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**C A L L E G U A S C R E E K W A T E R S H E D
M A N A G E M E N T P L A N**

Calleguas Creek Nitrogen Compounds and Related Effects TMDL

Results of Special Study on Type and Extent of Algae Impairments in Calleguas Creek and Mugu Lagoon

submitted to:

L O S A N G E L E S R E G I O N A L W A T E R Q U A L I T Y C O N T R O L B O A R D

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7.0 Discussion of Results

Following is a detailed discussion of the results of the various studies conducted and conclusions that can be drawn from the analyses.

7.1 FIELD AND LABORATORY METHODS

7.1.1 Sample Processing Steps for Chlorophyll a Analysis for Benthic Algae

The Sample Processing Comparison (Section 3) indicates that the widely used method for processing field samples of benthic algae in streams, and the approach embodied in the U.S. EPA's RBP for stream periphyton, can underestimate the chlorophyll *a* content of filamentous macroalgae by at least 28%. The direct extraction method utilized for this study in lieu of the RBP method has several advantages. In addition to increased accuracy, several customary and time consuming sample processing steps (illustrated in Figure 4) are eliminated by the direct extraction method, leading to reduced labor in the field and laboratory. The savings in time and labor costs could translate into the ability to collect larger numbers of field samples per site. Given the inherently high natural spatial variability of benthic algae, the increased precision provided by higher sample sizes would be beneficial. In addition, the direct extraction method provides an opportunity to sample algae colonizing loose substrates such as sand and fine gravel, or other materials which are not easily sampled using common tools. Because the RBP approach requires one to scrub algae off of substrates into containers of water, and because this step is impractical for sand and other loose substrates, these substrates are often omitted in field surveys. The syringe sampler, which performs well on a variety of stream substrates, is easily incorporated into a project utilizing direct extraction; the detachable scouring pads may be placed directly in acetone for pigment extraction without any sample processing.

One potential drawback of the direct extraction method is that it is not possible to subsample the benthic algae for other analyses – such as ash-free dry weight or carbon or nitrogen content. However, because non-algal components of periphyton (bacteria, fungi, detritus, invertebrates) contribute to these other parameters, they are unreliable indicators of algal biomass, and (not surprisingly) are not under consideration as potential indicators of algal impairment in the regulatory setting. Although these other data (ash-free dry weight, C, N) may be useful for other aspects of scientific investigation, they are rarely reported - and more rarely used - in evaluations of algal impairment.

Because field samples containing filamentous algae can contain several grams of algal tissue during bloom conditions, it is important to use a sufficient amount of acetone (or other extractant), for a sufficient amount of time, during direct pigment extraction. During this study, field samples were extracted in 250 ml of 90% acetone per field sample for 48 hours. Results of the second laboratory test (Section 3, Test 2, Figure 7) suggest that use of larger volumes of extractant (> 250 ml) may be necessary to fully extract the chlorophyll from the type of samples obtained when thick mats of filamentous algae occur in the field. This issue could be addressed in a straightforward manner in a future study.

Because many potential targets being discussed for benthic algae are based on biomass measurements that were obtained using the RBP method, the use of these targets may not be appropriate. The chlorophyll levels so far reported from streams containing filamentous algae may be underestimating the actual amount of biomass present. This means that statistical approaches relating nutrient loads to algal biomass may be predicting significantly less biomass from particular loadings than actually occurs in the field. Also, the biomass levels that have been measured in streams classified as "impaired" or "unimpaired", or assigned to different trophic states, may be too low. The results of this study suggest that the RBP approach should be further evaluated and more thoroughly compared to alternative approaches which avoid rough handling of algal tissue and potentially cell-rupturing field or laboratory steps. Also, reported relationships between percent cover and algal biomass should be interpreted in light of the sample processing protocols that were used to obtain chlorophyll data.

7.1.2 Percent Cover Method Comparison

The study found that percent cover data obtained using the grid viewer method and the point-transect method were statistically indistinguishable (Section 4.7.3). This suggests that either approach is a valid choice in a monitoring program. Both approaches have drawbacks and advantages in the field. In both approaches, the wetted width of the stream must be measured at each transect position. Consequently, no savings in time are afforded by choosing one method over the other with regards to this field step. Evaluation of macroalgal thickness is problematic when grid viewers are used to score algae; a measuring device must be compared to algal colonies at multiple points within the grid view –which cannot be done while holding the grid viewer at the surface of the water. Sun glint seriously impedes use of the grid viewer, as does shade and deep or turbid water. Where substrates protrude from the water, such as in riffles, the grid viewer cannot be submerged, and alternative approaches are necessary. Grid viewers are not commercially available, and must be constructed by hand.

The only equipment needed for the point-transect method is a thin measuring stick (such as a foot-long graduated bamboo skewer). The measuring stick is less cumbersome to carry in the field than a grid viewer and has a dual use: (1) to blindly pinpoint where algae are evaluated at each target location along the transect (avoiding operator bias), and (2) to assess the thickness of algal colonies. However, more calculations are needed to establish sampling locations along transects when using the point transect method. Also, algae are evaluated closer to stream margins in the point transect method, where access can be more challenging owing to riparian vegetation, aquatic plants, and other obstacles.

7.2 RELATIONSHIPS BETWEEN PERCENT COVER, BIOMASS, AND DO

The RBP percent cover protocol does not generate scores for thickness categories of macroalgae. However, the results of the study suggest that if a goal of a percent cover survey is to infer algal biomass from percent cover, it is important to evaluate the thickness of macroalgal growths at individual sampling points. Simple scores for the presence or absence of macroalgae, without regard to the length or thickness of algae at sampling points, overestimated algal biomass (see Figure 16). A scoring system including only macroalgal length strata also performed poorly in this regard. A scoring system that separated macroalgae into length *and* thickness categories allowed the derivation of a variable which well-predicted algal biomass: "percent cover of long-(med+thick) macroalgae". In the survey, scores for this single percent cover variable, which can be expressed in words as follows:

"filamentous macroalgae >10 cm in length occurring in masses ≥ 2 cm thick," would have sufficed as an indicator of algal biomass. However, this result might not have been obtained in the same stream reaches during other seasons if macroalgae with a different growth morphology than *Cladophora* dominated biomass, or if thick biofilms were present.

Additionally, percent cover of benthic algae did not predict nocturnal DO minima in stream reaches. None of the field-scored or derived percent cover variables evaluated were related to mean nocturnal DO minima. This suggests that percent cover data are of limited value for evaluating the risk of DO impairment in streams. However, on the reach level, percent cover (of "all macroalgae") was able to predict the *magnitude* of diel shifts in DO, or ΔDO (Figure 27). Although this is an interesting result from a scientific perspective, it less useful from a regulatory perspective because ΔDO cannot be linked directly to risk of DO impairment. The time series data showed that when in-stream photosynthesis and respiration causes large diurnal shifts in DO, high ΔDO values are not necessarily paired with nocturnal levels of DO below the thresholds established for protection of aquatic life.

7.3 EVALUATION OF POTENTIAL ALGAL IMPAIRMENTS

The results of the study demonstrate that algae is present during at least part of the year in all reaches surveyed. However, the impact of the algae on beneficial uses and potential impairments due to the algae is more difficult to assess. The potential impacts on recreation (REC-1 and REC-2) and aquatic life beneficial uses are discussed in this section.

7.3.1 Recreational Uses

Currently, no algal targets exist for the CCW that can be used to assess algal impairments of recreational uses. Additionally, there is no general scientific consensus on numeric targets that can be used to define impairment of recreational beneficial uses due to algae. As a result, an assessment of whether or not algae exceeds thresholds for protection of recreational uses in CCW is not feasible. However, numerous literature values exist that can provide context for the values observed in the CCW.

Percent cover of "nuisance" algae (usually filamentous macroalgae) can be used as an indicator of algal impairment of recreational uses. Typically, impairments of recreational uses of a waterbody due to algae stem from "how the waterbody looks", which may or may not be well correlated to the biomass of algae present. Judgements regarding how much of the stream bottom needs to be covered by macroalgae for a reach to be unaesthetic or otherwise less suitable for recreational uses are inherently subjective and problematic in a regulatory setting.

A number of studies related percent cover measurements to algal biomass or estimated the algal biomass that caused "nuisance conditions" independently from percent cover. Welch et al. (1988) found that greater than 20% coverage of bottom surface area with filamentous green algae typically corresponded to biomass levels above 100 mg chl-a/m^2. Biggs (2000a) found that 120 mg chl-a/m^2 related to 20% cover by filamentous greens, but recommended a threshold of 30% cover for protection of recreational uses. There is some consensus in the literature that "nuisance conditions" can be expected if levels of benthic algal biomass exceed 100-200 mg chl-a/m^2 (Welch et al., 1988; Dodds et al., 1998; Sosiak, 2002; Dodds and Welch, 2000; USEPA, 2000a, Biggs 2000a, TetraTech 2006). The California Nutrient Numeric Endpoints guidance document (NNE, TetraTech 2006) suggests a boundary for seasonal maximum benthic algal biomass between 100-150 mg chl-a/m^2 for protection of recreation and most other uses, depending on the risk category the site falls into. The EPA's Nutrient Criteria Technical Guidance Manual for Rivers and Streams (EPA 2000, Chapter 7) recommends a 150-mg chl-a/m^2 threshold.

With the exception of Site 10 in May, percent cover of filamentous macroalgae was near or above 50% in CCW stream reaches during both monitoring events. In April, benthic algal biomass was above 100-mg chl-a/m^2 at all sites, and above 150 mg chl-a/m^2 in Revolon Slough. However, the relationship between percent cover and algal biomass in CCW appears to differ from the relationships generated by the studies above. The regression equations obtained from the April survey data can be used to predict biomass on the basis of different percent cover values. The regression equation obtained using percent cover of only long-(med+thick) macroalgae - which was the best predictor of algal biomass in the study (Figure 17c) - was used to predict biomass values for percent cover ranging from 10-100% (Table 16). Because the biomass data reflect the higher chlorophyll yields of the direct extraction method, Table 16 also provides estimates of the biomass values that would have been obtained from field samples had the RBP method been used (i.e., assuming a 28% reduction in chlorophyll *a* yield). In the CCW reaches studied, percent cover targets in the range of 20-30% would have been associated with chlorophyll levels two to three times higher than the chlorophyll thresholds that have been proposed in the literature. Attainment of biomass levels of 150 mg chl-a/m^2 would have necessitated a percent cover of long-(thick+med) macroalgae well below 5%, at least during the spring bloom conditions that were present in April in CCW.

Table 16. Biomass Predicted by Percent Cover of Long-(thick+med) Macroalgae in Stream Reaches of Calleguas Creek Watershed

	mg chl-a/m^2	
Percent Cover	**via direct extraction**	**estimated RBP[(a)]**
10%	265	191
20%	431	311
30%	598	431
40%	765	550
50%	931	670
60%	1098	790
70%	1264	910
80%	1431	1030
90%	1597	1150
100%	1764	1270

(a) Direct extraction results discounted by 28%, see Part 1.

As a result, although the algae monitoring results were higher than literature values considered for protection of recreational uses, potentially different dynamics in the CCW make it difficult to assess whether or not these values represent an impairment. Further, watershed-specific analysis would be required to evaluate whether or not the observed algal biomass and percent cover can be considered to be impairing recreational uses in the CCW.

7.3.2 Aquatic Life Uses

Unlike recreational uses, aquatic life beneficial use impacts can be approximated by evaluating DO levels using established water quality objectives. Although algae is not the only factor that can lower DO levels, DO levels that are consistently above the water quality objectives indicate that algae is not impairing aquatic life beneficial uses.

The Basin Plan objective for DO in inland surface waters in the Los Angeles Region reads as follows (emphasis added):

> "At a minimum…the mean annual dissolved oxygen concentration of all waters shall be greater than 7 mg/L, and no single determination shall be less than 5.0 mg/L, *except when natural conditions cause lesser concentrations*. The dissolved oxygen content of all surface waters designated as WARM shall not be depressed below 5 mg/L *as a result of waste discharges*."

Although algae (through photosynthesis and respiration) can influence changes in pH and DO, it is important to note that other factors, such as bacterial respiration, chemical oxygen demand, stream flow, water temperature, depth, turbulence, density stratification, solar radiation, and buffering capacity, can also influence these indicators. When DO and pH both follow the diurnal pattern expected from the interplay between photosynthesis and algal respiration, it is appropriate to consider algal biomass (in the water column or the benthos) as a contributor to nocturnal DO minima. However, because submerged aquatic plants can produce a similar diurnal pattern of DO and pH, caution should be used when attributing these patterns to algal biomass when the ecosystem also contains submerged aquatic plants. Consequently, although algae likely play a role in controlling nocturnal DO minima, other natural conditions could cause low oxygen concentrations.

During the course of the study, individual 15-minute incremental measurements below 5.0 mg/L were observed at four out of six stream sites. However, the duration of the exceedances is also important, especially for diel monitoring, when evaluating potential DO impairments. The State listing policy for DO states, in part:

> "For depressed dissolved oxygen, if measurements of dissolved oxygen taken over the day (diel) show low concentrations in the morning and sufficient concentrations in the afternoon, then it shall be assumed that nutrients are responsible for the observed dissolved oxygen concentrations *if riparian cover, substrate composition or other pertinent factors can be ruled out as controlling dissolved oxygen fluctuations*. When continuous monitoring data are available, the seven-day average of daily minimum measurements shall be assessed…" (SWRCB 2004, emphasis added)

If the average of the daily minimum measurements is considered, only two sites in CCW were below 5.0 mg/L: Revolon Slough and Lower Conejo Creek. The Lower Conejo Creek site mean was just below 5.0 mg/L (4.98 mg/L) which is within the range of error (± 0.1 mg/L) of the instrument. As a result, the only site where average daily minimum DO can be conclusively stated to be below 5.0 mg/L during the study was Revolon Slough.

During the diel DO monitoring study, percent cover measurements were also collected at the monitoring locations. Although no percent cover category for benthic algae (lumped or otherwise) showed a relationship with mean minimum daily DO (Figure 25), the percent cover information provide some interesting observations.

- Individual nocturnal DO readings at Site 12, which had the most profuse growths of filamentous algae during the May survey,[20] ranged from 5.05-5.92 mg/L during the deployment. The lack of correspondence between high algal abundance (80% cover by macroalgae) and DO impairment at this site was not an artifact of high flow; among sites surveyed in May, flow was lowest at Site 12, and was less than one-fourth the flow in Revolon Slough where DO was driven lower at night. The fact that high percent cover of macroalgae was not paired with dissolved oxygen impairment indicates that percent cover of macroalgae should not be used to infer the risk of DO impairment in CCW.
- DO impairment did not occur during the latter stages of decomposition of a profuse filamentous algal bloom (Site 10, mean nocturnal minimum DO = 5.13 mg/L).
- The only site where DO excursions at night pushed the mean nocturnal minimum below the Basin Plan threshold was Revolon Slough, where the mean minimum DO was 4.04 mg/L. However, Revolon Slough had the lowest benthic algal biomass in the April survey (125 mg chl-a/m^2, Figure 11) and the lowest percent cover scores for long-(med+thick) macroalgae in April and May (Figure 12 and Figure 13).
- As discussed in Section 5, the unusual shape of the diel curves for pH and DO in Revolon Slough strongly suggest that processes unrelated to photosynthesis and algal respiration were contributing to DO patterns there. Also, ΔDO at Revolon Slough deviated from the statistically significant relationship between ΔDO and algal abundance (flow weighted or otherwise) that was obtained using the data from the other sites (Figure 27). The fact that this was also the only site where DO thresholds were clearly exceeded during the survey demonstrates that DO impairments must be assessed based on all factors, not just benthic algal biomass.

Algal biomass does not appear to be causing impairments of aquatic life based on low DO measurements in the CCW stream reaches evaluated. Although some excursions below the 5.0 mg/L Basin Plan objective were observed, the average daily minimum concentration during the study period was below 5.0 mg/L at only one site (Revolon Slough), and based on the shape of the diel curves for DO and pH, factors other than algal abundance appear to be affecting DO patterns there. Percent cover values as high as 80% (macroalgae) were not accompanied by mean nocturnal DO concentrations less than 5.0 mg/L. As a result, during the study period, aquatic life impairments in the stream system do not appear to have occurred as a result of algal blooms.

The Basin Plan objective for pH in inland surface waters states that pH is not to deviate from 6.5 to 8.5 as a result of wastes discharged and that ambient pH levels shall not be changed more than 0.5 units from natural conditions as a result of waste discharge. Observed maximum pH levels exceeded 8.5 in five of the six reaches monitored, but met the Basin Plan objectives at all times downstream of Hill Canyon WWTP, a major discharger to Conejo Creek. The scope of the study did not evaluate whether or not the pH changes in the waterbody were a result of waste discharge. Additionally, the natural pH conditions have not been determined for this waterbody. As a result, it is not possible to determine if the observed pH levels constitute an exceedance of water quality objectives in the waterbody.

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[20] Site 12 was 42% covered by long-(med+thick) macroalgae, and 80% covered by all macroalgae in May, see Figure 14)

To better assess whether the observed pH levels could be considered a potentially problematic byproduct of algal growth, thresholds specific to photosynthetically driven pH levels were reviewed. The NNE recommends a photosynthetically driven maximum pH of 9.0 or less as indicative of unimpaired conditions for a warm water stream, and a maximum pH of 9.5 or less as indicative of impaired warm water conditions. Photosynthetically driven mean maximum pH did not exceed 9.0 at any of the CCW stream sites studied.

7.3.3 Mugu Lagoon Aquatic Life Assessment

The Basin Plan objective for DO discussed above also applies to Mugu Lagoon. However, in the lagoon, an assessment of "natural conditions" is important and also challenging. DO levels and algal biomass can change as a result of the physiographic setting, salinity regime, frequency and timing of freshwater inflows, magnitude of tidal forcing, sediment load, stratification, and residence time. As a result, DO criteria are still under development for California estuaries (McLaughlin & Sutila 2007). As yet, there are no threshold values for macroalgal or phytoplankton biomass that are linked to specific DO values in estuaries because other factors, such as water residence time and bathymetry, alter DO response to algal blooms (McLaughlin & Sutila (2007).

Time of day and the spring/neap tide cycle exerted a greater influence on DO at the Mugu Lagoon sites than the low/high tide cycle. Over the course of the ten day deployment, low tides corresponded with oxygen minima *and* maxima at some point, as did high tides. During neap tide, near-anoxic conditions persisted at night through tidal transitions in sites where tidal creeks drained intertidal mudflats. The hypoxia was less pronounced at the M Street site where larger volumes of water were exhanged between tides in a deeper channel. These time series suggest that low tide (at night) does not necessarily present a higher risk of hypoxia than high tide in Mugu Lagoon. A longer time series, during which high tide would have eventually coincided with pre-dawn hours closer to a spring tide, would have helped to confirm whether or not Mugu Lagoon has DO patterns that are different in this regard than other California estuaries where diel monitoring has taken place. Longer residence times of water in intertidal habitat close to neap tide, and consequently longer immersion periods for intertidal macroalgae, may underlie the contrast in DO patterns between spring tide and neap tide periods. Hypoxic events were also more pronounced during neap tides in Upper Newport Bay (Nezlin et al. 2006). As a result, the presence of algae, tidal conditions, and potentially other factors, may need to be considered to determine whether or not impairments are occurring in Mugu Lagoon.

Information from guidance literature for California estuaries was utilized to assess whether the observed DO levels in Mugu Lagoon may be of concern. Two approaches are currently proposed by the U.S. EPA to determine indicator endpoints: 1) an *effects based approach*, in which scientifically determined responses by biota to specific biological, physical or chemical indicators are compiled, and thresholds selected based on management criteria, and 2) the *percentile approach* (McLaughlin & Sutila, 2007). DO thresholds for a range of physiological and behavioral effects for many pelagic and benthic estaurine species are known. However, the effects-based approach for determining algal impairments in estuaries is currently hampered by lack of data linking specific levels of macroalgal biomass to low DO or other potential impairments. When 3.0 mg/L was used as the DO threshold defining hypoxia in Upper Newport Bay, Nezlin et al. (2006) found that combined abundance (percent cover) of macroalgae (*Ceramium* and *Ulva* spp.) explained less than 1% and 14% of the variability in the frequency of hypoxic DO readings in bottom waters and surface waters, respectively. When linear regression was performed using only *Ulva* spp. abundance, these numbers were 1% and 37%, respectively (none of the relationships were significant). However, use of 5.0 mg/L as the DO threshold improved the relationships; in that case, *Ulva* spp. alone explained 75% and 57% of the variability in the frequency of hypoxic readings in bottom waters and surface waters, respectively. These results suggest that macroalgal abundance in Upper Newport Bay may be linked to mild hypoxia (3-5 mg/L), but poorly predicts more severe hypoxic events (< 3.0 mg/L).

The percentile approach offers two ways to establish numeric criteria for DO. The first is to identify the 75th percentile of a frequency distribution of compiled data for pristine "reference sites" within one class of estuaries. The second is to identify the 25th percentile of a frequency distribution of compiled data from a probability based sample of systems in the same estuary class. As a demonstration of the latter approach, McLaughlin & Sutila (2007) calculated the 25th percentile of combined monitoring data (6+ months of hourly data) from four perenially tidal lagoons in California (Elkhorn Slough, San Diegito Lagoon, San Elijo Lagoon, and Tijuana Estuary); the exercise yielded an example numeric target DO of 3.7 mg/L. Mugu Lagoon also falls into the perennially tidal lagoon category described in Sutila et al. (2007). According to Sutila et al. (2007), 23 of the 32 estuaries in Southern California belong to this subclass.

For comparison purposes, an analogous exercise was performed using combined 15-min DO data from the three Mugu Lagoon sites monitored in this study. The resulting 25th percentile for DO was 2.64 mg/L (N = 2808 readings). This result is higher than the mean daily minimum DO for ten diel cycles, which was below 2.0 mg/L at all three sites. The deployment occurred when (visual) estimates of macroalgal coverage were high (75-100%) in intertidal habitat connected by tidal exchanges of water to the instrument locations. A longer study, and quantitative estimates of percent cover or biomass, would be necessary to evaluate whether the 25th percentile of low DO readings would change seasonally in Mugu Lagoon, and whether such changes could be associated with algal abundance - including subtidal macroalgae and phytoplankton, which were not surveyed in our study. Although the impact of algal abundance on DO levels could not be assessed in this study, the diel monitoring results indicate low DO may be of concern in the lagoon and should be investigated further. However, a full assessment of the data should only be considered after DO criteria for estuaries are established in California, and the magnitude and duration of DO minima can be fully evaluated.

The Basin Plan objective for pH in bays and estuaries states that pH is not to deviate from 6.5 to 8.5 as a result of wastes discharged and that ambient pH levels shall not be changed more than 0.2 units from natural conditions as a result of waste discharge. The time series data indicate large diurnal shifts in pH in the Mugu Lagoon, especially when tidal range was relatively high. pH exceeded 8.5 most of the time at the M Street Bridge site, and exceeded 9.0 during the late afternoon when tidal range was high. Apparently photosynthesis-driven increases in pH above 8.5 occurred in both tidal creeks as well. The fact that the lowest DO readings of the time series in Mugu Lagoon (which were during the neap tide) were not accompanied by the lowest pH readings could be viewed as evidence that processes other than respiration were consuming oxygen at night during the neap tide that do not tend to lower pH (as algal respiration does). However, as in streams, the judgement about when algae driven pH maxima or nocturnal DO minima are *natural* in an estuary (i.e., would be expected with background loadings or in reference conditions) versus when they are the indirect result of nutrient discharges (mediated through algae or aquatic plants), is difficult when well-matched reference sites are lacking.

Consequently, it is not possible to evaluate whether the pH levels observed in Mugu Lagoon constitute an exceedance of Basin Plan objectives. Additionally, pH is not usually evaluated as an indicator of algal impairment in estuaries. Unlike for streams, guidance on maximum photosynthesis-driven pH thresholds has not been developed for estuaries. As a result, it cannot be determined whether potential photosynthesis-driven pH impairments occurred in Mugu Lagoon during the study.

7.4 CONCLUSIONS

The analysis of different sample processing methods demonstrated that direct extraction of filamentous macroalgae in acetone results in higher values of chlorophyll *a* than the traditionally used RBP sample processing approach for obtaining pigment extracts. Consequently, regional and national data sets may include many underestimates of benthic algal biomass. Targets for biomass or percent cover and statistical relationships produced using these data sets may not be appropriate for systems containing large amounts of filamentous algae.

The two methods for measuring percent cover used in the study (point-transect and the grid-viewer method) performed very similarly in CCW. As a result, either method can be utilized for determining percent cover in CCW.

A relationship between percent cover and biomass was discovered for one category of benthic algae assessed (long med-thick macroalgae), but not for any other categories or groupings assessed. Relationships between percent cover and DO minima were not found, but percent cover was related to the magnitude of diurnal DO shifts (ΔDO).

The study demonstrated that algal biomass during the spring bloom period was present in all reaches of the CCW and Mugu Lagoon, although different reaches contained different proportions of the types of algae evaluated. Recreational use impairments cannot be fully assessed at this time due to the lack of numeric thresholds specific to the CCW. Information gathered from this study indicate that thresholds for algal biomass (chl-a/m2) suggested in the literature for protection of recreational uses in streams may not be appropriate for CCW because relationships between algal biomass and percent cover may be different in CCW than those reported from other stream systems.

DO levels can be utilized to assess potential aquatic life impairments related to algae. In the stream system, nocturnal DO minima were only consistently below the water quality objective in one reach and the study results do not indicate an aquatic life impairment due to algae exists in any reach. For Mugu Lagoon, measured DO levels may be of concern, but could not be fully evaluated due to the lack of criteria specific to estuarine environments. Further evaluation will be needed when the DO criteria for California estuaries are completed. No conclusions can be drawn about the potential relationship between algal biomass and the measured DO levels in Mugu Lagoon.

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Appendix A. Chlorophyll Extraction Procedure and Analysis of Pigment Extracts Using Turner Designs 10-AU Digital Fluorometer

SUPPLIES

250 mL Polypropylene bottles

A/E glass fiber filters

Forceps

Rubber policemen

Sharpie

Composition book

13 x 100 mm borosilicate glass vials

1 Liter volumetric flask

Pasteur pipet

Pipet bulbs

1 L PP Nalgene bottle

Aluminum foil

Test tube rack

Parafilm

Squirt bottle

EQUIPMENT

Fluorometer

Vacuum pump

Vacuum flask

Tubing

Filter apparatus

REAGENTS

90% HPLC Grade Acetone (use a 1L volumetric flask)

10% Hydrochloric acid (HCl)

90% Acetone

- Add 100 mL of Nanopure DDI into a 1 L volumetric flask.
- Bring to volume with acetone – use a disposable Pasteur pipet
- Pour contents into a 1 L Nalgene bottle

10% HCl

- Add 10 mL HCl into a 100 mL volumetric flask
- Bring to volume with Nanopure DDI

Notes:

Do not pre-fill bottles with acetone – it evaporates quickly

Record sample information on log sheet in composition book and assign consecutive numbers to samples/blanks prior to processing.

PIGMENT EXTRACTION

Note: work should be performed under subdued lighting.

1) Place algae, sand, filters, scouring pads, or gravel substrates from each film canister into a 250mL polypropylene (PP) bottle.
2) Rinse residual canister contents or water onto a 25mm A/E glass fiber filter in filter housing.
3) Filter residual contents until dry without prolonging filtration. Note: Do not exceed 20 psi on vacuum pump.
4) Remove filter with forceps and place in 250mL PP along with other sample contents.
5) Add 250 mL of 90% HPLC Grade acetone. Cap tightly. Prepare one 250mL 90% acetone filter blank (treated in the same manner as the samples).
6) Cover with aluminum foil, or otherwise keep dark. Place upright in the freezer for 48 hours. Log time in a labbook.
7) Shake samples after 24 hours. **Do not shake 4 hours prior to reading – particulate matter will be resuspended.** Note time in labbook.

Note: **Log everything! Record all information in labbook. Make a note if any problems arise during filtration (e.g., torn filter, leaking filter housing, etc.). Rinse filter towers and frits thoroughly with Nanopure between samples.**

FLUOROMETRIC ANALYSIS

1. Allow fluorometer to warm up for 1 hour before analyzing samples.

2. Remove samples from freezer, shake, then centrifuge for 3 minutes @ 3600 rpm.

3. Decant acetone blank into 13x100 mm borosilicate glass test tube to about 90% full. Place test tube in fluorometer test tube holder. Record value for blank. With a sharpie, mark orientation of test tube and return to rack to be read again after all samples have been analyzed.

4. Decant sample into 13x100 mm borosilicate glass test tube. Place test tube in fluorometer test tube holder. With a sharpie, mark orientation of test tube.

5. Once the reading has stabilized, record the value in the labbook under "Initial Fluorometer Reading" (Rb – before acid). Be sure and record the Gain value in the labbook as well.

6. Add 2 drops of 10% HCl to the sample test tube.

7. Cover with Parafilm, and invert upside down several times to mix. Place test tube in same orientation as before acid addition in fluorometer test tube holder.

8. Once the reading has stabilized, record the value in the labbook under "Final Fluorometer Reading" (Ra – after acid). Be sure and record the Gain value in the labbook. Repeat steps 15-19 for all samples.

9. Run acetone blank for the last time. Record value for blank in labbook under "Final Acetone Blank Fluorometer Reading".

Note: If a dilution must be performed because the detector is saturated, be sure and note the ratio of dilution and volumes used. Usually a 1:1 dilution (e.g., 5 mL sample : 5 mL 90% acetone – 2x dilution) is sufficient.

10. When all samples are complete, pour extracts into waste container. Place test tubes into glass waste container. Rinse all Falcon tubes and caps 3xs with Nanopure. Place tubes in styrofoam rack upside down overnight to dry. Loosely cap tubes to allow further dessication and avoide dust.