magnitude and frequency of algal blooms.

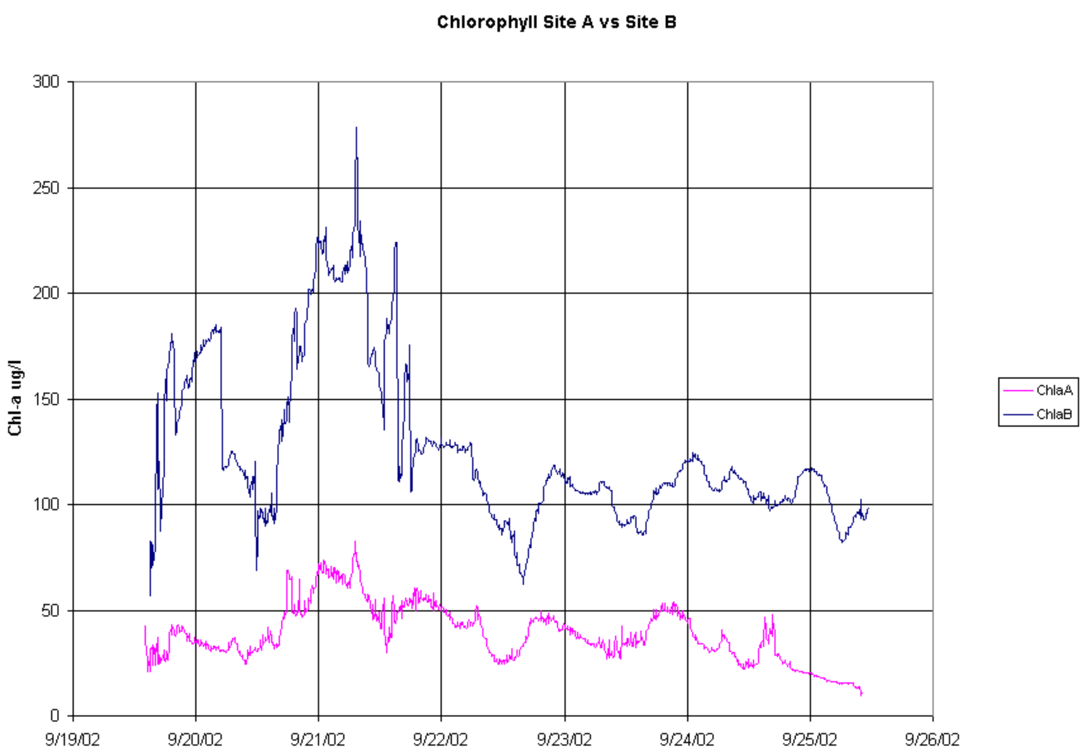
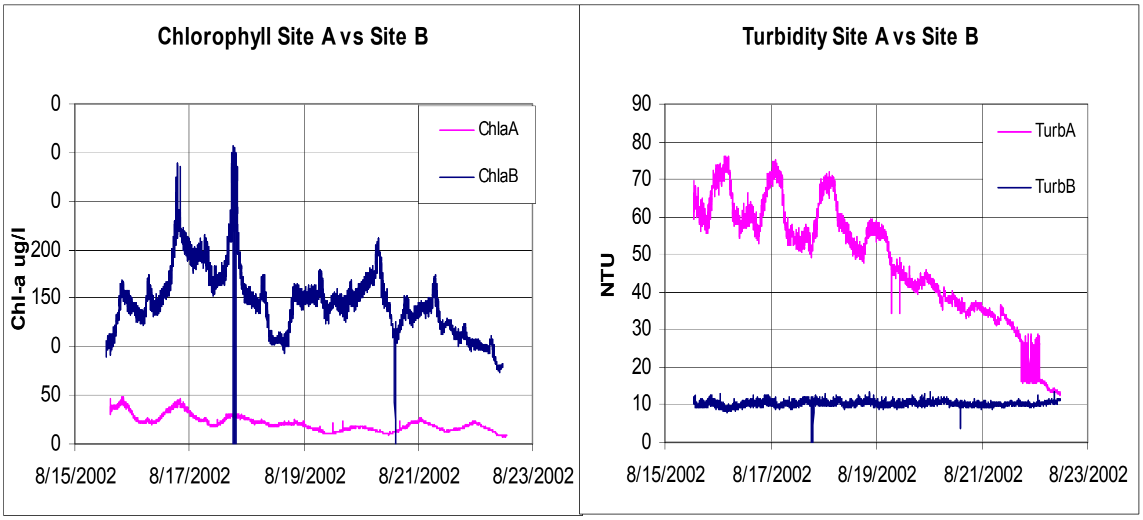


Figure H3.2 : Example of data from a two-week experimental deployment of a SCUFA sonde at the entrance and exit of the San Luis Drain. The data shows that algal chlorophyll concentration increases in the drain and that chlorophyll-a concentration can change significantly over short periods of time.

The next two sets of graphs plot chlorophyll-a and turbidity at Sites A and B for an earlier period between August 15, 2002 and August 23, 2002. Figure H3.3 shows a similar chlorophyll-a concentration increase between Sites A and B to that in Figure H3.2 – in which the chlorophyll-a concentration more than doubles along the 28 miles of channel. Figure H3.4 shows one of the factors that contributes to this increase which is a decrease in turbidity. Water that enter the drain at Site A contains a moderate sediment load as a result of the unlined earthern ditches the water flows through in transit to the San Luis Drain. Once in the San Luis Drain, the velocity diminishes as the flow cross-section expands and the flow gradient diminishes. The Drain cross-section increases again at about Check 19 further slowing flow velocity. Stokian sediment settling, which occurs as the drain water passes between Check structures, results in a decrease in drain sediment turbidity. As sediment settles out of the water column - light penetration increases resulting in greater potential algae growth per unit length of the drain channel. In Figure H3.4 the turbidity decreases dramatically after August 19 at Site A – however no corresponding increase shows up in the chlorophyll concentration at Site A in Figure H3.3. This would suggest that turbidity decreases due to sediment settling is a more important factor than any increase in turbidity due to enhanced algal growth at Site A.

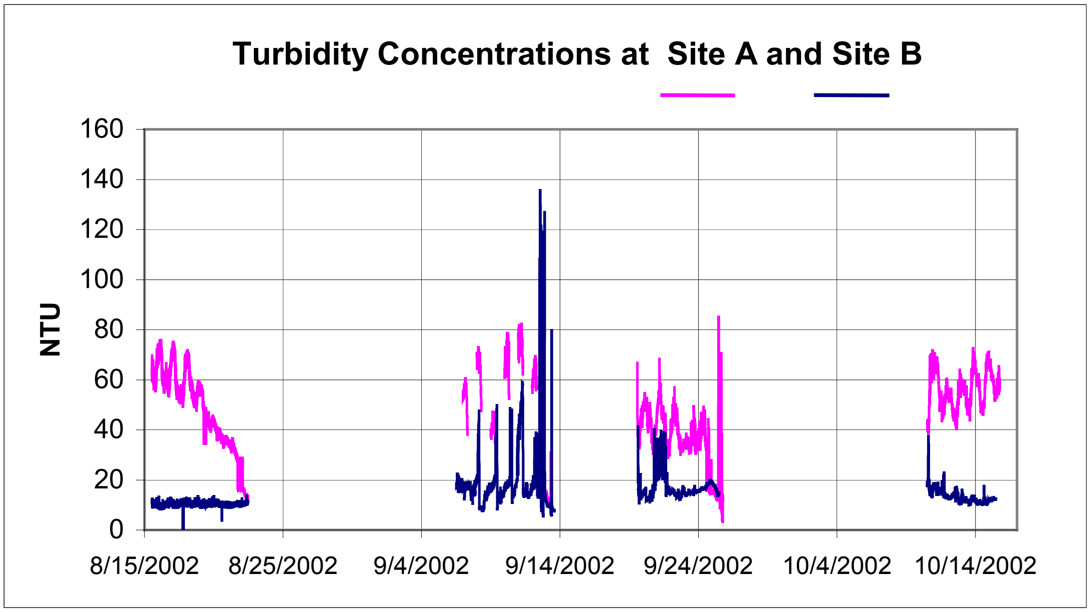


Figures H3.3 and H3.4. Comparison of Chlorophyll-a and turbidity concentrations at Sites A and B on the San Luis Drain.

The step decline in turbidity concentration shown in Figure H3.4 appears to somewhat of an anomaly. In Figure H3.5 a longer time series plot is shown for all the deployments of the SCUFA sonde in the San Luis Drain. Turbidity concentrations are shown to be quite variable and appear to show a similar range of high and low turbidity values during the period of deployment. Sediment fluctuations in the influent drain water at Site A is likely a result of various ditch cleaning operations within the Grasslands agricultural sub-Basin.

H 4. Analysis of diurnal trends in algal production

Given the apparent increase in algal biomass over the 1.5 to 3 day travel time (a function of flow) within the San Luis Drain, a question was raised as to whether a diurnal signal could be recognized in the time series data. If it is assumed that the Drain acts like a plug flow reactor with minimal horizontal dispersion and mixing the hypothesis can be advanced that photosynthetic diurnal growth in the Drain would show maximum concentrations during the mid-day to late afternoon period and minimum concentrations during the night. To test this hypothesis graphically, a plot has been made of chlorophyll-a concentration over time for a period between August 15 and August 20, 2002m indicating the noon to 6:00 p.m. time period which might be associated with periods of maximum algal growth.



Figures H3.5 Chlorophyll-a and turbidity concentrations at Sites A and B on the San Luis Drain for various deployments during 2002.

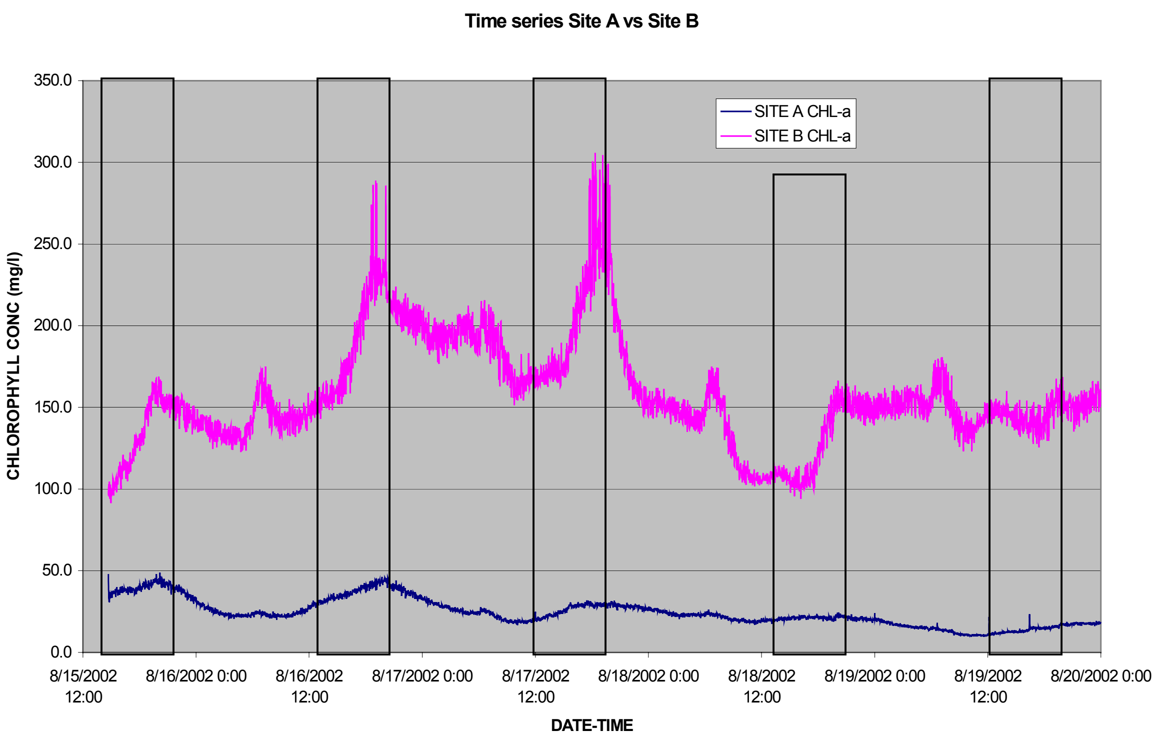


Figure 3.6. Time series of chlorophyll-a concentrations at Sites A and B on the San Luis Drain showing periods during which diurnal algal growth rates are expected to be at a maximum.

The highlighted areas do show positive gradients of algal production which might give weight to the hypothesis that there is a diurnal periodicity to algae growth.

However maximum chlorophyll concentrations appear to coincide with 8:00 p.m rather than 6:00 p.m, which is unexpected since the afternoon sun strikes the drainwater at an increasing acute angle as the hour approaches dusk – irradiating an uncreasingly smaller volume of drain water in the San Luis Drain. During August 16, algal growth rates remain high during the night hours.

The eighteen check structures and culverts that are encountered by the drain water in transit along the San Luis Drain tends to increase longitudinal dispersion. This phenomenon has been observed during several dye studies conducted in the early 1990’s which plotted the shape of the dye plume as water passed between Sites A and B on its course between the Main Drain (upstream of Site A) to Crows Landing on the San Joaquin River.

H 5. Summary

The set of experiments, conducted in the San Luis Drain and described in this supplement to the Quinn and Tulloch Final Report on San Joaquin River diversions and drainage (Quinn and Tulloch, 2001), were primarily to assess the utility of the SCUFA sondes for the anticipated 2003 CALFED Directed Action study. The author’s experience with these units has been positive and a number of SCUFA units have been recommended in the monitoring plan recommended to CALFED. Although initial deployment will be as autonomous units that are serviced every 2 weeks – a longer term objective is that these or similar units be integrated into the SCADA or real-time water quality monitoring systems of San Joaquin Basin water districts.

H 6. References

Quinn N.W.T and A. Tulloch. 2002. San Joaquin River diversion data assimilation, drainage estimation and installation of diversion monitoring stations. Final Report. CALFED Project #: ERP-01-N61-02

American Public Health Association. 1995. Standard Methods of the Examination of Water and Wastewater, 19th Edition. American Public Health Association, Washington, DC.

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