SEASONAL AND DILUTION RATE IMPACTS ON *NANNOCHLOROPSIS*
OCEANICA PRODUCTIVITY IN ALGAE RACEWAY PONDS

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ABSTRACT

Seasonal and Dilution Rate Impacts on *Nannochloropsis Oceanica*

Productivity in Algae Raceway Ponds

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Biomass growth of the alga *Nannochloropsis oceanica*, cultivated outdoors in six pilot-scale raceway ponds, was monitored over the course of 1.5 years, at two different dilution regimes each season, to establish the effects on algal biomass productivity and concentration of dilution rate, pond water temperature, and solar radiation. The 4.5-m² ponds were located in a mild, mid-latitude, coastal region (central California). Experimental conditions were operated in duplicates or triplicates with a consistent artificial seawater medium, pond depth, pH range, paddle wheel mixing speed, and replete nutrient conditions for the duration of the study. Two cultivation regimes were used to regulate pond biomass concentration: batch growth and a three-times-per-week dilution with a resulting dilution rate of 0.21/d. For the ranges of input variables tested, productivity (g/m²-d) was positively correlated to both pond water temperature and solar radiation. However, the data scatter in the correlations was substantial, indicating the existence of other major influences on productivity. A dilution regime consisting of three dilutions per week and a dilution rate of 0.21/d resulted in the higher productivities compared to batch cultivation for all seasons tested. With high light intensity (200-300 W/m²) and warm water (18.3°C daily average), the highest productivity was 11.4 g/m²-d with a resulting biomass concentration

of 0.15-0.20 g/L. With low light intensities (150-200 W/m²) and cool water (16.6°C daily average), the highest productivity was 6.9 g/m²-d with a resulting biomass concentration of 0.10-0.15 g/L.

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I. INTRODUCTION

To reduce dependence on fossil fuels, the U.S. Department of Energy (DOE) is promoting development of cost-competitive biofuels, in particular transportation fuels, from non-food biomass resources. Increased domestic biofuels production could potentially offset petroleum consumption (Office of Energy Efficiency & Renewable Energy, 2016). One potential biofuel feedstock is microalgae biomass, which could potentially displace as much as 17% of U.S. petroleum used for transportation (Wigmosta, Coleman, Skaggs, Huesemann, & Lane, 2011). The DOE stated in their *National Algal Biofuels Technology Roadmap* that the target annual average biomass production for algae is 30-60 g/d per m² of algae pond surface area to produce 10 million gallons of oil feedstock on roughly 800-2600 acres of pond surface area, but these target productivities have seldom been achieved in outdoor ponds, even for short time periods (U.S. DOE, 2010).

To maximize algal biomass productivity in raceway ponds, several main variables can be manipulated: algae strain, pH, pond depth, paddle wheel mixing speed, nutrient concentrations and types, and dilution rate. Due to engineering and economic constraints, only strain and dilution rate are practical to adjust widely to maximize productivity (Lundquist, Woertz, Quinn, & Benemann, 2010). However, algal strains with sufficiently high productivity potential will need to be discovered or developed. Until then, learning to optimize dilution rate to optimize the growth rate of existing candidate production strains would be useful

preparation. To study the effect of dilution rate, a pure algae culture was cultivated in pilot-scale raceway ponds that were operated within the optimal ranges for pH, depth, paddle wheel mixing speed, and nutrient concentrations.

The algae strain *Nannochloropsis oceanica* was selected for this study due to the stability of its cultures, sufficient growth rate, and value as an aquaculture feed (Weissman & Tillett, 1989) (Benning, 2014). *N. oceanica* is part of the Eustigmatales order and within the family of Eustigmataceae (Hibberd, 1981). *N. oceanica* is green and unicellular with coccoid cells ranging from 2- μm to 5- μm in diameter. This species is primarily found in marine ecosystems but can also be found in brackish and fresh waters (Hong-Po, et al., 2013).

The pH of ponds is influenced by photosynthesis and respiration of algae cells. During the day, algae assimilation of dissolved carbon dioxide ($\text{CO}_{2\text{aq}}$) via photosynthesis typically exceeds dissimilation of CO_2 via respiration. As the CO_2 concentration decreases, the carbonate equilibrium shifts leading to an increase in hydroxide ion (OH^-) concentration and consequently higher pH. This process is reversed in low-light and night conditions, where respiration exceeds photosynthesis, which leads to a net release of CO_2 into the water and lower pH (Tucker & D'Abramo', 2008). To maintain pH in the ideal range for algae growth, generally between 7-9 (Food and Agriculture Organization of the United Nations, 1996), cultivation reactors are typically equipped with CO_2 sparging. For large-scale raceway ponds, it is not cost effective to sparge CO_2 through multiple

diffusers and usually only one or two diffuser locations are used (Lundquist, Woertz, Quinn, & Benemann, 2010).

The water depth influences several variables such as temperature changes, the concentration of algal biomass, and the ability to accomplish mixing in raceways. For large raceways approaching 4 ha in size, the optimum depth for efficiency of paddle wheel mixing is about 30 cm (Weissman & Tillett, 1989). For deeper ponds, higher energy consumption is not economical, and for shallower ponds, mixing of long channels is inefficient. Thus the choice of raceway depth is limited to about 30 cm, and depth is not a variable to adjust in these paddle wheel-mixed raceway productivity experiments.

Paddle wheel mixing minimizes cell sedimentation in raceways, brings algae cells to the pond surface for exposure to light, and promotes gas exchange with the atmosphere such as dissipation of dissolved oxygen (beneficial to algae productivity) and loss of dissolved CO₂ (detrimental to process economics). The optimum channel velocity for productivity is likely to be strain dependent with velocities used ranging between 5-40 cm/sec (The National Institute of Oceanography, 2008). Velocities of 20-30 cm/sec have been suggested to be typical (Rogers, et al., 2014) (Weissman & Tillett, 1989).

According to Monod growth kinetics, the near-maximum specific growth rate is achieved when nutrient concentrations approach specific high levels. To

achieve the highest algal biomass productivity, required nutrients should remain replete with respect to strain half-saturation constants.

With the practical range of the above variables being limited, dilution rate remains as the most flexible productivity-related variable. Dilution rate regulates the culture concentration by removing a specific amount of biomass, thereby lowering the concentration of algae cells in the pond and increasing the amount of light available per remaining cell. The Beer-Lambert Law shows that, at low cell concentration, the amount of light penetrating the culture medium is related to the concentration of the cells. Also, within limits, higher light exposure increases culture productivity. Diluting the ponds is therefore potentially beneficial to algal biomass productivity. If the culture is too dense, then all light is adsorbed within the first few centimeters, which might not be used efficiently by the cells and is therefore wasted. If the culture is too dilute, then cells may be exposed to excessive light that can damage the photosynthetic abilities of the cell (photo-inhibition). Therefore, optimizing dilution rate, which regulates cell culture concentration, can maximize productivity. Dilution rate (D) can also be expressed as hydraulic residence time (HRT), a term more commonly used in the environmental engineering field. The mathematical inverse of dilution rate is HRT.

The objective of the present research was to determine the optimal dilution rate for maximum productivity of a potential production strain, *N. oceanica*, in a mid-latitude coastal climate. The methods described herein may be used for

optimization of dilution rate for other strains, locations, seasons, and possibly even transient weather conditions.

This study took place in San Luis Obispo, California, (latitude: 35° 16' 58" N) from the fall of 2013 to the spring of 2015 as part of the Algae Testbed Public-Private Partnership (ATP³) led by Arizona State University and funded by the Bioenergy Technologies Office, U.S. Department of Energy. A total of seven experiments were carried out within 1.5 years with ponds operated in either duplicates or triplicates of one another. The goals of these experiments were to determine the effects of season (solar radiation and pond water temperature) on biomass productivity and to determine the effect of dilution schedule on biomass productivity. Six pilot scale raceway ponds were used to cultivate the pure culture of *N. oceanica*. The ponds were operated at a constant depth of 25 cm, with a paddle wheel speed of 7.45 rpm, at a pH of 7-8, and with replete nutrient concentrations. The alga was grown in 35-ppt artificial salt water. Two cultivation regimes were observed throughout these experiments, which included batch cultivation and a three-times-per-week dilution with a dilution rate of 0.21/d. Seasonal changes such as solar radiation levels and pond water temperature were observed throughout the study as well.

II. METHODS

The following section outlines the experimental plan and how the study was done, including a description of the raceway ponds used, their operation, media preparation, algal biomass cultivation, analytical testing procedures, and equations used to analyze the results.

Experimental Design

A series of seven experiments (Table 1) were executed to determine the effect of dilution rate and seasonal variations on productivity in pilot-scale raceway ponds. All water quality testing, including optical density, ash free dry weight, nitrate, phosphate, and microscopy, and photobioreactor algal growth were conducted at the Civil and Environmental Engineering Department, California Polytechnic State University, San Luis Obispo. All pond experiments took place at the City of San Luis Obispo Water Resource Recovery Facility. However, no wastewater was used in the studies described. The goals of these experiments were to determine the effects of season (solar radiation and pond water temperature) on biomass productivity and to determine the effect of dilution schedule on biomass productivity.

The two dilution regimes (Table 1) were chosen to observe their effect on productivity over various seasons: (1) batch cultivation, with inoculation cell concentration of ~ 0.05 g/L and final of ~ 0.3 g/L, and (2) semi-continuous cultivation, with three dilutions per week and a dilution rate of 0.21/d (HRT = 4.7

days). Other dilution rates were used in some experiments (indicated by asterisks in Table 1) but insufficient data was collected to make conclusions.

The algal biomass concentration range of 0.05-0.3 g/L was selected because a concentration less than 0.05 g/L did not provide the pond with enough initial biomass to successfully start the ponds under all conditions, especially low light intensities experienced during colder months. The high concentration of 0.3 g/L was selected due to excessive respiration losses at higher concentrations. It was also observed during data analysis that the slope of the growth curve begins to flatten after this concentration, indicating that the culture is reaching the stationary phase of growth, which is undesirable when trying to maximize productivity (Harmon, Dempster, & McGowen, 2015).

Table 1: List of experiments conducted during the study with respective, time periods, and dilution types.

Experiment #	Start Date	End Date	Dilution Schedules
1	10/18/2013	11/8/2013	Batch
2	11/11/2013	12/4/2013	Batch
3*	4/4/2014	5/28/2014	Batch
4**	6/13/2014	7/23/2014	Batch; 3 dilutions/week, 0.21/d
5	9/16/2014	10/27/2014	Batch; 3 dilutions/week, 0.21/d
6	12/19/2014	1/26/2015	Batch; 3 dilutions/week, 0.21/d
7***	5/6/2015	6/5/2015	Batch; 3 dilutions/week, 0.21/d

* Also had 3 dilutions/week, dilution rate = 0.11/d

** Also had 3 dilutions/week, dilution rate = 0.15/d

*** Also had 3 dilutions/week, dilution rate = 0.21/d using ammonia as nitrogen source.

Raceway Ponds

Six identical raceway ponds (Figure 1; Table 2) were operated in duplicate or triplicate. Ponds were operated with a paddle wheel speed of 7.45 rpm (variable frequency drive setting of 20 Hz) and a depth of 25 cm.

Figure 1: Typical raceway pond used in this study, constructed of fiberglass tanks and stainless steel paddle wheels. The long axis of the ponds was oriented east-west with the paddle wheels on the north side.

Table 2: Raceway Tank Dimensions.

Pond Part	Dimension
Total Pond Area	4.48 m ²
Illuminated Pond Area*	3.90 m ²
Pond Operating Volume	1025 L
Tank Length	3.51 m
Tank Width	1.52 m
Tank Depth	0.41 m
Paddle Wheel Diameter	0.88 m
Paddle Wheel Width	0.66 m

*Illuminated pond area is the total pond area minus the paddle wheel area that would shade the pond with the sun directly overhead. The illuminated pond area was used to calculate productivities in this study (Equation 2 and Equation 3).

In-situ Sensors

YSI 5200A Multiparameter Monitoring and Control Instruments were used to measure and record pH, temperature, dissolved oxygen, salinity/conductivity, and oxidation-reduction potential at 15-minute intervals.

Light Intensity

The average 24-hour total solar radiation measurements were obtained from the California Irrigation Management Information System (CIMIS) using data collected from the San Luis Obispo station (Id: 052) and were averaged between

sample times. Solar radiation was measured using pyranometers at a height of 2 meters above the ground.

Algal Strain Cultivation

A strain of *Nannochloropsis oceanica*, obtained from Cellana LLC (Kona, Hawaii), was used as the model organism. It had been isolated from the north coast of the island of Hawaii. For the present experiments, this strain was scaled-up in laboratory photobioreactors (PBRs) to volumes large enough to inoculate the raceways.

Media Preparation

For 800-mL PBRs, the culture medium was prepared using deionized water, and for 15-L PBR panels, city tap water was used. Tap water contained 170 mg CaCO_3/L alkalinity and minimal measured free chlorine. Artificial seawater was prepared using 42 g/L of Instant Ocean salts (Product No. SS15-10) to give a salinity of 35 ppt. Nutrients were added as per a modified F/2 culture media recipe (Guillard & Ryther, 1962) to give soluble nitrogen and phosphorus concentrations for columns and panels of 140 mg $\text{NO}_3\text{-N/L}$ and 19.4 mg $\text{H}_2\text{PO}_4\text{-P/L}$ and concentrations for raceway ponds of 52.5 mg $\text{NO}_3\text{-N/L}$ and 7.3 mg $\text{H}_2\text{PO}_4\text{-P/L}$. Culture media was supplemented with trace metals to concentrations of 2.5 $\mu\text{g Cu/L}$, 3.7 $\mu\text{g S/L}$, 2.5 $\mu\text{g Mo/L}$, 5.0 $\mu\text{g Zn/L}$, 2.5 $\mu\text{g Co/L}$, 50 $\mu\text{g Mn/L}$, 3.4 mg EDTA/L, and 0.65 mg Fe/L (Rosov, Cardello, Dempster, Harmon, & McGowen, Modified F/2 Media, 2015).

Artificial seawater used to refill ponds after dilutions was prepared and stored on-site. A day prior to dilution, artificial seawater was disinfected using 12% bleach at a concentration of 1 mL bleach/L salt water. Before ponds were refilled, chlorine levels were tested and any residual was de-chlorinated with 500 g/L sodium thiosulfate solution. Once ponds were refilled, media components were added to ensure ponds were nutrient-replete.

Inoculum Production

To propagate seed culture for pond inoculation, *N. oceanica* was transferred using aseptic techniques from 1-L containers shipped from Cellana LLC to autoclaved 800-mL cylindrical glass columns with conical bottoms and topped-off with autoclaved modified F/2 medium to achieve an initial concentration of approximately 50 mg AFDW/L (ash-free dry weight). The column was sealed to prevent contamination using an EPDM rubber stopper with a capillary tube inserted through the center and a cotton-plugged vent hole. The columns were placed on a custom light rack with a bank of twenty fluorescent lamps (Philips F40T12 Hg, 40 W). Carbon dioxide-enriched air was filtered (0.2- μ m pores; VWR, Part No. 28145-477) and delivered to the cultures through the capillary tubes. A gas mixer (Matheson, Model No. 665) controlled the ratio of carbon dioxide (CO₂) to air, with the proportion of CO₂ adjusted to maintain media pH between 7.0 and 8.0. Columns were operated under 24-hour light. Algal biomass concentration was monitored via optical density (OD).

For scale-up, once column cultures approached a concentration of approximately 1 g/L, they were examined under the microscope for the presence of contaminant species. Columns with no observed contaminant species were used to inoculate fourteen 15-L square Plexiglass[®] panel PBRs. *N. oceanica* was transferred from the columns using aseptic techniques to disinfected panels and then brought to volume with modified F/2 media. Cultures were mixed by sparging filtered air (0.2- μ m pores; VWR, Part No. 28145-477) through aeration squares constructed from PVC pipe (2.6-cm internal diameter), which spanned the bottom length of the panels with 1-mm diameter holes spaced roughly 2 cm apart. Panel pH was maintained between 7.0 and 8.0, and was continuously illuminated. Biomass concentration was monitored by optical density.

After reaching the late-linear growth phase with a sufficient amount of biomass to inoculate ponds to an initial concentration of 40 mg AFDW/L, panel cultures were inspected for contaminant species. Panels with no observed contaminant species were distributed equally among the six-raceway ponds. Prior to inoculation, ponds were disinfected with a 12% bleach solution and filled to a depth of 25 cm with 35-ppt artificial seawater. Modified F/2 media components were added to each pond (Rosov, Cardello, Dempster, Harmon, & McGowen, Indoor Seed Production in Columns and Panels, 2015).

Pond Operations

Every weekday, the pH, salinity, and temperature sensors mounted in the ponds were checked against separately calibrated handheld meters. Evaporation

losses were made-up with tap water. Grab samples were collected to monitor algal growth and nutrient uptake Mondays, Wednesdays, and Fridays.

After several days of growth outdoors, when a minimum concentration of ~ 0.3 g AFDW/L was reached, a dilution schedule was initiated to achieve the desired dilution rate or hydraulic retention time (HRT). Ponds were diluted on a semi-continuous basis with a dilution either once-per-week ("batch") or three-times-per-week. During the dilution, the ponds were drained to the desired depth using a pump. Paddles remained on during draining to maintain a homogeneous mixture until desired depth was almost reached, then paddles were turned off to accurately reach desired depth. Ponds were refilled with artificial seawater and nutrients. AFDW concentrations were maintained between 0.05 g AFDW/L (initial) and 0.3 g AFDW/L (final). On harvest days, grab samples were taken before and after harvest to monitor algal concentrations and nutrient levels.

III. ANALYTICAL METHODS

Several analytical tests were conducted on pond water samples to monitor growth, nutrient uptake, and contamination by microorganisms: optical density (OD), ash free dry weight (AFDW), nitrate, phosphate, and microscopy.

Optical Density (OD)

Optical density (OD) was used to estimate the algal biomass concentration using a spectrophotometer at a wavelength of 750 nanometers. Samples were diluted using 0.45- μ m filtered, 35-ppt saltwater diluent to achieve an OD (absorbance) less than 1.0. Each pond sample was measured in triplicate and the averages reported. The standard operating procedure described in *Determining Optical Density* (APPENDIX A) was followed.

Ash Free Dry Weight (AFDW)

Ash-free dry weight (AFDW) was used as an estimate of the algal biomass concentration in the ponds. This method measured the total particulate biomass in a cultivation sample, which included non-algal microorganisms and other contaminants such as dead cells and debris. The *Gravimetric Method for Determination of Dry Weight (DW) and Ash Free Dry Weight (AFDW)* (APPENDIX A) standard operating procedure was followed using 4.7-cm diameter, 1.2- μ m-pore size glass microfiber filters (VWR, Cat No. 516-0083).

Sample Preparation for Nitrate and Phosphate

Prior to nitrate and phosphate analysis, pond samples were filtered using 1.2- μm -pore size glass microfiber filter (VWR, Cat No. 516-0083) stacked on top of a 0.45- μm -pore size cellulose filter (Fisher Scientific, Cat No. 09-719-2E) to remove any particulate matter that could interfere with the accuracy of the test.

Nitrate

Nitrate concentrations in the ponds were monitored via zinc reduction to ammonium and subsequent quantification by conductivity change using a Timberline TL-2800 Ammonia/Nitrate Analyzer, following *Nitrate Concentration Measurement* (APPENDIX A) standard operating procedure.

Phosphate

Phosphate concentrations in the ponds were measured using a PhosVer colorimetric assay (Lovibond, Lot Code: N-08-B) at an absorbance of 890 nanometers following *Phosphate Concentration Measurement* (APPENDIX A) standard operating procedure.

Microscopy

Samples pulled from the columns and panels during the scale-up phase were analyzed under light microscope (Olympus CX 41, Model 6M12454) for invasive species such as other algal strains and microorganisms that could outcompete or hinder the productivity of the model, following *Quick Contamination Check Microscopy Bench Method For Project ASU* (APPENDIX

A). Columns or panels containing traces of invasive species were discarded prior to pond inoculation. Pond samples were monitored two times per week.

IV. CALCULATIONS AND DATA ANALYSIS

Hydraulic Retention Time

The hydraulic retention time or hydraulic residence time (HRT) is a measure of the average length of time that a soluble compound remains in a bioreactor (Metcalf & Eddy, 2003). HRT is the inverse of dilution rate. Two dilution regimes were evaluated over the course of this study.

The batch cultivation regime comprised an initial batch growth period following inoculation from laboratory cultures and subsequent 1-week batches inoculated from the ponds themselves. The sequential batches could also be considered low-dilution rate, semi-continuous cultures (dilution rate = 0.11/d, HRT = 9.1 d).

For the initial batch cultivation from lab inoculum, a lag phase, typically of 5 days duration, was observed. Data from the lag phases were excluded from analysis.

The second cultivation regime was a semi-continuous dilution three times per week (dilution rate = 0.21/d, HRT = 4.7 days). The HRT was calculated using Equation 1.

$$HRT = \left[\frac{(V_p) \times 7 \text{ days/week}}{(V_h)(N_h)} \right]$$

Equation 1: Hydraulic Retention Time.

Where HRT is the hydraulic retention time in days; V_h is the volume of the pond water removed for the dilution or full draining of the ponds in units of L/dilution; V_p is the operating volume of the ponds in units of L; and N_h is the number of dilutions per week in units of dilutions/week.

Operator error occasionally resulted in non-target HRTs. When the error was greater than 10%, data from the timeframe was excluded from the present analysis. Figure 2 shows the cultivation regimes and breakdown of the lag phase, batch phase, and steady state growth phase for better understanding.

Figure 2: Biomass concentration vs. time graph to show different cultivation regimes and breakdown of lag phase (1), batch phase (2), and steady state growth phase (3).

Productivity

Areal productivity (g/m²-d AFDW) was the main output metric used, but it was calculated using two different methods. The first, referred to as harvest-productivity (Equation 2), was calculated using the AFDW concentration at the time of dilution. Harvest-productivity shows the influence of a cultivation regime on productivity. The second method, referred to as slope-productivity (Equation 3), was calculated using the change in AFDW concentration between grab samples (typically 2-3 days apart). Slope-productivity shows the influence of daily weather changes (e.g. solar radiation) on productivity, and it also allows productivity to be calculated over smaller pond concentration ranges. Both harvest-productivity and slope-productivity for semi-continuous dilutions (“batch,” one-time-per-week dilution, dilution rate = 0.11/d; and three-times-per-week, dilution rate = 0.21/d) were calculated after steady state was reached, which is defined as the time after the first dilution was conducted following the first batch cultivation of the experiment (Figure 2). The 5-day lag phase was determined by observation of AFDW vs. time graphs created from the collected data (graphs not shown).

$$P = \left[\frac{(AFDW_f) \times (V_h)}{(T_f - T_i)} \right] \div (SA_p)$$

Equation 2: Harvest-productivity.

Where P is the productivity of the algae biomass in units of g/m²-day; $AFDW$ is the measured biomass concentration of the pond at the time of dilution or full

draining of the pond in units of g/L; V_h is the volume of the pond removed for the dilution or full draining of the ponds in units of L; T_f is the time that the dilution or full draining of the pond occurred in units of days; T_i is the time that the previous dilution occurred in units of days; and SA_p is the illuminated pond surface area in units of m^2 .

Harvest-productivities for the batch cultivation were calculated from 5 days after the ponds were initially inoculated until the end of the batch growth when cell concentration reached approximately 0.3 g AFDW/L at which time the culture was first diluted. The initial 5 days after inoculation were excluded because *N. oceanica* PBR cultures were acclimating to their new pond environment, resulting in a lag phase noticeable in the data. The influence of the lag phase was evident in the low correlation between solar radiation and productivity ($R^2 = 0.13$) when including the lag phase, compared to an $R^2 = 0.57$ when it was excluded (data not shown).

$$P = \left[\frac{(AFDW_2 - AFDW_1) \times (V_p)}{(T_2 - T_1)} \right] \div (SA_p)$$

Equation 3: Slope-productivity.

Where P is the productivity of the algae biomass in units of g/m^2 -day; $AFDW_2$ is the measured biomass concentration of the pond at the time when a grab sample was collected from the ponds for an AFDW test in units of g/L; $AFDW_1$ is the measured biomass concentration of the pond at the time when the previous grab sample was collected from the ponds for an AFDW test in units of g/L; V_p is the

volume that the ponds were operated at in units of L; T_2 is the time when a grab sample was collected from the ponds for an AFDW test in units of days; T_1 is the time when the previous grab sample was collected from the ponds for an AFDW test in units of days; and SA_p is the illuminated pond surface area in units of m^2 .

Productivity values were averaged among duplicate and/or triplicate ponds operated under the same conditions and harvesting schedules.

V. RESULTS AND DISCUSSION

The following section outlines some of the relationships observed between seasonal variations and productivity, dilution rate and productivity, and culture concentration and productivity. The productivities presented in this section were calculated using both the harvest-productivity (Equation 2) and slope-productivity (Equation 3) depending on relationship that is being observed.

Effects of Solar Radiation and Pond Water Temperature on Algal Productivity

Productivities were calculated using the slope-productivity equation (Equation 3) and plotted as a function of the average 24-hour solar radiation in Figure 3. The slope-productivity equation was used to explore the influence of daily changes in solar radiation levels on productivity. Linear regression lines were fit to the data for both of the observed dilution schedules (batch and three dilution per week, dilution rate = 0.21/d) to represent the correlation between productivity and solar radiation. Productivity increased with solar radiation for ponds operated under both dilution schedules (Figure 3; Table 3), but the 3x/week dilution cultures had a greater sensitivity to radiation than the batch cultures (slope = 0.051 vs 0.034). For a given solar radiation, the 3x/week dilution correlation was almost always at a higher productivity than the batch mode correlation.

Figure 3: Slope-productivity as a function of average 24-hour solar radiation for the two dilution rates, both operated from October 2013 to June 2015.

Table 3: Summary of correlations and slopes between slope-productivity and solar radiation.

Dilution Schedule	Slope	R ²
Batch	0.034	0.56
3 times/week, dilution rate = 0.21/d	0.051	0.67

Productivity increased dramatically with pond water temperature between 6 and 21°C for both dilution regimes (**Figure 4**; Table 4). As with radiation, the 3x/week dilution productivity was more sensitive to temperature than the batch

mode, although temperature and radiation are highly correlated to each other. Unlike in the radiation correlation, the 3x/wk dilution correlation to temperature always gave a lower productivity than the batch correlation, for a given temperature.

Figure 4: Slope-productivity as a function of pond water temperature for the two dilution rates, both operated from October 2013 to June 2015.

Table 4: Summary of correlations between slope-productivity and pond water temperature.

Dilution Schedule	Slope	R ²
Batch	0.78	0.49
3 times/week, dilution rate = 0.21/d	0.95	0.58

Theoretically, algal biomass productivity should be higher when pond water temperatures are higher, up to an optimum temperature beyond which productivity would decrease. In the literature, the highest specific growth rate for *N. oceanica* was achieved at an optimum temperature of 28°C (Sandnes, Kallqvist, Wenner, & Gislerod, 2005), but this temperature was not reached during this study. However, the productivity still increased as water temperature increased, showing that the ponds followed the expected theory.

Even under controlled nutrient concentrations, pH, depth, and mixing speed within narrow ranges, with a single strain of algae, huge variability of algal productivity was observed which couldn't be attributed to solar radiation and pond water temperature alone. So the considerable scatter in both the light and temperature correlations indicates that additional variables were very important to productivity. Grazing by algae predators or inconsistency of the radiation and temperature from day to day and light/temperature history effects might contribute to the scatter. For the oddly low productivities of 2.04 g/m²-day and 6.20 g/m²-day with solar radiation of 349 W/m², the microscopy from the day before productivities were calculated indicated flagellates in pond samples. High values, such as the ones with productivities between 17.6 g/m²-day and 18.8 g/m²-day, could be due to simultaneous high solar radiation (roughly above 320 W/m²) and high water temperature (roughly 18-20°C). Other sites through the Algae Testbed Public-Private Partnership (ATP³) measured average productivity ranges of 7-13 g/m²-day during the spring and summer to <2-7.5 g/m²-day during

the fall and winter using *N. oceanica*, which are comparative to the averages measured in this study during those seasons (Dirks & McGowen, 2015).

Effects of Dilution Schedule on Harvest-productivity

The productivity was also calculated using the harvest-productivity equation (Equation 2) to provide a different view of the effects that dilution regime had on productivity. The productivities (Figure 5) were averaged from the replicate ponds operated under the same dilution regime.

A dilution schedule of three per week (dilution rate = 0.21/d) yielded the highest overall harvest-productivity compared to the “batch” dilution rate. The highest calculated harvest-productivity during the batch dilution schedule was 13.5 g/m²-day, with an average harvest-productivity of 9.0 g/m²-day calculated by averaging all productivity values shown in Figure 5 from January through September. The highest calculated harvest-productivity during the three dilutions per week was 16.0 g/m²-day, with an average harvest-productivity of 12.5 g/m²-day calculated by averaging all productivity values shown in Figure 5 from June through September.

Figure 5: Harvest-productivities averaged from replicate ponds as a function of time of year for the two dilution regimes, with error bars representing the standard deviation among replicate ponds.

However, the data collected for the two dilution regimes does not cover the entire calendar year, preventing conclusions on annual average productivity. During seasons with a lower light intensity (September-February), the dilution schedule of three times per week with dilution rate = 0.21/d might lead to cell washout due to lower productivities. However, during seasons with higher light intensity (March-August), higher dilution rates might increase biomass productivity. Many other dilution schedules to optimize algal biomass productivity could be conceived of for future study.

To show how dilution regime influenced biomass concentration, the biomass concentrations from Experiment #4 (June-July 2014) were averaged among replicate ponds and plotted as a function of time (Figure 6). The batch cultures maintained higher biomass concentrations, ranging over roughly 0.10-

0.45 g AFDW/L on average (Figure 6). In contrast, the three times per week cultures maintained a lower biomass concentration range, roughly 0.10-0.25 g AFDW/L. Therefore, to optimize algal biomass productivity, the data should be examined with respect to biomass concentration within the ponds.

Figure 6: Biomass concentrations from Experiment #4 averaged from replicate ponds. The batch mode of cultivation led to higher average biomass concentrations than the three times per week mode.

Effects on Productivity of Algal Biomass Concentrations at Different Solar Radiation Levels

By viewing the productivity with respect to algal biomass concentration and 24-hour solar radiation levels, the optimal culture concentration was determined as a function of irradiance. This relationship was important because, given an average amount of seasonal-dependent solar radiation an ideal biomass concentration could be found to optimize light distribution, and hence productivity. The link between productivity, concentration, and solar radiation is

shown in Figure 7. For reference, 150 W/m^2 corresponds to a cloudless winter day at San Luis Obispo, and 300 W/m^2 is representative of a cloudless summer day. The slope-productivity equation (Equation 3) was used to calculate productivity in relation to operating biomass concentration. The slope-productivity equation allows productivity to be calculated over smaller pond concentration ranges without the direct influence of dilution schedule. The productivities (Figure 7) were averaged based on the respective algal concentration range determined from the measured AFDW and solar radiation levels to show how concentration, solar radiation, and productivity are related. The biomass concentration ranges observed were broken down into increments of 0.05 g/L ; starting at 0.05 g/L and ending at 0.45 g/L , the lowest and highest biomass concentrations observed during the study, respectively.

The higher level of solar radiation generally resulted in higher algal biomass productivity (Figure 7). For an average 24-hour solar radiation between $200\text{-}300 \text{ W/m}^2$, the highest average productivity ($11.4 \text{ g/m}^2\text{-day}$) corresponded to algal concentrations of $0.15\text{-}0.20 \text{ g/L}$. For the $200\text{-}300 \text{ W/m}^2$ solar radiation range, concentrations below 0.15 g/L experienced lower productivities, which might have been due to photo-inhibition of the algal cells caused by high light intensity and low concentration or the lower population of algae within the ponds. For the same solar radiation range, algae cell concentrations above 0.20 g/L had lower productivities as well. This lower productivity might be due to inefficient use of the light caused by the absorption and inefficient use of light in the top few

centimeters by the dense algae biomass, combined with high respiration from the high algal concentration.

Figure 7: Averaged slope productivities are grouped based on algal concentration ranges, differing by 50 mg, and their respected 24-hour solar radiation levels to show influence of solar radiation and concentration on productivity.

For an average 24-hour radiation of 150-200 W/m², the algal concentration with the highest productivity was between 0.10-0.15 g/L, with an average productivity of 6.9 g/m²-day. At concentrations less than 0.10 g/L, these cultures might be affected by photo-inhibition, as shown by the lower productivity; however, 0.05-0.10 g/L is a low culture concentration and therefore low productivity could be influenced by the lack of cells within the system. Productivity was dramatically lower with higher culture concentrations, potentially due to excessive respiration.

For an average 24-hour solar radiation between 100-150 W/m², the highest productivity (5.4 g/m²-day) culture had an algal concentration of 0.25-0.30 g/L. At higher radiation levels, there seemed to be a pattern where the higher the solar radiation levels, the denser the algal culture could be, and with lowering light conditions the concentration should be lower to allow adequate light to be supplied to algae cells. However, at this low 100-150 W/m² radiation, there seems to be an opposing pattern, where culture densities need to be higher to be more productive. Therefore, this concludes that with varying solar radiation levels over the year, the optimal density to maximize productivity would vary as well.

VI. CONCLUSIONS

This study, using the alga *N. oceanica* cultivated in six identical pilot scale raceway ponds, allowed several conclusions to be drawn about how to optimize algal biomass growth. The first goal was to determine to what extent the system followed the expected positive relationships between areal productivity and solar radiation and pond water temperature. This pattern was seen for both radiation and temperature. However, even with important variables (pH, depth, mixing speed, nutrient levels and type, and strain) held relatively constant, solar radiation and pond water temperature were not the only major influences on productivity, which was shown in the large scatter of the productivity data points on radiation and temperature correlation graphs. Possible influences on productivity that were not addressed were the presence of invasive organisms and the history of solar radiation and pond water temperatures, which might affect productivity.

The second goal was to determine the effect of the two observed dilution regimes on algal biomass productivity to find which regime maximized productivity given seasonal conditions. Of the two dilution regimes used in this study, the highest productivity was achieved using three dilutions per week with a dilution rate = 0.21/d. During the warmer months of the year, this dilution schedule outperformed the batch cultures, with a highest calculated productivity of 16.0 g/m²-day. However, neither of these dilution regimes spanned the entire calendar year, so it is hard to conclude if the three times per week with a dilution

rate = 0.21/d would be better than batch for all seasons. To accurately determine which dilution regime is better overall; both should be tested over an entire year. Also, other dilution regimes/schedules might have resulted in even higher productivities over the entire year and/or specific seasons, but these were not examined.

The data also showed what biomass concentrations corresponded to the various productivity levels seen with respect to seasonal solar radiation levels. It was found that with a high solar radiation of 200-300 W/m², the highest productivity cultures (11.4 g/m²-day) had cell concentrations of 0.15-0.20 g/L. Lower solar radiation levels led to lower maximum productivities and lower culture concentrations.

The results of this study might be predictive of productivities and culture concentrations of *N. oceanica* cultures in similar climates. Three dilutions per week with a 0.21/d dilution rate appear to give higher productivity than sequential batch cultures.

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APPENDIX A: Supplemental Materials

Determining Optical Density

Overview:

This procedure describes how to prepare samples for optical density (OD) measurements and reading the absorbance at 750 nm on a spectrophotometer. OD readings must be between 0.1 and 1.0 to be accurate.

As of 6/18/2015, this procedure has been updated to

Materials:

- Cuvette appropriate for your spectrometer (site-specific)
- Spectrophotometer (site-specific)
- Pipettes capable of accurately dispensing 300 μ L- 5 mL volumes (site-specific)
- Corresponding pipette tips
- f/2 culture media or seawater containing 35 g/L of sea salt
- At least 2-5 mL of algae for each sample to be run (site-specific)
- 2-5mL centrifuge tubes or test tubes

Procedure:

1. Decide on the dilution for your sample. If you know your sample will have an absorbance above 1.0, then it should be diluted. If the approximate absorbances are unknown, then the samples should be run without being diluted. A dilution may be performed later if the absorbance is above 1.0. If dilutions are necessary, prepare a dilution using the sample dilution procedure below.
2. Set up your spectrophotometer to run samples following the Instrument Operating Procedure for your instrument so you may run your samples immediately after they are prepared. To measure the absorbance at an OD of 750 and 680 nm, configure the Shimadzu UV-1700 spectrophotometer as follows:
 - a) Turn on the spectrophotometer with the switch on the left. The spectrophotometer will go through a ~10 minute initialization and warm up process.
 - b) If all internal QA/QC criteria are met, push F1 for PARAMS
 - c) Push F2 for SET, input the file number 4 which corresponds to ASU OD and press enter
 - d) Photometric (multi wavelength) should come up with preloaded settings. Verify that wavelength 1 is 680 nm and wavelength 2 is 750 nm.
 - e) Rinse a non-scratched cuvette thoroughly with diluent, insert the cuvette filled with diluents into the spec such that the optical surface matches the pathway of light from the spec. To do so, the arrow on the cuvette should be to the left or right. If the cuvette does not have an arrow, it is multidirectional and the clearest sides should be oriented in the pathway of the light beam. Consult supervisor if unsure.
 - f) Press F1 for BaseCorr, this will zero the spec to the diluents at each wavelength.
3. Mix algae sample thoroughly by blending the sample using the immersion blender until the sample is homogeneous. Ask a supervisor if unsure how to operate the immersion blender safely. Dispense the homogenized sample into a falcon tube, where dilutions should be performed. Mix each sample before reading by capping and inverting the container several times before immediately pipetting appropriate volume (site-specific) into one cuvette.

4. Once the sample has been placed in the cuvette, insert the cuvette into the spec and press start. Record the absorbance of the sample at both wavelengths.
5. Continue analyzing all samples using the same cuvette, rinsing with diluent in between each pond.
6. Verify the spec is calibrated correctly throughout the analysis by inserting a cuvette with just diluent and also observing the spec blank value for any drifting.
7. Record absorbance measurements and dilutions on the ASU Production Data Sheet

Sample Dilution Procedure (if necessary):

A few considerations before diluting a set of samples:

- The diluents should be the same fluid that your samples are grown in. For *Nannochloropsis* sp. or *Desmodesmus*, use F/2 media or seawater containing 35g of sea salt/L.
 - Decide which dilution is needed. For example, dense algae often require a 1:10 dilution.
1. Obtain 2-5 mL capped centrifuge tube or test tubes and arrange them on a rack such that three replicates can be performed for each sample. (i.e., you will have three tubes for each sample that have identical dilute samples in them; this means that the dilution must be performed three separate times for each pond sample – you **cannot** dilute one time and split it into three cuvettes.)
 2. Thoroughly agitate your samples and pipette the appropriate amount into duplicate test tubes. (i.e., pipette 500 μ L algae into 5 mL of f/2 or salt water for a 1:10 dilution)
 3. Next pipette the appropriate amount of diluents into each test tube. (i.e., pipette 5 mL of f/2 media or salt water for a 1:10 dilution)
 4. Cap each tube and invert several times to mix thoroughly and follow the procedure above from step No.2 to finish preparing and analyzing your samples.

Measuring the Absorbance Blank:

Before starting the Optical Density Test, a measurement of the absorbance of the blank (culture medium or fresh/saltwater diluent) must be done. The blank ensures that the spectrophotometer is not including the absorbance of the culture medium in the results of the test. Procedure:

1. Fill cuvette with culture medium or diluent and place in spectrophotometer then close lid
2. Push "BaseCorr" (F2) on spectrophotometer (wait until the spec zeros)
3. Remove cuvette and close the lid
4. Record absorbance value (this may be negative or positive)

Zero Check:

During the test, a zero check should be performed periodically to see if the spectrophotometer is drifting during the test. This is done by filling a cuvette with diluent (like when measuring the absorbance blank) and placing it in the spectrophotometer. Ideally the absorbance reading should be **0.000** since it is the same diluent used when zeroing the spectrophotometer. An acceptable difference for the zero check is **0.003**. If this value is exceeded the cuvette should be cleaned, checked for bubbles, or replaced with a clean new cuvette.

Determination of Total Solids and Ash in Algal Biomass

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Laboratory Analytical Procedure (LAP)

Issue Date: February 4, 2014

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1. Introduction

- 1.1 Algal biomass samples may contain a high and varying percentage of moisture, which can change rapidly when the sample is exposed to ambient humidity levels.
- 1.2 In addition, algal biomass samples may contain varying percentages of ash, depending on the species and the presence of growth media in the sample.
- 1.3 The following procedure describes the methods used to determine the moisture-free total solids and ash content of a freeze-dried algal biomass sample. A traditional convection and vacuum oven drying procedure are covered for total solids content, and a dry oxidation method at 575°C is covered for ash content.
- 1.4 This Laboratory Analytical Procedure is based on and is substantially similar to terrestrial feedstock analytical protocols, although at lower temperatures, *Determination of Total Solids in Biomass and Total Dissolved Solids in Liquid Process Samples* [1] and *Determination of Ash in Biomass* [2]. Minor algal-biomass-specific modifications were made.
- 1.5 Portions of the total solids method are similar to ASTM E1756-01 and T412 om-02 [3].
- 1.6 Portions of the ash method are substantially similar to ASTM E1755-01 [3].

2. Scope

- 2.1 This procedure is intended to determine the amount of total solids remaining after either 60°C atmospheric pressure oven or 40°C vacuum oven drying of an algal biomass sample previously prepared (e.g., freeze drying, spray drying, etc.). This method is not intended for biomass slurries or prepared samples with a moisture content of greater than 10%.
- 2.2 This procedure is intended to determine the ash content of an algal biomass sample, corrected for moisture content determined during either the 60°C atmospheric pressure or 40°C vacuum oven drying method.

3. Terminology

- 3.1 *Algal Biomass Sample* – Algal biomass prepared and dried by freeze drying, spray drying, etc., ensuring moisture is <10% and is ground/homogenized to a particle size <1 mm. This prepared biomass is referred to as “as-received.”
- 3.2 *Oven Dry Weight (ODW)* – The weight of biomass mathematically corrected for the amount of moisture present in the sample.
- 3.3 *Total Solids* – The amount of solids remaining after heating the sample as described in section 10.1 until a constant weight is reached. Conversely, the moisture content is a measure of the amount of

water (and other compounds volatilized at 60°C atmospheric pressure or 40°C vacuum drying) present in the sample.

- 3.4 *Ash* – The inorganic residue remaining after dry oxidation at 575°C.
- 3.5 *Constant Weight* – The weight that is achieved after sequential measurements that show a difference no larger than 0.5 mg after placing the sample back in the oven or furnace for at least 1 hour, and reweighing.

4. Significance and Use

- 4.1 The results of the chemical analyses of algal biomass samples are typically reported on a dry weight basis. The total solids content of a sample is used to convert the analytical results obtained from subsequent methods on an as-received basis to an ODW basis.
- 4.2 The ash content is a measure of the inorganic and mineral content of an algal biomass sample and is used in conjunction with the ODW to report analytical results on an ash-free, ODW basis.

5. Interferences

- 5.1 The total solids procedure is not suitable for algal biomass samples that have not been dried or that contain a significant amount of moisture (see section 3.1).
- 5.2 The ash procedure is not suitable for samples that have not first been dried in an oven to correct for moisture content.
- 5.3 If measurements are taken before a constant weight is reached, data may be biased.

6. Apparatus

- 6.1 Analytical balance, accurate to at least 0.1 mg (e.g., Mettler Toledo XP205 DeltaRange)
- 6.2 Desiccator containing dry desiccant
- 6.3 Porcelain crucibles (ideally weighing less than 10 g) or equivalent, e.g., aluminum weigh boats (see section 10.1.5)
- 6.4 Convection drying oven, set to 60°C ± 1°C
- 6.5 Muffle furnace, equipped with a thermostat, set to 575°C ± 25°C or equipped with an optional ramping program (an alternative to pre-igniting the sample)
- 6.6 Ashing burner, ignition source, tongs, and clay triangle with stand (if not using a ramping program on the muffle furnace, see section 10.2.1 for pre-ignition)

7. Reagents and Materials Needed

7.1 Reagents

7.1.1 None

7.2 Materials

7.2.1 None

8. ES&H Considerations and Hazards

8.1 Use appropriate safety measures when handling an open flame.

8.2 When placing crucibles in an oven or furnace, use appropriate personal protective equipment, including heat resistant gloves. ALWAYS use tongs to handle hot crucibles.

9. Sampling, Test Specimens and Test Units

9.1 Samples must be dried (see section 3.1) before being placed in a drying oven.

9.2 Care must be taken to ensure a representative and homogenous sample is taken for analysis.

9.3 The ash procedure should only be completed on samples that have first been dried in an oven to correct for moisture content.

10. Procedure

10.1 Total Solids

10.1.1 Pre-condition crucibles in the 575°C muffle furnace overnight to remove any combustible contaminants.

10.1.2 After conditioning is complete, remove crucibles from the 575°C furnace and cool to room temperature in a desiccator (preferably under vacuum).

10.1.3 Using gloves, tweezers, or tongs (to prevent adding weight from hand oils), weigh each crucible. Record the crucible weight in a lab notebook to the nearest 0.1 mg.

10.1.4 Weigh out 100 ± 5 mg (or appropriate quantity based on section 14.4) of prepared algal biomass into the pre-weighed crucible. Record the weight of the crucible and sample in a lab notebook to the nearest 0.1 mg. Include an empty crucible as the method control.

- 10.1.5 Aluminum weigh boats are acceptable as an alternative sample container; a ramping oven following the program shown in section 10.2.2.1 should be used, and any pre-combustion steps (section 10.2.1) for ash determination should be disregarded.
- 10.1.6 Place the samples into a convection drying oven at $60^{\circ}\text{C} \pm 1^{\circ}\text{C}$ at atmospheric pressure or $40^{\circ}\text{C} \pm 1^{\circ}\text{C}$ under vacuum and dry for at least 18 hours. Remove the samples and allow them to cool to room temperature in a desiccator.
- 10.1.7 Weigh the crucible and oven-dried sample and record the weight in a lab notebook to the nearest 0.1 mg to constant weight.

10.2 Ash

NOTE: Use the same sample that was used for total solids determination for the ash procedure detailed below.

NOTE: If a muffle furnace with a ramping program is not available, samples must be pre-ignited first or you will start a fire in the furnace.

- 10.2.1 Ashing the samples using pre-ignition followed by dry oxidation in the 575°C muffle furnace
 - 10.2.1.1 Using an ashing burner and a clay triangle on a stand, heat the crucible containing the oven-dry sample until smoke appears.
 - 10.2.1.2 Immediately ignite the smoke and allow the sample to burn (re-ignite the smoke if necessary) until no more smoke or flame appears.
 - 10.2.1.3 Allow the crucible to cool on a suitable surface before placing it in the muffle furnace.
 - 10.2.1.4 Place the cool sample in the muffle furnace at $575^{\circ}\text{C} \pm 25^{\circ}\text{C}$ for 24 ± 6 hours. Handle the pre-ignited samples with care while placing them in or taking them out of the furnace to prevent sample loss.
 - 10.2.1.5 Remove the ashed samples from the muffle furnace and allow them to cool to room temperature in a desiccator.
 - 10.2.1.6 Weigh the crucible and ashed sample and record the weight in a lab notebook to the nearest 0.1 mg to constant weight.
- 10.2.2 Ashing the samples using a muffle furnace equipped with a ramping program
 - 10.2.2.1 Ramping program: Ramp from room temperature to 105°C
 - Hold at 105°C for 12 minutes

- Ramp to 250°C at 10°C/minute
- Hold at 250°C for 30 minutes
- Ramp to 575°C at 20°C/minute
- Hold at 575°C for 180 minutes
- Allow temperature to drop to 105°C
- Hold at 105°C until samples are removed

10.2.2.2 Place the crucibles in the muffle furnace and start the ramping program.

10.2.2.3 Remove the ashed samples from the muffle furnace and allow to cool to room temperature in a desiccator.

10.2.2.4 Weigh the crucible and ashed sample and record the weight in a lab notebook to the nearest 0.1 mg to constant weight.

11. Calculations

11.1 Calculate the percent total solids on a dry weight basis as follows:

$$\%Total\ Solids = \frac{(Weight_{crucible+dry\ sample} - Weight_{crucible})}{weight_{sample\ as\ received}} \times 100$$

If desired, the percent moisture can also be calculated:

$$\%Moisture = 100 - \left(\frac{(Weight_{crucible+dry\ sample} - Weight_{crucible})}{weight_{sample\ as\ received}} \times 100 \right)$$

Calculation of the ODW of a sample is done as follows:

$$ODW_{sample} = \frac{(Weight_{air\ dried\ sample} \times \%Total\ Solids)}{100}$$

11.2 Calculate and record the percent ash on an ODW basis as follows:

$$\%Ash = \frac{(Weight_{crucible+ash} - Weight_{crucible})}{ODW_{sample}} \times 100$$

11.3 To report or calculate the relative percent difference (RPD) between duplicates, use the following calculation:

$$RPD = \left(\frac{(X_1 - X_2)}{X_{mean}} \right) \times 100$$

where:

X_1 and X_2 = measured values

X_{mean} = the mean of X_1 and X_2

- 11.4 To report or calculate the root mean square deviation (RMS) or the standard deviation (STDEV) of the samples, use the following calculation:

$$RMS = x_m = \text{mean} = \sqrt{\left(\frac{\sum_{i=1}^n x_i}{n} \right)^2}$$

$$RMS\text{deviation} = \sigma = \text{stdev} = \sqrt{\frac{\sum_{i=1}^n (x_i - x_m)^2}{n}}$$

where:

x_m = the root mean square of all x values in the set

n = number of samples in set

x_i = measured value from the set

12. Report Format

- 12.1 Report the results as the percent total solids (or percent moisture), and cite the basis used in the calculations.
- 12.2 Report ash as a percent of the ODW of the sample.
- 12.3 For replicate analyses of the same sample, report the average, standard deviation, and %RPD.

13. Precision and Bias

- 13.1 An inherent error in any moisture determination involving drying of the sample is that volatile substances other than water may be removed from the sample during drying.

14. Quality Control

- 14.1 *Reported Results* – Report results with two decimal places. Report the average, standard deviation, and %RPD.
- 14.2 *Replicates* – Run all samples in duplicate, at minimum.
- 14.3 *RPD Criterion* – Each sample must reproduce total solids and ash content at $\pm 1\%$ wt.

14.4 *Sample Size* – 50 ± 2.5 mg or 100 ± 5 mg, based on available sample. If expected ash content is $>10\%$, then $25 \text{ mg} \pm 2.5$ mg of material can be used. Caution: the final ash weight must be above the minimum weight of the balance.

14.5 *Sample Storage* – All samples should be stored in an airtight container in a -20°C freezer.

15. Appendices

15.1 None

16. References

[1] Sluiter, A.; Hames, B.; Ruiz, R.; Scarlata, C.; Sluiter, J.; Templeton, D. *Determination of Total Solids in Biomass and Total Dissolved Solids in Liquid Process Samples: Laboratory Analytical Procedure (LAP)*. 9 pp.; NREL/TP-510-42621. Golden, CO: National Renewable Energy Laboratory, 2008. <http://www.nrel.gov/docs/gen/fy08/42621.pdf>

[2] Sluiter, A.; Hames, B.; Ruiz, R.; Scarlata, C.; Sluiter, J.; Templeton, D. (2008). *Determination of Ash in Biomass: Laboratory Analytical Procedure (LAP)*. 8 pp.; NREL/TP-510-42622. Golden, CO: National Renewable Energy Laboratory, 2008. <http://www.nrel.gov/docs/gen/fy08/42622.pdf>

[3] *2003 Annual Book of ASTM Standards*, Volume 11.05. Philadelphia, PA: American Society for Testing and Materials, International.

[4] Van Wychen, S.; Laurens, L.M.L. *Determination of Total Solids and Ash in Algal Biomass*. NREL/TP-5100-60956. Golden, CO: National Renewable Energy Laboratory. 2013. <http://www.nrel.gov/docs/fy14osti/60956.pdf>

SOP: Nitrate Concentration Measurement

Last revised: 11/20/2015 by CS (added more troubleshooting)

INTRODUCTION

There are numerous analytical methods for nitrate determination. For the current quarter, we will be using an automated nitrate reduction method with the Timberline TL-2800 automated ammonia analyzer. For a description of the principals of operation, please refer to the Timberline training materials in P:\AlgDig\Methods & data forms & instruments & equipment\Nitrogen - N\Timberline (Ammonia and NO₃). There are several very helpful documents in there, please review before using the analyzer.

SAFETY CONSIDERATIONS

A strong base, ~5% w/V potassium hydroxide, is used in this analysis, as well as a boric acid buffer, a weak acid. Diethylenetetraaceticacidpenta (DTPA), a chelator, is a component in the caustic solution. Samples either fresh or salt water water with trace nutrients. A lab coat and goggles are required when preparing the caustic solution. It should be noted that strong bases are particularly damaging to the eyes upon contact. Be prepared to rinse eyes thoroughly in the eye wash station if there is any potential for eye exposure. You are required to adhere to CalPoly San Luis Obispo's rules and regulations regarding laboratory use.

It should be noted that the TL2800 belongs to the soil science department, who are currently loaning it out to us. Please notify your supervisor if there are any irregularities in its performance.

MATERIALS AND INSTRUMENTATION

- Culture media sample, 0.45 um filtered
- DI Water
- Timberline TL-2800 automated ammonia analyzer
- "Caustic Solution": 3.6% potassium hydroxide, 1% DTPA solution in DI water
- 50% w/V potassium hydroxide (**Add Part Number**)
- Diethylene triamine pentaacetic acid (DTPA)
- Magnetic stir plate
- 100 ml graduated cylinder
- Reagent bottles, various sizes
- "Buffer Solution": 250 ppm boric acid solution, pH adjusted to 6.9 with ammonium hydroxide
- 1 M ammonium hydroxide

- Disposable dropper
- 10,000 mg/L boric acid stock solution
- “Ammonia analyzer effluent” waste container
- 1 L beaker filled with DI water

SOLUTION PREPARATION

Autosampler rinse:

Discard any remaining DI water from the 1L autosampler rinse beaker. Replenish with ~800 ml of DI water.

Caustic solution:

Check the caustic solution bottle. You will need at least 250 ml of the Caustic Solution for standards, CVS's, and triplicate P1-P6 samples. Assume you will need ~500 ml if running a complete post-harvest set. The “Caustic Solution” (3.6% potassium hydroxide, 1% DTPA solution in DI water) can be prepared as follows. A lab coat, gloves, and goggles (not eyeglasses) are required for this step

1. Rinse the previously labeled 1 L reagent bottle with ~20 ml of tap water. Swirl in bottle, then dispose rinse in the “Ammonia analyzer effluent” waste container.
2. Rinse bottle 3x with hot tap water, 3x with RO water, and a final rinse with DI water. These rinses can go down the drain.
3. Add a stir bar to the bottle.
4. Fill the 1 L reagent bottle with ~700 ml DI water. No need to be exact here.
5. Add 72 +/- 2 ml of 50% w/V potassium hydroxide to a 100 ml graduated cylinder. Pour this into the DI water containing reagent bottle. Rinse the graduated cylinder 3x with DI water, with each rinse poured into the reagent bottle.
 - The 50% w/V KOH can be found in the blue chemical cabinet
6. Measure 10 g of diethylene triamine pentaacetic acid (DTPA). Transfer this into the reagent bottle. Rinse the weigh boat 3x with DI water, with each rinse transferred into the reagent bottle.
7. Fill the reagent bottle to the 1 L mark with DI water.
8. Stir solution with magnetic stir plate until solids are completely dissolved.

Boric Acid “Buffer Solution”:

As with the caustic solution, you will need at least 250 ml of the Buffer Solution for standards, CVS's, and triplicate P1-P6 samples, or 500 ml for a pre and post-harvest set. As more time is required to make this solution, it is prepared in 2 L batches.

1. Rinse the previously labeled 2 L reagent bottle with ~500 ml of tap water. Swirl in bottle, then dispose rinse in the “Ammonia analyzer effluent” waste container.
2. Rinse bottle 3x with hot tap water, 3x with RO water, and a final rinse with DI water. These rinses can go down the drain.

3. Add a stir bar to the bottle.
4. Fill bottle with ~1.8 L DI water.
5. Add 25 ml of 10,000 mg/L boric acid stock solution per L of Buffer Solution. If preparing 2 L, add 50 ml to the 2 L reagent bottle.
6. Rinse this graduated cylinder 3x with DI water, with each rinse transferred into the 2 L reagent bottle.
7. Set the reagent bottle on a stir plate, stirring vigorously.
8. Calibrate a pH meter at 4, 7, and 10. Rinse the probe thoroughly, then place the probe in the 2 L reagent bottle, such that the top of the probe remains above the water line.
9. Add ~2 ml of 1 M ammonium hydroxide to the 50 ml graduated cylinder. Add ~48 ml of DI water to the graduated cylinder.
 - Perform this step in a fume hood.
 - This solution will be used to pH adjust the Buffer Solution to 6.9.
10. With the Buffer Solution stirring, add the diluted ammonium hydroxide solution dropwise until reaching a pH of 6.90.
11. When the pH is stable at 6.90, remove the pH sensor, and fill the reagent bottle to the 2 L mark. Mix thoroughly, and set by the Timberline for future use.

STANDARD PREPARATION

Standard stock solutions can generally be found in the 192-209 (Vista) refrigerator. Instructions for stock solution preparation are given below, although will mostly likely already be prepared. However, you will need to prepare a set of diluted standards prior to each analysis.

Preparation of primary standards:

1. 1 to 2 days prior to standard stock solution preparation, place ~4 g of sodium nitrate into the 105 °C oven (aluminum weigh boats are good for this). This will remove any water that the raw chemical may have absorbed. If preparing a CVS, place 4 g from a *different* stock of sodium nitrate. Record the manufacturer, part number, and lot number.
2. After 1-2 days, weigh **3.036 g** of sodium nitrate.
3. Add ~250 ml of diluent (either 0.45 um filtered 35 ppt synthetic ocean water (phosphate and nitrate free) or DI water) into a clean 500 ml volumetric flask.
4. Add the measured sodium nitrate into the 500 ml flask. Rinse the weigh boat with several washes of diluent.
5. Shake to dissolve.
6. Bring to volume to the 500 ml mark, measuring from the meniscus.
7. Invert several times to mix.
8. Transfer into a clean, dry, 500 ml reagent bottle.

This will give a 1000 mg N/L solution. Identify one bottle as the standard, and the other as a CVS.

Preparation of diluted standards:

A set of standards are prepared before every analysis in order to develop a relationship between peak area and nitrate concentration. As we gain more knowledge regarding the stability of the diluted standards, we will likely prepare these weekly.

1. Consult the table below, and choose a set of micropipettes suitable for dispensing the required volume. Add the volumes specified below of the 1000 mg N/L primary stock solution to a 50 ml volumetric flask.

Table 1: Standard preparation calculations.[illegible]

***Use the nitrate standard labeled “CVS” to prepare this solution.**

- a. Test a 10 – 100 uL micropipette at 50 uL. Adjust the micropipette until the dispensed volume is within 1% of the target value.

- b. Test a 100 – 1000 uL micropipette at 100 uL. Adjust the micropipette until the dispensed volume is within 1% of the target value.
2. Start with the lowest concentration standard. Rinse the 50 ml volumetric flask with diluent. Pipette the specified volume of 1000 mg N/L standard into the 50 ml flask, then bring to volume with the appropriate diluent.
3. Transfer prepared standard level into an appropriately labeled 50 ml centrifuge tube.
4. Repeat at Step 2 for the next standard level, no need to rinse the volumetric flask if working from low to high.
5. After all standard levels have been prepared, rinse the volumetric flask thoroughly with diluent before preparing the CVS.
6. Place the standards in the autosampler standard tray, in order from lowest (left) to highest (right) concentration.

ANALYZER SETUP

1. Turn on the Timberline ammonia analyzer computer by pressing the power button on the front of the device. The computer monitor is connected directly to the analyzer. Verify the monitor power switch is on.
2. Turn on the Timberline ammonia analyzer using the switch on the right hand side of the TL-2800.
3. Turn on the Cetac autosampler by flipping the power switch located on the rear of the autosampler unit.
4. If the Timberline analysis software is already open but the Timberline ammonia analyzer or the Cetac autosampler are off, close the window. Make sure the Timberline ammonia analyzer is off, as well as the Cetac autosampler then repeat steps 2 and 3 before proceeding to open the software again. This is done to prevent any disconnection between the software and the analyzer that could lead to false analysis of samples.
5. On the computer, open the Timberline analysis software.
 - A dialogue will appear asking to reset the autosampler. Do not change any of the values, hit OK. The autosampler should return to its home position, and the rinse water will begin to recirculate.
 - Ensure that there are at least 800 ml of FRESH deionized water connected to the autosampler tubing.
6. Inspect the autosampler rinse and sample delivery tubing. **If tubing appears discolored (i.e. black, green, ...), consult your supervisor for cleaning or replacement.**
7. Remove the influent lines labeled “Caustic” and “Buffer” from the deionized water container. Insert the “Caustic” line into the KOH solution and the “Buffer” line into the 250ppm boric acid solution.
 - Ensure that the Buffer and Caustic lines are in the proper solutions, that the lines reach the bottom of the containers, and that there are no obstructions in the lines.
8. Ensure that the Caustic and Buffer *effluent* lines are placed in the appropriate waste container, and that the container is not overfull.

- Transfer contents into the “Ammonia Analyzer Waste” container if the effluent storage bottle is more than 3/4 full.
9. Navigate to File/Load setup parameters from method file, then choose Desktop/Lundquist Analysis/Project ASU/XXXXXX.
 - This loads the appropriate run settings. Choose the appropriate format for either a pre or post-harvest sample set.
 10. Verify that the appropriate gain and post-attenuation factors are selected, and that the gain factor matches the switch setting on the front of the TL-2800 unit.
 11. This will turn on the pump to begin circulating DI, Buffer solution, and Caustic solution, through the unit, as well as load the appropriate gain and post-attenuation settings, and toggle the instrument to “Total N” mode.
 12. Allow at least 5 minutes to equilibrate once all solutions are pumping through the analyzer before starting the calibration run.

CALIBRATION

1. Place the 50 ml centrifuge tubes containing the diluted standard in the front of the autosampler. Place tubes in order from low to high N concentration, moving from left to right.
2. Inspect the “Cell Voltage” box on the Timberline software. Press the “Zero Cell” button repeatedly until the system equilibrates at a value near zero (~0.001). If the cell voltage continuous to rise or fall, the machine has not yet equilibrated.
3. Once the voltage cell has equilibrated, click on the “Calibration Samples” tab. Verify that the calibration sample list matches the set of standards you have prepared.
4. Start the calibration run. Watch the first sample as it enters the machine. Verify that the sample goes through the zinc reduction column. If not, click “Cancel Run”, and restart the sequence and consult the “Troubleshooting Common Issues” section.
5. While waiting for the standard curve to run, prepare your sample set and spike as described below.
6. When calibration run completed, verify that your peak areas approximately match those below.

Table 2: Expected sensor response, 3.6% KOH and 1% DTPA, Gain = 100, Post-attenuation=2.

<i>Standard Level</i>	<i>Target concentration, mg NO₃-N/L</i>	<i>Expected peak area, +/- ~15%</i>	<i>Expected peak height +/- ~15%</i>
1	1	100	0.238
2	2	200	0.461
3	5	500	1.114
4	10	1000	2.097

SAMPLE AND SPIKE PREPARATION

Samples need to be 0.45 um filtered, which will most likely be done before you. To preserve the life of the zinc reduction column, we'll be diluting each sample by 10x. The zinc reduction

column has a finite lifetime – i.e. at some point the zinc will be unable to reduce high NO₃- samples in a consistent manner. As the nitrate concentration in our samples should always be greater than ~30 mg N/L, a 10x dilution will put our sample in the range of our standard curve. While it is generally preferred to avoid diluting samples to avoid introducing error, in this case the Timberline offers sufficient sensitivity in the 1 – 10 mg/L range that the dilution is a sufficient compromise. Samples are diluted as follows:

1. Arrange a set of sample tubes in a wire test tube rack. Each sample is measured in triplicate.
 - A wire rack allows you to see any volume pipetted into the vial to avoid confusion (unlike a foam rack)
2. Calibrate a 101 – 1000 ul pipette to 1000 ul.
3. Calibrate a 1 – 10 ml pipette to 9 ml.
4. Dispense 1000 ul of sample into the respective test tube.
 - Between samples (i.e. Pond 1 vs. Pond 2, rinse the tip 3x with DI water. No need to rinse between replicates)
5. Dispense 9 ml of the appropriate diluent into each tube. Note that this serves as the mixing step. Be careful that the sample does not splash out while dispensing.

Matrix Spike:

You will need to prepare a spike for each the pre and post-harvest (if applicable) sample set. Open the “201X_0M_0D_Timberline_Nitrate_Initials” spreadsheet located in AlgDig/Project ASU/(Current Experiment)/Nitrate-Timberline folder. Green rows are entered by the user, white rows are calculated by the spreadsheet.

1. Enter the sample that was chosen to be spiked in cell D21. Any sample can be chosen here, and a different sample should be chosen every week.
2. Calculate the average diluted nitrogen concentration of the given sample's triplicate set (Cell D22). As you have not yet measured this concentration, enter your best guess. Consult the previous day's analysis for a rough estimate. If the ponds have been growing, and soluble nitrogen transformed into cell biomass organic nitrogen, the current value should be less than the previous value.
3. Cell D23 calculates a target concentration to achieve for your spike. As a rule of thumb, this is 1.5x greater than your sample concentration. Verify that your spiked concentration will be below the highest concentration in your standard curve.
4. Cell D24 contains the concentration of standard used to spike your sample. This should not change.
5. Cell D25 contains the volume of your diluted sample. In our case, this should always be 10 ml.
6. Adjust the standard volume in cell D26 until the expected concentration (D27) is with ~5% of the target concentration (D23).
7. As before, dilute the sample chosen to be spiked. However, before adding the 9 ml of diluent, add the volume of 1000 mg N/L standard determined above. This will allow all three components to mix while the 9 ml of diluent is added.

Record the volume of 1000 mg N/L standard used in the Run Log. Proceed to the next step.

LOADING SAMPLES

Load samples as follows:

Table 3: Sample list

Tube Location	Sample	Tube Location	Sample
A1	6 mg N/L CVS A	B1	P4b
A2	P1a	B2	P4c
A3	P1b	B3	P5a
A4	P1c	B4	P5b
A5	P2a	B5	P5c
A6	P2b	B6	P6a
A7	P2c	B7	P6b
A8	P3a	B8	P6c
A9	P3b	B9	SPIKE
A10	P3c	B10	6 mg N/L CVS C
A11	6 mg N/L CVS B	B11	
A12	P4a	B12	

1. Check the calibration run. Verify that the peak areas approximately match those in Table 2.
2. Assuming everything looks good. Press the button labeled “Start Sample Run”.
3. Each sample requires ~2:45 minutes to run.
4. When the run completes, view the “Saved Data” tab. Before proceeding, check the following to verify a successful run:
 - Are your CVS values all approximately 6 mg N/L? If one is way off, or if they consistently decrease throughout the run, this may mean the reduction tube requires replacement. Cap and place your 10x diluted samples in the refrigerator, and contact your supervisor.
 - Do your triplicate sets all fall relatively close to one another? If the RSD exceeds 10% for any triplicate set, save the 0.45 um filtrate of that sample for analysis during the next day of Timberline NO3-N analysis.
5. Save the .tdf file in the Desktop/Lundquist Analysis/Project ASU/(Current experiment) folder in the appropriate format.
6. As the Timberline computer is not connected to the internet or the public drive, save the data as a .CSV file onto a flash drive, and transfer this to a computer with AlgDig access. Save this file in AlgDig/Project ASU/(Current Experiment/Nitrate – Timberline/Raw Timberline Files.

DATA ANALYSIS

1. Open the Timberline data analysis excel temple. Select the “Raw Data” tab
2. Data/Import text file/(navigate to your transferred file in the Raw Timberline Files folder).

- Delimitate data by comma
3. Copy/paste the standard areas into the appropriate cells.
 4. Copy/paste tube names and sample areas into appropriate cells.
 5. Check your RSD's. They should be below 10%, ideally below 5%.
 6. Check your CVS. Did it always come back within 10% of the expected value?
 7. Enter the data for your spike. Did it pass?
 8. Assuming your data set passed QAQC, copy the appropriate table and paste it into the production spreadsheet.
 9. Enter the predicted CVS a, b, c, Concentrations in the "Timberline Standard and CVS Log" spreadsheet in P:\AlgDig\PROJECT ASU\Analytical Method Info...Misc studies. Notify your supervisor if there are any surprises.

SHUTDOWN PROCEDURE

1. Remove the caustic and buffer lines from their respective flasks and insert into a DI water container.
2. Select "Pump On" and flush the system with DI water for 15 minutes. Leave the lines in the DI water when finished.
3. Turn off the autosampler and analyzer, including pump switch.
4. Before rinsing sample tubes, ensure that they are in the correct order as specified in the software. Mislabeling or misplacing sample tubes is a common error.
5. As long as samples are not acidified, the diluted sample can be disposed down the drain.
6. Organize tubes back into the color matching foam rack
7. Rinse 3x with hot tap water, followed by a 3x RO water rinse. Tap upside down on bench to remove excess water, and set in nitrate kit to dry.

DETERMINING GAIN AND POST-ATTENUATION FACTORS

The Timberline features adjustable gain and post-attenuation factors. This allows the user to analyze a wide range of sample concentrations. The appropriate gain and post-attenuation factors must be chosen such that the full range of the conductivity sensor's output signal is utilized. The gain and post-attenuation factor manipulate the conductivity sensor's raw voltage signal as follows:

Voltage displayed in software

$$= (\text{Raw voltage from conductivity sensor}) * \frac{\text{Gain}}{\text{Post attenuation factor}}$$

The conductivity sensor saturates at a voltage of 2.08 V. If the voltage is saturated, either the gain must be lowered, or post-attenuation factor increased. The optimal gain and post attenuation factor depend on the background signal (i.e. voltage contribution from the DI water, buffer, and caustic solutions), and your highest level standard. Recommended gain and post-attenuation factors for methods developed to-date are given below, and can be used as a rule of thumb:

<i>Highest standard concentration, mg N/L</i>	<i>N form</i>	<i>Caustic solution, wt% KOH</i>	<i>Buffer, mg/L boric acid</i>	Gain	Post-attenuation
10	NO ₃ ⁻	3.6%, 1% DTPA	250	100	2
100	NH _x	5% NaOH	250	10	2

For new analyses, the appropriate gain and post-attenuation factor are determined as follows:

1. Turn on the Timberline pump, put the sucker straws into the appropriate containers, and give the machine 5-10 minutes to stabilize.
2. Verify that the Timberline front panel gain setting matches that on the software.
3. Press the “Zero Cell” button repeatedly until a value close to zero appears (~0.01 or so).
4. Place your highest concentration standard in the first standard position. If performing a separate standard run, this is the left-most position in the standard 50 ml centrifuge tube holders.
5. In the Timberline software, click “Start Run” and observe the graph of cell voltage as it is being analyzed. The cell voltage graph should form a smooth peak.
 - If the peak is truncated, adjust the Gain and Attenuation settings to a lower value. Gain changes the readings by a factor of 10, while attenuation divides the gain by as set factor. So a gain of 1000 and an attenuation of 4 would result in an amplification of 250x.

TROUBLESHOOTING COMMON ISSUES

Did the Cetac autosampler stop running? If so, go to File/Reset Autosampler. The autosampler rinse pump automatically stops after ~ 5 minutes of inactivity.

Not getting a peak on a nitrate sample? Check the top of the zinc reduction tube column. If stagnant, the sample may not be passing through the zinc reduction column. Toggle from the “Run Total N” to “Run Just Ammonia”, then back to the “Run Total N” option.

Listen for a click near the zinc reduction column. You should see signs of fluid flow at the top of the zinc reduction tube, which indicates your sample should now be passing through the column.

Not getting a peak? Only a sinusoidal squiggle? This often occurs if the software was turned on before the TL-2800 analyzer. Close the software, then restart.

If the computer gives a “invalid system disk, replace disk and press any key” error, make sure there is no flash drive plugged in to the monitor. This will keep it from starting up correctly. To fix, remove flash drive and restart the timberline computer or press escape on the next screen.

SOP: Phosphate Concentration Measurement

I. INTRODUCTION

Phosphate quantification using the Lovibond Vario Phos 3 colorimetric assay

Before performing analysis, read the MSDS and original manufacturer's instructions (Lovibond 531550 Vario Phos 3 – found in Project ASU – Reference supporting documents). To measure the concentration of phosphate in a given culture sample, the *PhosVer* colorimetric assay is used as follows.

II. MATERIALS AND INSTRUMENTATION

- Culture sample, 0.45 um filtered
- DI Water
- Lovibond 531550 Vario Phos 3 F10 ml (Lot Code: N 08 B)
- Spectrophotometer (absorbance is measured at 890 nm)
- 50 ml falcon tubes
- Phosphate Waste Container
- Glass Cuvette
- 50 ml vials with screw lid
- Vortex mixer
- Timer
- Culture sample
- 0.45 um filters (either 47 mm filter paper type, Fisher: PN 09-300-71; or filter cartridge type, Fisher PN: 14-555-268)
- Luer-lok syringe, 60 ml. Fisher PN: 13-689-8 (only if filter cartridges are used for 0.45 um filterint)
- 1.1 um filter paper, 47 mm (used as a prefilter before 0.45 um)
- Artificial sea salt (Instant Ocean™, Oceanic™), or phosphate free natural sea water
- 500 ml volumetric flask
- 500 ml storage containers
- Timers
- Microscope lens paper
- Soft-bristled pipe cleaner

III. PROCESS DESCRIPTION

Preparation of Samples:

Sample preparation:

Interferences that augment the absorbance at 890 nm must be removed prior to analysis. With algal samples, turbidity due to the algal suspension is the greatest interference. This is removed as follows: (Note: Centrifuging is typically not necessary since our cultures are not very dense).

1. Place 50 ml sample into an acid washed 50 ml centrifuge tube.
2. Balance each centrifuge tube to constant weight.
3. Centrifuge at 10,000 rpm for 10 minutes.
4. Place a 0.45 μm , 47 mm diameter filter on the filter support. Place a 1.1 μm filter on top.
5. Turn the vacuum pump on, and filter directly into a clean, acid washed, 50 ml vial with screw top lid. Assuming no dilutions are needed, 10 ml are required per replicate, with additional filtrate needed for the nitrate analysis (10 ml per replicate for nitrate, samples analyzed in triplicate)

Analyze samples immediately for best results. If this is not possible, store samples at 4 °C for up to 48 hours.

Dilutions:

Samples must be diluted to achieve an anticipated phosphate concentration below the upper limit of the calibration curve 2.5 mg/L PO_4^- (0.815 mg/L P). For reference, the initial phosphate concentration of the pond should be 20 mg/L PO_4^- (6.56 mg/L P).

The diluent should approximate the matrix expected in the filtered sample – i.e. it must have the same conductivity and background salt (i.e. phosphate, iron, trace metals) makeup, although cannot contain soluble forms of phosphorus. To prepare diluent, add artificial sea salts (Instant Ocean or Oceanic, for example – natural seawater may contain trace phosphate) until achieving the same conductivity as the culture sample (+/- 2 ppt). Add all culture media components to concentrations present in the culture media recipe, except nitrate. Filter the diluent through a 0.45 μm filter.

Samples can either be diluted in bulk (i.e. 10 ml sample in 90 ml diluent for a dilution factor of 10, then dispense 10 ml of the diluted sample into the 15 ml screw capped vial), or directly into the 50 ml screw capped vials (i.e. 1 ml of sample into 9 ml diluent) for a 10-fold dilution. However, the sample volume exposed to the powder pillow reactant must remain constant at 10 ml. If performing dilutions directly to 10 ml, the following table can be used.

Table 1: Recommended dilution factor and sample volumes for anticipated phosphate (PO₄⁻) concentrations.

Expected PO ₄ - concentration, mg/L	Dilution factor required to measure 1 mg/L of PO ₄ -	Volume of sample to add to achieve DF, ml	Volume of diluent, ml
0	1	10.000	0.000
2	2	5.000	5.000
4	4	2.500	7.500
6	6	1.667	8.333
8	8	1.250	8.750
10	10	1.000	9.000
12	12	0.833	9.167
14	14	0.714	9.286
16	16	0.625	9.375
18	18	0.556	9.444
20	20	0.500	9.500
22	22	0.455	9.545
24	24	0.417	9.583

Preparation of standards:

- Add ~500 mg of sodium phosphate monobasic anhydrous (NaH₂PO₄) into a labeled aluminum weigh boat and place in a 105 C drying oven for at least two hours.
- Add ~250 ml of 0.45 um filtered 35 ppt synthetic ocean water (phosphate free) into an acid washed 500 ml volumetric flask.
- Add 158 mg of sodium phosphate monobasic anhydrous (NaH₂PO₄) into the 500 ml volumetric flask. This will give a 250 mg/L PO₄³⁻ solution. Record the actual mass of sodium phosphate monobasic added. (Note: Must use monobasic for standard prep in salt water, otherwise precipitate will form).
- Bring to the 500 ml mark using the filtered diluent prepared earlier.
- Prepare 0.1, 1, 2, and 2.5 mg/L PO₄⁻ standards. To prepare, add the volumes specified below of the 250 mg/L PO₄⁻ stock solution to a 25 ml volumetric flask. Add the diluent prepared above until reaching the 25 ml mark.

Target concentration, mg/L PO ₄ ⁻	Volume to prepare, ml	Volume of 250 mg/L PO ₄ ⁻ to add to 25 ml volumetric flask, ml
0.1	25	Add 2.5 ml of the 1 mg/L stock solution
1	25	0.100
2	25	0.200
2.5	25	0.250
1.5 (CVS)	25	0.150*

*Prepare the 1.5 mg/L PO₄⁻ solution using the secondary 250 mg/L standard solution.

- Prepare the 1.5 mg/L PO₄⁻ calibration verification standard (CVS) as described in the table above, using the secondary phosphate solution. This standard is used to confirm our standard curve successfully predicts the phosphate concentration.

- g) Add 10 ml of standard to a 50 ml centrifuge tube and analyze as described in the next section.

Expected standard OD's are reported below:

Table 2: Range of expected OD's for phosphate standards. Analyses were measured using Lovibond PN531550.

Standard concentration, [mg/L PO ₄ -3]	OD (in salt water matrix, average of N runs)	(give time period over which these were averaged)	OD (in fresh water matrix, average of N runs)	(give time period over which these were averaged)
0.0	0.008			
0.1	0.04			
1.0	0.252			
2.0	0.489			
2.5	0.623			
1.5 CVS				

Measuring the Absorbance Blank:

Before starting the Phosphate Concentration Test, a measurement of the absorbance of the blank (culture medium or fresh/saltwater diluent) must be done. The blank ensures that the spectrophotometer is not including the absorbance of the culture medium in the results of the test. Procedure:

1. Fill cuvette with culture medium or diluent and place in spectrophotometer then close lid
2. Push "auto zero" on spectrophotometer (wait until the spec zeros)
3. Remove cuvette and close the lid
4. Record absorbance value (this may be negative or positive)

Zero Check:

During the test, a zero check should be performed periodically to see if the spectrophotometer is drifting during the test. This is done by filling a cuvette with diluent (like when measuring the absorbance blank) and placing it in the spectrophotometer. Ideally the absorbance reading should be **0.000** since it is the same diluent used when zeroing the spectrophotometer. An acceptable difference for the zero check is **0.003**. If this value is exceeded the cuvette should be cleaned, checked for bubbles, or replaced with a clean new cuvette.

Reaction with PhosVer reagent:

- a) Consult the MSDS to ensure proper PPE are adorned.

- b) Fill glass cuvette with culture medium and measure absorbance blank at **890 nm**. Remove cuvette from spectrophotometer, clean, and place in a readily accessible location.
- c) If you expect the phosphate concentration to be above the detection limit of the assay (2.5 mg L^{-1} , or $0.026 \text{ mM PO}_4^{3-}$), dilute the sample as needed. When appropriate dilutions have been made, pipette exactly 10 ml into a 15 ml capped vial.
- d) Carefully open a PhosVer packet with scissors, and pry open with metal tweezers.
- e) Transfer all PhosVer contents into sample vial and quickly fasten cap. Invert vial and set on table to mix. Not all solids will dissolve.
- f) Set timer for **two** minutes, let sample sit undisturbed. A blue color will form if phosphate is present.
- g) After the two minute reaction period, pipette 2 ml of solution into the cuvette, and measure absorbance at 890 nm. Convert the spectrophotometer reading into phosphate concentration using the standard curve.

Spiked sample preparation

The “Spike Calculation” section in the data sheet is used for this analysis.

- a) Enter data into appropriate data sheet. Determine the phosphate concentration of your samples.
- b) Select a sample to spike. Choose a sample such that if the diluted concentration is increased by 1.5x – 2x, the measured concentration of the diluted sample will be below the highest point of your calibration curve.
- c) Adjust the “250 ppm standard volume” cell until the “expected concentration” cell is nearly equal to the “target concentration”. Generally this value should be under 1 ml.
- d) Prepare 10 ml of sample using the same dilution as before.
- e) Pipette the volume specified in the “sample volume” cell of the diluted sample into a 50 ml centrifuge tube labeled “Spike”.
- f) Pipette the volume listed in the “standard volume” cell of 250 mg/L PO_4^{3-} standard into the “Spike” labeled 50 ml centrifuge tube.
- g) Check the sample volume. It should be equal to 10 ml.
- h) Add a Phosver reagent packet, and react as specified above. Convert the optical density into phosphate concentration, and enter this value into the “measured concentration” cell.
- i) The spike passes if recovery is between 85 – 115%.

Clean up

The phosphate assay generates waste that required collection and proper disposal. Notify your supervisor if anything is missing or is not clean. Use the cleanup instructions as follows:

- a) After analyzing your samples, pour contents of the centrifuge tube into a collection beaker.
- b) Rinse the centrifuge tube and cap 3x with a DI squeeze bottle, with each rinse going into the collection beaker.

- c) With all traces of reactant/sample removed, rinse the tubes 3x with hot tap water, followed by 3 rinses of reverse osmosis water.
- d) Place all caps in a large beaker. Rinse beaker/caps 3x with hot tap water, then 3x with reverse osmosis water.
- e) Place centrifuge tubes upside down in their respective space in the test tube rack. Leave caps in the drying rack to air dry.
- f) Rinse the cuvette out several times with DI water, dab dry upside down on a paper towel, make sure the outside is clean, and place back in the cuvette case. Place the cuvette case on the aluminum blocks in the ASU cabinet.
- g) Rinse standard beakers and any volumetric flasks 3x with hot tap water and 3x with reverse osmosis water. Place back in nutrient test kit box.
- h) Place all contents back into the nutrient test kit box and return to cabinet.

IV. QUALITY ASSURANCE/QUALITY CONTROL

Triplicate samples (perform at least one triplicate per set) are used to assess assay variability. The relative standard deviation (standard deviation divided by the average) should be less than 5%.

Sample spikes are used to determine matrix effects. Use the instructions in the spreadsheet.

V. NOTES

Use instructions in “phosphate standard curve” excel spreadsheet to develop the OD to phosphate concentration correlation. Note that the standard curve will depend on the culture media used in the stock solution. Use the “Phosphate Standard Curve” excel file, also found in the SOP documentation folder, for samples in f/2 culture media at 35 ppt. For added accuracy, repeat measurements for standard curve development three times, and report the precision at each concentration level.

VI. INTERFERENCES

Table copied from Hach method 8048.

Interferences		Concentration in f/2 + seawater (mg/L)
Substance	Interference Levels and Treatments	
Aluminum	Greater than 200 mg/L	-
Arsenate	Interferes at any level	-
Chromium	Greater than 100 mg/L	0.0002
Copper	Greater than 10 mg/L	0.00339
Hydrogen Sulfide	Interferes at any level	-
Iron	Greater than 100 mg/L	0.65681
Nickel	Greater than 300 mg/L	0.0066
Silica	Greater than 50 mg/L	-
Silicate	Greater than 10 mg/L	-
Zinc	Greater than 80 mg/L	0.01
Turbidity (large amounts) or color	May cause inconsistent results.	-
pH, excess buffering	Highly buffered samples or extreme sample pH may exceed the buffering capacity of the reagents and require sample pretreatment. pH 2-10 is recommended	-

VII. REVISION HISTORY

6/24/2015, bjc: Somewhere along this SOP's history, sodium phosphate monobasic monohydrate was used instead of sodium phosphate monobasic anhydrous. There may be periods before 6/24/2015 when 181 mg of NaH_2PO_4 were used to prepare the standard, which gives an actual concentration of 286.6 mg PO_4 -/L. 158 mg of sodium phosphate monobasic anhydrous gives the target standard stock solution concentration of 250 mg PO_4 -/L.

QUICK CONTAMINATION CHECK MICROSCOPY BENCH METHOD FOR PROJECT ASU

I. Background:

Microscopy is the process of observing microorganisms under a microscope. For the ASU project, it is used to check for contaminants in the pond samples.

Before starting, please review how to correctly create a slide and the parts of a microscope.

Figure 1 explains how to correctly create a wet mount slide (Messengale).

Figure 2 shows a standard microscope with parts labeled (Parts).

Test Duration: ~ 2hr

Equipment:

4" X 6" Lens Paper (Fisher cat# 11-996)
Plain Microscope Slides (Fisher cat# 12-550-A3)
Cover Glass
Type A Immersion Oil (non-drying)
10-100 uL pipette
Pipette tips
Paper Towels

Instrument and Location:

Vista lab (192-209)
Olympus CX41 Microscope
Infinity Analysis Software

AlgDig & Microscopy Folder Location:

- My Computer/ Public\$/AlgDig/Project ASU/UFS LRB1201 Summer 2014/Quick Contamination Check and Micrographs

II. Preparing Workspace:

1. Safety goggles **MUST** be worn at all times. Gloves are optional; since the analysis is done on a **NO GLOVE COMPUTER**, wearing one glove is highly recommended.
2. All materials should be in the drawers labeled microscopy in the Vista lab. Turn the microscope on – the power button is on the right hand side of the microscope above the light knob.
3. Open the Infinity Analysis Software. Under the Start button, click All Programs, click Infinity Software, and choose Infinity Analyze.
4. Place a folded paper towel in front of the computer. All wet work should be done over the paper towel to minimize spills and keep area clean.

Preparing Software:

1. Create a folder in My Computer>Public\$>AlgDig>Project ASU>UFS LRB1201 Summer 2014>Quick Contamination Check and Micrographs>Pond Micrographs. Name it DDMMYYYY_Initials.
2. Open the spreadsheet with contaminant counts located in My Computer>Public\$>AlgDig>Project ASU>UFS LRB1201 Summer 2014>Quick Contamination Check and Micrographs>CP_Summer2014_LRB1201_Initials.

III. Calibrating the Microscope to Take Images:

This step must be done before taking images using the 40x lens or the 100x lens

****Remember to recalibrate at the appropriate magnification if switching back and forth between lenses****

400x magnification

- i. In INFINITY ANALYSIS, open hemacytometer at 400x recalibration image in My Computer>Public\$>AlgDig>Project ASU>UFS LRB1201 Summer 2014>Quick Contamination Check and Micrographs>hemacytometer_at_400x_rec calibration
- ii. Click on the Measure tab at the top right-hand side of the program, then click calibrate.
- iii. Carefully choose a point at an intersection. Drag the cursor over four smaller boxes. Try to click on the exact same spot as the first point, but on the new intersection. As shown here:

- iv. A specification box will show up. Set the length to **200 micron** and the magnification to **40**. Click Okay.
- v. Click on the measure tab at the top of the program, then click Point-to-Point. Click on a spot and drag the cursor down to the bottom of the box. If the annotation that pops up verifies 200um, you are now ready to take images at 400x. As shown here:

1000x magnification *Immersion oil must be used at this magnification*

****Immersion oil damages all other lenses****

- i. In INFINITY ANALYSIS, open hemacytometer at 1000x recalibration image in My Computer>Public\$>AlgDig>Project ASU>UFS LRB1201 Summer 2014>Quick Contamination Check and Micrographs>hemacytometer_at_1000x_rec calibration
- ii. Click on the Measure tab at the top right-hand side of the program, then click calibrate.
- iii. Carefully choose a point at an intersection. Drag the cursor over one box. Try to click on the exact same spot as the first point, but on the new intersection. As shown here:

- iv. A specification box will show up. Set the length to **50 micron** and the magnification to **100**. Click Okay.
- v. Verify the calibration. Click on the measure tab at the top of the program, then click Point-to-Point. Click on a spot and drag the cursor down to the bottom of the box. If the annotation that pops up verifies 50 um, you are now ready to take images at 1000x. As shown here:

IV. Quick Contamination Check Procedure:

DO FOR EACH POND:

Prepare Slide:

1. Shake up pond water sample, vigorously.
2. Pipette two 10uL drops, about half an inch to an inch apart, onto a slide.
3. Cover the sample drops with the cover slips.
 - i. Put edge of cover glass perpendicular to slide near sample.
 - ii. Move the cover glass towards the drop, until it touches the pond water.
 - iii. Drop the cover glass on the pond water, covering your sample.
 - a. If bubbles appear on slide, repeat steps i-iii again.
 - iv. With a paper towel or lens paper, soak up extra water around the cover glass.

Be careful to not touch or press down on the cover glass.
 - v. If algae sample is tampered (by touching it), repeat steps i-iv again.

100x magnification Analysis:

1. Turn microscope phase to open ("O") phase (the dark phase is another good option).
2. Turn revolving nosepiece to 10x. This will be the yellow, **smallest** lens.
3. Place slide on microscope stage.
 - i. Adjust coarse and fine adjustment knobs to focus on the sample specimens.

These can be found on the right-hand side of the microscope.
 - ii. Adjust the lighting to make sure the sample specimens are not too dark or washed out. The lighting knob is found on the right-hand side of the microscope above the coarse and fine adjustment knobs.
4. Randomly scan 3 passes on each slide (these 6 passes will scan 6 μ l in total). One pass from each the top 1/3, middle 1/3 and bottom 1/3 of the slide will be performed. As shown below:

5. When a contaminant is detected, focus under 40x objective to identify different species or contaminant category. *Images of contaminants or algae should only be taken at 40x objective or 100x objective. At least one image per pond should be taken.

400x magnification Analysis:

1. Turn revolving nosepiece to 40x. This will be the blue **medium** sized lens.
2. Adjust coarse and fine adjustment knobs until sample specimens are present.
3. REMEMBER TO CALIBRATE BEFORE TAKING IMAGES! (see section III. above)
4. Take images of the contaminants and/or algae specimen by clicking Capture on the left-hand side of the program.
5. Record size of the algae or contaminants. Do this by clicking on Measure, then click either Point-to-Point or Circle From 3 Points.
 - a. If contaminants are detected, refer to the Quick Contamination Check Slides PDF located in My Computer>Public\$>AlgDig>Project ASU>Standard Operating Procedures>Quick_Contamination_Check_Slides for help identifying the contaminants.
6. Annotate the image. Do this by clicking on Annotate, then click Text.
 - a. Observations include but are not limited to amount of bacteria or different types of bacteria present, potential types of contaminants such as flagellates, ciliates, amoeba, etc., clumping of cells. The date and lens objective used should be included in the annotation. If there are no contaminants detected, take an image of algae cells and annotate appropriately.

- b. Click Save As. Make sure in the “Save as type:” box that JPEG is selected. Save your images with descriptive titles, including Pond number, any contaminants seen, date, and lens objective used.
- c. Images should be saved in the file created for that day’s microscopy in My Computer>Public\$>AlgDig>Project ASU>UFS LRB1201 Summer 2014>Quick Contamination Check and Micrographs>Pond Micrographs>DDMMYYYY_INitials.

An example for contaminant image at 40x Objective with annotation is shown below:

Image title: Pond6_potential cilia contaminant_29July2014_40x

7. Record the total number of contaminants counted for each slide scanned in the spreadsheet with contaminant counts. The spreadsheet will automatically calculate the total number of organisms observed per 1 ml of sample for each contaminant category.
8. Record any observations or notes about the pond or conflicts that came about in the contaminant count spreadsheet in the “Other Notes” column.

1000x magnification Analysis:

WARNING: Do NOT use immersion oil on any lens other than 1000x. Immersion oil damages all other lenses.

1. Turn revolving nosepiece to 100x. This will be the black and white, largest lens.
2. Place slide on microscope stage.

- i. Add a drop of immersion oil to the top of the slide.
 - ii. Adjust coarse adjustment knob until the lens touches the surface of the oil.
 - iii. Adjust fine adjustment knob until sample specimens are present.
3. REMEMBER TO CALIBRATE BEFORE TAKING IMAGES! (see section III. above)
4. Take images of the contaminants and/or algae specimen by clicking Capture on the left-hand side of the program.
5. Record size of the algae or contaminants. Do this by clicking on Measure, then click either Point-to-Point or Circle From 3 Points.
 - a. If contaminants are detected, refer to the Quick Contamination Check Slides PDF located in My Computer>Public\$>AlgDig>Project ASU>Standard Operating Procedures>Quick_Contamination_Check_Slides for help identifying the contaminants.
6. Annotate the image. Do this by clicking on Annotate, then click Text.
 - a. Observations include but are not limited to amount of bacteria or different types of bacteria present, potential types of contaminants such as flagellates, ciliates, amoeba, etc., clumping of cells. The date and lens objective used should be included in the annotation. If there are no contaminants detected, take an image of algae cells and annotate appropriately.
 - b. Click Save As. Make sure in the "Save as type:" box that JPEG is selected. Save your images with descriptive titles, including Pond number, any contaminants seen, date, and lens objective used.
 - c. Images should be saved in the file created for that day's microscopy in My Computer>Public\$>AlgDig>Project ASU>UFS LRB1201 Summer 2014>Quick Contamination Check and Micrographs>Pond Micrographs>DDMMYYYY_Initials.

An example for contaminant image at 100x Objective with annotation is shown below:

Image title: PanelC_contaminants_22July2014_100x

7. Record the total number of contaminants counted for each slide scanned in the spreadsheet with contaminant counts. The spreadsheet will automatically calculate the total number of organisms observed per 1 ml of sample for each contaminant category.
8. Record any observations or notes about the pond or conflicts that came about in the contaminant count spreadsheet in the “Other Notes” column.
9. Wipe off immersion oil from lens when finished.

Final Clean Up:

1. Glass slides should be disposed of in the broken glass box.
2. Clean up area: throw paper towels, used lens paper, and used pipette tips away.
3. Wipe down work area.
4. Turn off microscope and place cover back on it.
5. Exit out of Infinity Analysis Software

ANNUAL WATER QUALITY REPORT

WATER TESTING
PERFORMED
IN 2014

Presented By

Este informe contiene información muy importante sobre su agua potable. Tradúzcalo o hable con alguien que lo entienda bien.

PWS ID#: 4010009

Our Mission Continues

We are proud to present once again our annual water quality report covering all testing performed between January 1 and December 31, 2014. Most notably, last year marked the 40th anniversary of the Safe Drinking Water Act (SDWA). This rule was created to protect public health by regulating the nation's drinking water supply. We celebrate this milestone as we continue to manage our water system with a mission to deliver the best-quality drinking water. By striving to meet the requirements of SDWA, we are ensuring a future of healthy, clean drinking water for years to come.

Please let us know if you ever have any questions or concerns about your water.

Note about Fluoride

Our water system treats your water by adding fluoride to the naturally occurring level to help prevent dental caries in consumers. State regulations require the fluoride levels in the treated water be maintained within a range of 0.7 - 1.3 ppm, with an optimum dose of 0.8 ppm. Our monitoring showed that the fluoride levels in the treated water ranged from 0.1 - 0.9 with an average of 0.62 ppm. Information about fluoridation, oral health, and current issues is available from <http://www.swrcb.ca.gov/drinking-water/certlic/drinkingwater/Fluoridation.shtml>.

Important Health Information

Some people may be more vulnerable to contaminants in drinking water than the general population. Immunocompromised persons such as those with cancer undergoing chemotherapy, those who have undergone organ transplants, people with HIV/AIDS or other immune system disorders, some elderly, and infants may be particularly at risk from infections. These people should seek advice about drinking water from their health care providers. The U.S. EPA/CDC (Centers for Disease Control and Prevention) guidelines on appropriate means to lessen the risk of infection by *Cryptosporidium* and other microbial contaminants are available from the Safe Drinking Water Hotline at (800) 426-4791 or <http://water.epa.gov/drink/hotline>.

Community Participation

City Council meetings are held on the first and third Tuesdays of each month at 6:00 p.m. at City Hall, 990 Palm Street, San Luis Obispo, California. A public comment period is held at the beginning of each meeting.

Use Water Wisely

As drought conditions continue to grip the region and the State, it is important for all of us to remember to continue to use water wisely. Rainfall during the past two years has been substantially less than normal, with 2013 being the driest since rainfall records have been kept in the county. Although the community's investment in a multi-source water supply is keeping the water supply situation relatively stable for the coming years, changing weather patterns and extreme drought conditions could be the "new normal" for the Central Coast.

The State Water Resources Control Board has mandated the curtailment of outdoor watering during the upcoming year and has adopted regulations aimed at preventing water waste. The mandatory three-day-a-week water restrictions will remain in effect through the summer and fall, and possibly longer depending on the rainy season this year.

Other mandatory regulations include:

- No washing down driveways or other hardscapes
- Irrigation runoff is prohibited
- Shut-off nozzles are required when washing vehicles
- Decorative fountains must recirculate water

For more information about these regulations or the services the Utilities Department provides, please visit our Web site at slowater.org or give us a call at (805) 781-7215.

Where Does My Water Come From?

The City of San Luis Obispo is fortunate to have several sources of water. The Salinas Reservoir (also known as Santa Margarita Lake, eight miles east of Santa Margarita), Whale Rock Reservoir (Cayucos), and Nacimiento Lake (16 miles northwest of Paso Robles) are our main supplies. The surface water from the three lakes is treated at the Stenner Creek Water Treatment Plant. At present, well water is used to meet a small percentage (2%) of the City's demand for water. The active well is the Pacific Beach Well #1 (Los Osos Valley Road). During 2014, our treatment plant and wells delivered 1.91 billion gallons of water to San Luis Obispo.

Substances That Could Be in Water

The sources of drinking water (both tap water and bottled water) include rivers, lakes, streams, ponds, reservoirs, springs, and wells. As water travels over the surface of the land or through the ground, it dissolves naturally occurring minerals and, in some cases, radioactive material, and can pick up substances resulting from the presence of animals or from human activity.

In order to ensure that tap water is safe to drink, the U.S. Environmental Protection Agency (U.S. EPA) and the State Water Resources Control Board (State Board) prescribe regulations that limit the amount of certain contaminants in water provided by public water systems. State Board regulations also establish limits for contaminants in bottled water that must provide the same protection for public health. Drinking water, including bottled water, may reasonably be expected to contain at least small amounts of some contaminants. The presence of contaminants does not necessarily indicate that water poses a health risk.

Contaminants that may be present in source water include: **Microbial Contaminants**, such as viruses and bacteria, that may come from sewage treatment plants, septic systems, agricultural livestock operations, and wildlife; **Inorganic Contaminants**, such as salts and metals, that can be naturally occurring or can result from urban stormwater runoff, industrial or domestic wastewater discharges, oil and gas production, mining, or farming; **Pesticides and Herbicides**, that may come from a variety of sources such as agriculture, urban stormwater runoff, and residential uses; **Organic Chemical Contaminants**, including synthetic and volatile organic chemicals, that are by-products of industrial processes and petroleum production and that can also come from gas stations, urban stormwater runoff, agricultural applications, and septic systems; **Radioactive Contaminants**, that can be naturally occurring or can be the result of oil and gas production and mining activities.

More information about contaminants and potential health effects can be obtained by calling the U.S. EPA's Safe Drinking Water Hotline at (800) 426-4791.

Source Water Assessment

Assessments of the drinking water sources for the City of San Luis Obispo have been conducted. These sources include Salinas Reservoir, Whale Rock Reservoir, Nacimiento Lake, Pacific Beach Well, and Fire Station #4 Well. To request a summary of an assessment, contact Jeff Densmore, District Engineer, Santa Barbara District, at (805) 566-1326, or the City of San Luis Obispo at (805) 781-7215.

A copy of the complete assessment is available from the SWRCB Division of Drinking Water, 1180 Eugenia Place, Suite 200, Carpinteria, California, 93013, or the City of San Luis Obispo, 879 Morro Street, San Luis Obispo, California, 93401.

Lead in Home Plumbing

If present, elevated levels of lead can cause serious health problems, especially for pregnant women and young children. Lead in drinking water is primarily from materials and components associated with service lines and home plumbing. We are responsible for providing high-quality drinking water, but we cannot control the variety of materials used in plumbing components. When your water has been sitting for several hours, you can minimize the potential for lead exposure by flushing your tap for 30 seconds to 2 minutes before using water for drinking or cooking. If you are concerned about lead in your water, you may wish to have your water tested. Information on lead in drinking water, testing methods, and steps you can take to minimize exposure is available from the Safe Drinking Water Hotline or at www.epa.gov/safewater/lead.

QUESTIONS?

For more information about this report, or for any questions relating to your drinking water, please contact Dean Furukawa, Water Treatment Plant Supervisor, at (805) 781-7566 or dfurukawa@slocity.org.

Sampling Results

During the past year, we have taken hundreds of water samples in order to determine the presence of any radioactive, biological, inorganic, volatile organic, or synthetic organic contaminants. The tables below show only those contaminants that were detected in the water. The State requires us to monitor for certain substances less often than once per year because the concentrations of these substances do not change frequently. In these cases, the most recent sample data are included, along with the year in which the sample was taken.

We participated in the 3rd stage of the EPA's Unregulated Contaminant Monitoring Regulation (UCMR3) program by performing additional tests on our drinking water. UCMR3 benefits the environment and public health by providing the EPA with data on the occurrence of contaminants suspected to be in drinking water, in order to determine if EPA needs to introduce new regulatory standards to improve drinking water quality. Contact us for more information on this program.

REGULATED SUBSTANCES							
SUBSTANCE (UNIT OF MEASURE)	YEAR SAMPLED	MCL [MRDL]	PHG (MCLG) [MRDLG]	AMOUNT DETECTED	RANGE LOW-HIGH	VIOLATION	TYPICAL SOURCE
Aluminum (ppm)	2014	1	0.6	0.112	ND–0.21	No	Erosion of natural deposits; residue from some surface water treatment processes
Barium (ppm)	2014	1	2	0.043	ND–0.13	No	Discharges of oil drilling wastes and from metal refineries; erosion of natural deposits
Chlorine (ppm)	2014	[4.0 (as Cl ₂)]	[4 (as Cl ₂)]	0.67	ND–1.8	No	Drinking water disinfectant added for treatment
Chromium (ppb)	2014	50	(100)	3.57	ND–14.0	No	Discharge from steel and pulp mills and chrome plating; erosion of natural deposits
Control of DBP precursors [TOC] ¹ (% removal)	2014	TT	NA	26.2	21–33	No	Various natural and man-made sources
Fluoride ² (ppm)	2014	2.0	1	0.62	0.10–0.90	No	Erosion of natural deposits; water additive that promotes strong teeth; discharge from fertilizer and aluminum factories
Haloacetic Acids–Stage 2 ³ (ppb)	2014	60	NA	20.3	ND–31.0	No	By-product of drinking water disinfection
Gross Alpha Particle Activity (pCi/L)	2011	15	(0)	0.0145	ND–0.029	No	Erosion of natural deposits
Hexavalent Chromium (ppb)	2014	10	0.02	5.36	ND–13.0	No	Discharge from electroplating factories, leather tanneries, wood preservation, chemical synthesis, refractory production, and textile manufacturing facilities; erosion of natural deposits
Nitrate [as nitrate] (ppm)	2014	45	45	4.03	ND–6.3	No	Runoff and leaching from fertilizer use; leaching from septic tanks and sewage; erosion of natural deposits
TTHMs [Total Trihalomethanes] –Stage 2 ³ (ppb)	2014	80	NA	76.3	7.0–115	No	By-product of drinking water disinfection
Turbidity ⁴ (NTU)	2014	TT	NA	0.10	0.06–0.10	No	Soil runoff
Turbidity (Lowest monthly percent of samples meeting limit)	2014	TT	NA	100%	NA	No	Soil runoff
Tap water samples were collected for lead and copper analyses from sample sites throughout the community.							
SUBSTANCE (UNIT OF MEASURE)	YEAR SAMPLED	AL	PHG (MCLG)	AMOUNT DETECTED (90TH%TILE)	SITES ABOVE AL/ TOTAL SITES	VIOLATION	TYPICAL SOURCE
Copper (ppm)	2013	1.3	0.3	0.114	0/30	No	Internal corrosion of household plumbing systems; erosion of natural deposits; leaching from wood preservatives
Lead (ppb)	2013	15	0.2	0.9	0/30	No	Internal corrosion of household water plumbing systems; discharges from industrial manufacturers; erosion of natural deposits

SECONDARY SUBSTANCES

SUBSTANCE (UNIT OF MEASURE)	YEAR SAMPLED	PHG SMCL (MCLG)	PHG AMOUNT DETECTED	RANGE LOW-HIGH	VIOLATION	TYPICAL SOURCE
Aluminum (ppb)	2014	200	NS	ND–210	No	Erosion of natural deposits; residual from some surface water treatment processes
Chloride (ppm)	2014	500	NS	28–34	No	Runoff/leaching from natural deposits; seawater influence
Iron (ppb)	2014	300	NS	ND–400	No	Leaching from natural deposits; industrial wastes
Specific Conductance (micromhos)	2014	1,600	NS	652–830	No	Substances that form ions when in water; seawater influence
Sulfate (ppm)	2014	500	NS	30–115	No	Runoff/leaching from natural deposits; industrial wastes
Total Dissolved Solids (ppm)	2014	1,000	NS	360–460	No	Runoff/leaching from natural deposits

UNREGULATED CONTAMINANT MONITORING REGULATION 3 (UCMR3)

SUBSTANCE (UNIT OF MEASURE)	YEAR SAMPLED	AMOUNT DETECTED	RANGE LOW-HIGH
Chlorate (ppb)	2014	245	120–330
Molybdenum (ppb)	2014	2.55	ND–3.5
Strontium (ppb)	2014	363	260–420
Vanadium (ppb)	2014	3.7	3.0–5.2

¹ Total organic carbon (TOC) has no health effects. However, TOC provides a medium for the formation of disinfection by-products such as THMs and HAA5s. The City's TOC reduction requirement was 25% based on a running annual average calculated quarterly.

² The City currently adds fluoride to the treated water produced by the water treatment plant to achieve an optimum target residual of 0.8 ppm. Some limited areas in the City along Los Osos Valley Road receive a blend of surface water and ground water that may have a lower fluoride residual.

³ Regulatory compliance is determined based on the Locational Running Annual Average (LRAA).

⁴ Turbidity is a measure of the cloudiness of the water. We monitor it because it is a good indicator of the effectiveness of our filtration system.

Definitions

AL (Regulatory Action Level): The concentration of a contaminant which, if exceeded, triggers treatment or other requirements that a water system must follow.

MCL (Maximum Contaminant Level): The highest level of a contaminant that is allowed in drinking water. Primary MCLs are set as close to the PHGs (or MCLGs) as is economically and technologically feasible. Secondary MCLs (SMCLs) are set to protect the odor, taste, and appearance of drinking water.

MCLG (Maximum Contaminant Level Goal): The level of a contaminant in drinking water below which there is no known or expected risk to health. MCLGs are set by the U.S. EPA.

micromhos: A measure of electrical conductance.

MRDL (Maximum Residual Disinfectant Level): The highest level of a disinfectant allowed in drinking water. There is convincing evidence that addition of a disinfectant is necessary for control of microbial contaminants.

MRDLG (Maximum Residual Disinfectant Level Goal): The level of a drinking water disinfectant below which there is no known or expected risk to health. MRDLGs do not reflect the benefits of the use of disinfectants to control microbial contaminants.

NA: Not applicable

ND (Not detected): Indicates that the substance was not found by laboratory analysis.

NS: No standard

NTU (Nephelometric Turbidity Units): Measurement of the clarity, or turbidity, of water. Turbidity in excess of 5 NTU is just noticeable to the average person.

pCi/L (picocuries per liter): A measure of radioactivity.

PDWS (Primary Drinking Water Standard): MCLs and MRDLs for contaminants that affect health along with their monitoring and reporting requirements, and water treatment requirements.

PHG (Public Health Goal): The level of a contaminant in drinking water below which there is no known or expected risk to health. PHGs are set by the California EPA.

ppb (parts per billion): One part substance per billion parts water (or micrograms per liter).

ppm (parts per million): One part substance per million parts water (or milligrams per liter).

TT (Treatment Technique): A required process intended to reduce the level of a contaminant in drinking water.