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Effects of entrainment on marine microbiota in the Moss Landing Power Plant

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EFFECTS OF ENTRAINMENT ON MARINE MICROBIOTA IN THE MOSS LANDING POWER PLANT

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Master of Science

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Gala Virginia Wagner

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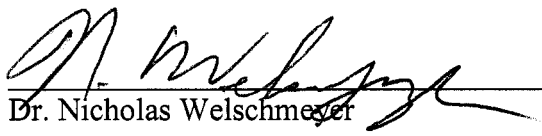

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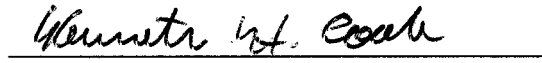

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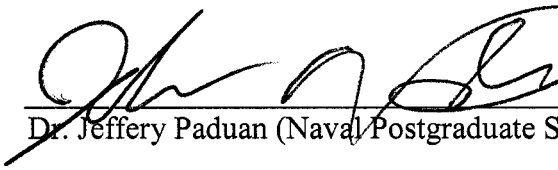

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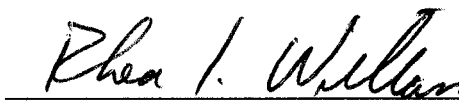

ABSTRACT

EFFECTS OF ENTRAINMENT ON MARINE MICROBIOTA IN THE MOSS LANDING POWER PLANT

by Gala V. Wagner

The impacts of once-through cooling to bacteria and phytoplankton passing through the Moss Landing Power Plant (MLPP) were investigated. Measurements were made along the cooling-water flow-path, such that conditions before intake, during entrainment and after discharge into Monterey Bay could be assessed. Bacterial growth was enhanced as a result of passage through the MLPP cooling system, as evidenced from increases in bacterial growth, frequency of dividing cells, and respiration in water sampled at the immediate exit from the power plant. Phytoplankton were negatively impacted after passage through the MLPP cooling system as shown by reductions in photochemical quantum efficiency (*Fv/Fm*), primary productivity, and increases in pheopigment/Chl *a* ratios at the power plant exit station. Thus, bacteria and phytoplankton were differentially impacted by once-through cooling transport. However, differential enhancement and inhibition effects on bacteria and phytoplankton, respectively, were not detected after discharge and dilution into local Monterey Bay waters.

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INTRODUCTION

Electric power plants account for 75 – 90% of the thermal pollution in the U.S. (Langford 1988; Laws 2000). Refineries, petrochemical plants, cokeries, and steel mills account for the remainder of thermal pollution. The production of energy by steam generation (heated water producing steam that turns turbines) requires large quantities of water, often estuarine or marine, to cool power plant condenser tubes. California alone has 21 (19 conventional and 2 nuclear) coastal power plants generating 23,910 megawatts of energy and using approximately 64.4 billion liters of coastal and estuarine waters per day for once-through cooling purposes (California Energy Commission 2004).

The increasing use of cooling water in the electricity generating industry leads to increasing use of estuarine and marine resources because cooling water is largely of marine origin (Laws 2000; Turnpenny and Coughlan 2003). Planktonic organisms entrained in the cooling water are exposed to the initial thermal increase within the power plant heat exchangers and may be negatively affected (Clark 1989; Langford 1990; Laws 2000; Turnpenny and Coughlan 2003). In addition, the thermal effluent that enters the surrounding marine environment may also affect planktonic organisms in the vicinity of the discharge site (Clark 1989; Langford 1990; Laws 2000).

Marine plankton (zooplankton, phytoplankton, and bacteria) are the base of the marine food web and are important for nutrient cycling in the ocean. Impacts on local populations of plankton (i.e. increased or decreased productivity, immigration, and emigration) can influence the dynamics and structure of more distantly located populations (Raimondi and Reed 1996). Changes in planktonic activities caused by

changes in environmental conditions can confer significant impacts on functions of coastal ecosystems (Choi et al. 2002).

The 1970s brought significant scientific focus to entrainment in power plant cooling water systems, subsequent thermal discharge and the potential negative effects on commercially important fish larvae, marine invertebrates, and the environment (Capuzzo 1980; Langford 1990). Zooplankton losses have been reported to range from 0% - 100% loss (Clark and Brownell 1973; Langford 1983). Ecological Analysis Inc. (1983) concluded that zooplankton entrainment mortality at Moss Landing Power Plant (MLPP) was 100% for all entrained organisms. More recently researchers described the entrainment effects of the new combined-cycle units on the local populations of fish larvae and *Cancer* spp. megalops using an empirical transport model (ETM) (Tenera 2000). Based on the ETM, the mean estimate of total entrainment mortality was 13.1% of the local population for larval fish, and 5.8% of the local population for *Cancer* spp. megalops (Tenera 2000). These researchers concluded that it is unlikely that populations of fishes and crabs would be adversely affected by the new MLPP combined-cycle cooling water intake system (Tenera 2000). To date the effect of the MLPP cooling system on phytoplanktonic and bacterial organisms has not been studied in detail.

The impact of entrainment on phytoplankton is generally negative and appears to result from thermal stress and/or chlorination, but it is unclear whether cells have actually been killed or just temporarily stressed (Laws 2000). Several studies have shown that primary productivity decreases 2 to 80 % in thermal effluent from power plants (38 %, Morgan and Stross (1965); 2 – 37 %, Fox and Moyer (1973); 26 %, Suchanek (1975);

30 %,Bienfang and Johnson (1980); 22%, Miller et al. (1976); 20 -80 %, Chang and Rossman (1985); 60 %, Servais and Billen (1989); 36%, Martinez-Arroyo et al. (1999); 38%, Guseva and Chebotina (2000)). Whereas others found that entrainment of phytoplankton had minimal impact on phytoplankton productivity (Dunstall 1985; Hirayama and Hirano 1970; Socal et al. 1999). Thus, the effects of thermal discharge appear to vary widely.

The impact of heated discharge water on phytoplankton seems dependent on both ambient sea temperature and the subsequent increase in temperature from the power plant. In the temperate northeastern Pacific and northwestern Atlantic oceans researchers found that an increase in temperature of 8 – 11 °C stimulated photosynthesis when ambient water temperatures were 16 °C or cooler, and inhibited photosynthesis when ambient water temperatures were 17 °C or warmer (Briand 1975; Morgan and Stross 1965). These findings were supported by Chang and Rossmann (1985) who found differential effects of temperature increase on phytoplankton as evidenced by the differential heating of cooling water, such that a portion of the water was heated above the tolerance level for phytoplankton while other portions of water were not subjected to inhibitory high temperatures.

Temperature increase may also affect the phytoplankton assemblage by eliminating stenothermal forms and increasing remaining eurythermal species (Naylor, 1965). Studies have found changes in phytoplankton assemblage by passage through the cooling system. Briand (1975) studied marine phytoplankton passing through the cooling system of two southern California coastal power plants and found that passage through

the condenser tubes enhanced the dominance of two heat tolerant species (*Asterionella japonica* and *Gonyaulaux polyedra*) while killing less heat tolerant diatoms (45.7% decrease in cell numbers) and dinoflagellates (32.8% decrease in cell numbers) when compared between intake and discharge sampling sites. Rossmann et al. (1980) found that at elevated temperatures, phytoplankton assemblages exhibited a decrease in species diversity, seen as a shift from a diatom dominated assemblage to one dominated by green algae. Saravanane et al. (1998) found increased dominance of *Thalassiosira,* a diatom genus, after passage through a power plant cooling system. Touliabah and Taylor (2004) also found dominance of *Thalassiosira* in effluent at 45 °C and domoic acid producing *Pseudo-nitzschia pseudodelicatissima* in effluent at 50 °C. This finding is particularly revelant to the Monterey Bay ecosystem since *Pseudo-nitzschia* spp. blooms are well documented locally (Scholin et al. 2000). However, it is unknown if the MLPP will affect harmful algal blooms. Corroborating laboratory experiments conducted by Goldman and Ryther (1976) and Ukeles (1961) further supported changes in the phytoplankton assemblage caused by thermal stress by incubating phytoplankton at increasing temperatures. The results of these lab experiments indicated that the maximum temperature for normal growth is variable between phytoplankton groups. However, field studies comparing intake and discharge sites found no significant change in phytoplankton assemblages caused by thermal stress (Martinez-Arroyo et al. 1999; Suchanek 1975).

Bacteria are generally regarded as the organisms most tolerant of temperature (Brock 1985). Mesophilic bacteria have an optimal growth rate at temperatures between

20 and 45 °C, and thermophilic bacteria have an optimal growth rate at temperatures between 45 and 70 °C (Brock 1985). High temperature (40 °C) has been shown to decrease bacterial production by 9 to 39 % (Choi et al. 2002). Shiah and Ducklow (1994) found that bacterial growth rates were positively correlated with incubation temperatures between 3 and 25 °C.

Thermal discharges have been found to cause changes in the abundance and populations of bacteria (Langford 1990). Miller et al. (1976) and Fox and Moyer (1973) found that the numbers of bacteria increased in the discharge of power stations. Suzdaleva (1998) found that bacterial populations from the intake water were significantly reduced when heated to temperatures higher than 50 °C, while the bacterial population from the discharge water was not significantly reduced when heated to temperatures up to 70 °C, suggesting the enhancement of thermophilic bacteria in the discharge water. Rankin et al. (1974) and Solski (1974) found that the abundances of bacterial populations were unchanged at thermal discharge sites compared with intake waters.

The focus of the research in this study is to investigate the impacts of the thermal increase and entrainment in the Moss Landing Power Plant (MLPP) on marine microbiota. The MLPP, operated by LS Power, is California's largest power plant and is capable of supplying the state with 2545 megawatts of electricity per day (California Energy Commission 2004). Moss Landing Power Plant is located in Moss Landing, California roughly midway between Santa Cruz and Monterey, California. Cooling water for the facility is drawn from two separate intake structures within Moss Landing harbor.

Historically, cooling water from five units was discharged into Elkhorn Slough, one of California's largest estuaries, but this practice was discontinued in 1995 (Tenera 2000). Currently MLPP discharges thermal effluent into Monterey Bay through two subsurface conduits 200 m from shore and approximately 7 m below the sea surface (Paduan 2002; Tenera 2000). The discharge into Monterey Bay is estimated at 2.54 - 4.56 billion liters per day, equivalent to half the volume of Elkhorn Slough (Genz 2003). The average temperature of the intake water is 13.9 °C. After a residence time of approximately 30 minutes in the power plant (Genz 2003), the average temperature of the discharged water is elevated to approximately 27.2 °C (Tenera 2000). Moss Landing Power Plant is permitted by the Monterey County Water Quality Resources Control Board to increase the ambient (intake) water temperature by 14.3 °C daily (Tenera 2000). Most power plants in the United States operate so that the effluent water is between 5 °C and 15 °C above ambient (Laws 2000). The discharge plume is turbulent, producing rapid mixing with surrounding seawater, and therefore rapid cooling of the discharge water (Paduan 2002).

With an increasing global demand for electricity there are increasing impacts on coastal marine ecosystems. In 1968 the thermal power stations in California had a total daily capacity of 17,000 MW discharging their thermal effluent into tidal areas (Adams 1969). The total capacity has increased 71% (currently 23,910 MW) in thirty-six years (California Energy Commission 2004). In 2001 MLPP upgraded their system producing 2545 MW from 1250 MW in 2000. The increase of electrical output by MLPP increased the volume of thermal effluent discharged into Monterey Bay by 37%.

Temperature increase by thermal effluent from MLPP may affect the assemblages of phytoplankton and bacteria by the elimination of some species with possible replacement by other species. The enhancement of pathogenic bacterial growth and/or the replacement of the natural phytoplankton assemblage with harmful algal blooms due to thermal increase and entrainment in the power plant could pose health hazards to humans, fish, marine birds and mammals in the Moss Landing area. To date the effect of the MLPP cooling system on phytoplanktonic and bacterial organisms has not been studied in detail. It was hypothesized that the temperature increase caused by the cooling water system of the power plant has a negative effect on phytoplankton biomass, productivity, and physiology and acts to promote bacterial growth.

OBJECTIVES

The goal of this study was to investigate the impacts of the thermal increase and entrainment in the Moss Landing Power Plant on marine microbiota (bacteria and phytoplankton) from Monterey Bay, California. The following objectives were examined through the course of research:

1) Determine the impact of the thermal increase and entrainment on the biomass and productivity of bacteria.
2) Determine the impact of the thermal increase and entrainment on the biomass, physiology, and productivity of phytoplankton.
3) Assess the spatial extent of the area impacted surrounding the discharge plume within Monterey Bay, California.

MATERIALS AND METHODS

Sampling

Surface water samples were collected in triplicate in darkened 1.0 L polycarbonate bottles (rinsed three times at each station before filling) at five sites chosen to represent the path traveled from the intake source in Moss Landing harbor to the discharge site in Monterey Bay (Fig. 1). The station sequence was as follows: Station 1 (36° 48.283'N, 121° 47.067'W) located in Moss Landing harbor directly in front of intake chambers for Units 6 and 7. Station 2 (36° 48.017'N, 121° 47.001'W) the surge chamber located on MLPP property which represented the closest access to heated water immediately leaving the plant. The surge chamber is a vertical tunnel (9.8 m in height, 4.9 m in diameter, Fig. 2). Samples were collected using a sampling bailer from the surface water in the surge chamber. Station 3 (36° 48.258'N, 121° 47.476'W) located in Monterey Bay, directly over the thermal discharge site located ~ 200 m from shore. Station 4 (36° 48.264'N, 121° 47.511'W) located in Monterey Bay, 100 m west of the discharge site and Station 5 (36° 48.262'N, 121° 47.680'W) located in Monterey Bay, 500 m west of the discharge site. Temperature was measured at each sampling site using a digital thermometer (Hanna instruments) accurate to ± 0.2 °C. Samples were collected and analyzed periodically from March 2004 to November 2005 (Table 1).

Bacterial analysis

Bacterial biomass was measured using two techniques, direct bacterial counts and colony growth. For direct bacterial counting, sample water (15 mL) from each site was

preserved with 1% gluteraldehyde and 1 to 5 mL was quantitatively filtered onto 0.02 μm Anodisc filters (used for the possibility of quantifying viruses), backed with 0.45 μm Millipore filters. The sample was filtered to dryness and removed while the vacuum was on. The dry filter was placed on top of 100 μL of working stock SybrGold ® solution on a sterile Petri dish and stored in the dark for 15 minutes. After the staining period, the filter was placed back on the filtration rack and rinsed with of 0.02 μm filtered de-ionized water. The filter was placed on a microscope slide with one drop of Slow Fade ®. A cover slip was placed over the filter. Slides were stored in the freezer (Noble 2001) until bacteria cells could be enumerated using an epifluorescent microscope under blue light excitation (Hagstrom et al. 1979; Sherr et al. 2001). Using the 1250x magnification (oil immersion objective lens) the mean cells per counting grid (at least 100 bacterial cells) were enumerated.

Bacterial colony growth was measured using unpreserved sample water. A quantitative volume of sample (10-25 μL) was streaked onto Difco ® marine agar plates in triplicate with steriloops and incubated at 25 °C for 24 hrs in the dark. The bacterial colonies were counted at the end of the incubation time (Wood 1965).

Bacterial productivity

Relative productivity of bacteria was estimated by determining the frequency of dividing cells (FDC) as described by (Hagstrom et al. 1979; Sherr et al. 2001). Frequency of dividing cells (FDC) provides an indicator of bacterial productivity (Newell, 1981) based on total bacteria enumeration, which can be contrasted to colony growth which only

reflects activity of those cells capable of cultivation on agar growth media (Azam 2001). Bacterial cells were enumerated as explained previously, noting paired cells and single cells, to calculate FDC as a percentage. This technique provides a means to estimate relative growth rates without the need for incubations.

Phytoplankton analysis

Biomass and degradation

Biomass and potential degradation of the phytoplankton were measured using high performance liquid chromatography. To concentrate the phytoplankton cells, sample water (100 mL to 500 mL) from each site was filtered onto a 0.7 μm Whatmann glass fiber filter (GF/F). To extract phytoplankton pigments, each filter was placed in a 2.0 mL micro-centrifuge tube with 1.2 mL of 90% acetone and stored at -20 °C. Extraction proceeded for a minimum of 24 hours in the dark. After extraction, the samples were vortexed, the filters were compressed to the bottom of the tube with a stainless steel spatula and centrifuged for approximately 1 minute in a microfuge to remove debris. The extract was sub-sampled (300 μL) and injected onto a Binary Gradient HPLC using a 1 mL sample loop. The column of the HPLC was Rainin Dynamax Microsorb C8, 25 cm long, 4mm diameter, 5 μm diameter silica particles. Solvents consisted of:

Solvent A (vol/vol): MeOH (50%): MeCN (25%): Pyridine 0.25M aqueous (25%)

Solvent B (vol/vol): Me CN (60%): MeOH (20%): Acetone (20%)

The pigments analyzed were chlorophyll a, chlorophyllides, and pheopigments using the method of (Zapata et al. 2000). The final concentrations for the specified pigments were calculated using equation 1.

$$\textit{Pigment concentration} = \frac{\textit{(HPLC area units * Dilution*Acetone extraction vol.)}}{\textit{(Pigment response factor*Sample Vol* Vol Filtered)}} \quad (1)$$

Where:

Pigment response factor = Chl *a* area units / weight of Chl *a* standard

Concentration of Chl *a* standard determined by spectrophotometric analysis.

Phytoplankton physiology

Chlorophyll fluorescence serves as an indicator of the photosynthetic reactions in the chloroplasts of green plants, including algae. The pulse amplitude modulated (PAM) fluorometer measures *in vivo* fluorescence (photochemical yield) (Genty et al. 1989; Schreiber and Bilger 1987). *In vivo* fluorescence is a useful way to assess the photosynthetic activity of phytoplankton, specifically the state of photosystem II.

Fluorescence measurements were made on triplicate samples (3 mL) from each site using a PAM fluorometer (Water-PAM emitter detector, H. Walz GmbH) with a photomultiplier detector on a photosensor module (H6779-01 Hamamatsu) with an optical filter set for low background signal ($\lambda > 710$ nm). Saturation pulses (0.8 sec) of strong white light (4000 μmol $m^{-2}s^{-1}$ photosynthetically active radiation, PAR) were supplied to the sample with a halogen lamp pulse source (FL 103, Walz). Saturation pulse light passed through a short-pass filter ($\lambda < 690$ nm) before entering the fiber optics through the 15 mm quartz cuvett into each sample. The fluorescence ratio,

Fv/Fm corresponding to photochemical efficiency, was determined using the saturation pulse technique (Schreiber et al 1998) on dark adapted samples. The technique is rapid, sensitive, and density-independent, allowing quantitative assessment of algal physiological status from variable fluorescence properties of photosystem II (Genty 1989). The saturation pulse method provides quantitative information on photochemical yield determined by:

$$YIELD = (Fm - F)/Fm = Fv/Fm$$

Where:

Fm = maximum fluorescence

F = minimum fluorescence

Fv = variable fluorescence

Fv/Fm = unit less ratio

Dark adaptation time = 60 seconds

The variable fluorescence ratio, *Fv/Fm*, produces values ranging from 0.0 to 0.7 (Genty 1989; Schreiber at al. 1998). Exponentially growing phytoplankton usually exhibit higher dark-adapted *Fv/Fm* values from 0.55-0.70. Decreases from the highest possible values of *Fv/Fm* have been shown to indicate physiological stress to the photoautotroph in both higher land plants and algae.

Laboratory heat experiments

Independent laboratory experiments were made to verify that elevated temperatures induce changes in photochemical yield (*Fv/Fm)* of phytoplankton.

Triplicate samples (10 mL) from the intake site and nine different phytoplankton cultures were incubated in 13 mL polypropylene tubes in a water bath for approximately 20 minutes. The experiments performed on each sample included a control and samples treated with increasing temperatures (15 °C, 20 °C, 25 °C, 30 °C, 35 °C, 40 °C, 45 °C, and 50 °C). Each treatment was kept in the dark at the ambient temperature of the source (intake or culture) before being placed in the water bath at the treatment temperature. At the end of the incubation period, photochemical yield was measured using the PAM. Recovery of the phytoplankton was assessed using the PAM fluorometer after a 24 hour incubation at 25 °C in the dark.

Community production and respiration

On three sampling dates (6/15/05, 9/14/05, and 11/1/05), net production and community respiration of the samples at Stations 1, 2, 3, and 4 were determined by measuring the concentration of dissolved oxygen. The concentration of dissolved oxygen was determined by an automated precise Winkler titration (Furuya and Harada 1995). With the use of a microvolume dispenser, an automatic dispenser model 665 Dosimat (Metrohm), with 0.005-N sodium thiosulfate as a titrant. The minimum volume of a titration was 0.005 mL. The end-point was detected potentiometrically. Duplicate surface water samples were collected in 2 L clear polycarbonate bottles. Sample water from each 2 L bottle was distributed to three 300 mL biological oxygen demand (BOD) bottles with polypropylene tubing to reduce oxygen introduction. One bottle was immediately fixed with manganese chloride and alkaline iodide to determine the initial

oxygen content, one bottle was wrapped in aluminum foil (dark bottle), and the third bottle was left uncovered (light bottle). The light and dark bottles were placed in an incubation tank with continuous sea water flow for a 24 hour incubation. At the end of 24 hours each bottle was fixed with manganese chloride and alkaline iodide. Algal net primary production rate was determined from the light bottle minus dark bottle; dark community respiration was determined from the initial oxygen level minus final dark bottle.

Statistical analysis

An Analysis of Covariance with a Tukey's pairwise comparisons test was used to detect differences among the stations for the following parameters: temperature, bacterial biomass, FDC, photochemical yield, community productivity, and community respiration (Zar 1999). Normality, equal variances, and independence were confirmed by analysis of residuals. The correlative relationships between temperature and the above parameters were analyzed.

Absolute values of parameters on any given date provided useful comparisons of biological conditions among stations. However, seasonal variation in biomass and production confounded useful comparisons among sampling dates. In order to remove seasonal variations in biomass and production among sampling dates, the results were normalized to Station 1 (the intake source water) for each biological parameter measured on a given date. Normalized values were scaled to 1.0 for Station 1 providing a "snapshot" of the relative variation in parameters measured at all five stations; mean

values of the relative changes in specific parameters could then be summarized for all sampling dates.

RESULTS

Temperature

Mean (± S.D.) water temperature before entering MLPP (Station 1) was 15.1 ± 1.8 °C. The power plant increased mean (± S.D.) temperature to 22.3 ± 2.3 °C in the surge chamber (Station 2), the mean (± S.D.) temperature at the discharge site (Station 3) was 18.4 ± 2.1 °C, the mean (± S.D.) temperature 100 m from the discharge site (Station 4) was 14.5 ± 2.0 °C, and the mean (± S.D.) temperature 500 m from the discharge site (Station 5) was 14.1 ± 1.9 °C (Figs. 3 and 4). The maximum temperature measured at Station 2 was 27.2 °C and at Station 3 was 24.6 °C (Fig. 3). The mean temperatures at Stations 2 and 3 were significantly higher ($p \leq 0.001$) than all other stations. Stations 1, 4, and 5 did not differ significantly ($p \geq 0.127$).

Bacteria

Biomass

Mean bacterial biomass from epifluorescence direct counts at the surge tank (Station 2) was significantly greater (mean = 4.2×10^5 cells/mL) than at the intake site (Station 1) (mean = 3.1×10^5 cells/mL) ($p \leq 0.001$) (Figs. 5 and 6). Station 2 was also significantly higher from the discharge site (Stations 3) ($p \leq 0.001$), and Station 4 ($p = 0.019$) but not Station 5 ($p = 0.530$). Stations 1, 4, and 5 (intake water vs. outer Monterey Bay stations) did not differ significantly ($p \geq 0.803$). There was not a significant relationship ($r^2 = 0.099$, $p = 0.090$) between average bacterial biomass and temperature

(Fig. 7). Generally, the levels of direct count total bacteria among stations on any given date did not differ by more than a factor of two.

The colony growth assessments on bacterial agar plates showed much larger ranges in bacterial activity among stations. On average the bacterial colonies at the surge tank (Station 2; mean (± S.D.) = $4.4 \times 10^{4} \pm 9.3 \times 10^{4}$) was 5 times as concentrated as the intake site (Station 1; mean (± S.D.) = $4.2 \times 10^{3} \pm 2.8 \times 10^{3}$) (Figs. 8 and 9). This indicates a significant increase in bacterial colonies in the power plant. The surge tank (Station 2) was significantly different ($p \leq 0.001$) from all other stations. Station 3 was $2.1 \times 10^{3} \pm 1.7 \times 10^{3}$ which was not significantly different from all other stations ($p \geq 0.567$). Stations 1, 4, and 5 (intake vs. outer Monterey Bay sites) did not differ significantly from each other ($p \geq 0.547$). There seems to be two different relationships between temperature and bacterial colonies (Fig. 10). There is a positive relationship between temperature and bacterial colonies (line 1) and a relationship which shows no change positive or negative between temperature and bacterial colonies (line 2). This suggests a differential reaction of bacterial colonies to temperature. These results indicate that bacterial colonies are enhanced by thermal increase and entrainment in the MLPP, but the bacterial colonies enhancement was not detectable at the Monterey Bay stations.

Bacterial productivity

The results indicated by percentage that the surge tank had twice as many dividing (productive) cells (Station 2; mean (± S.D.) = 29.7 ± 3.6 %) as the intake site (Station 1; mean (± S.D.) = 12.9 ± 2.4 %) (Figs. 11 and 12). The FDC of Station 2 (mean (± S.D.) = 29.7 ± 3.6 %) was significantly greater than all other stations ($p \leq 0.005$). The mean (± S.D.) FDC of the discharge site (Station 3) was 18.2 ± 8.6 %, which was significantly different from the intake (Station 1) ($p = 0.003$), but did not differ significantly from the outer bay sites (Stations 4 and 5) ($p \geq 0.744$). The FDC of Station 4 was a 15.1 ± 7.4 %, and Station 5 was 14.3 ± 7.3 %; Stations 1, 4, and 5 (intake vs. outer Monterey Bay sites) did not differ significantly ($p \geq 0.810$). There was a significantly positive relationship ($r^2 = 0.579$ $p \leq 0.001$) between temperature and FDC (Fig. 13). The results of bacterial productivity from FDC generally parallel those from bacterial colonies, although the absolute value of the growth enhancement is lower for FDC. Both methods yield results consistent with enhanced bacterial growth by thermal increase and entrainment in the MLPP.

Phytoplankton

Biomass and degradation

Phytoplankton biomass (Chl *a* μg/L) had both spatial and seasonal variation (Fig. 14). The phytoplankton biomass was 1.5 times greater at the intake site (Station 1) (mean ± S.D. = 3.4 ± 2.8 μg/L), than phytoplankton biomass at the surge tank (Station 2) (mean ± S.D. = 2.3 ± 1.9 μg/L) and lowest at the discharge site (Station 3) (mean ± S.D. = 1.2 ±

1.0 μg/L) (Fig. 15). The outer Monterey Bay sites (Stations 4 and 5) were variable with a mean (± S.D.) = 3.5 ± 2.4 μg/L at Station 4, and with a mean (± S.D.) = 6.4 ± 4.9 μg/L at Station 5 (Figs. 14 and 15). There was not a significant relationship ($r^2 = 0.026$, $p = 0.174$) between phytoplankton biomass (Chl *a*) and temperature (Fig. 16).

The ratio of pheopigments to Chl *a* provides a measure of the degree to which degradation of Chl *a* by phytoplankton cell death and/or grazing of cells has taken place, relative to algal cellular Chl *a*; it may also indicate the presence of sediment-derived particles which are dominated by pheopigment degradation products. The mean pheopigment to Chl *a* ratio was 2.1 times greater at the surge tank (Station 2) (mean ± S.D. = 1.03 ± 0.66) than at the intake site (Station 1) (mean ± S.D. = 0.48 ± 0.47) (Figs. 17 and 18). The mean (± S.D.) ratio of pheopigments to Chl *a* at the discharge site (Station 3) was 0.51 ± 0.37). The mean (± S.D.) ratio of pheopigments to Chl *a* at Station 4 was 0.26 ± 0.08 and at Station 5 was 0.21 ± 0.10. There was a significant relationship ($r^2 = 0.084$, $p \leq 0.011$) between the pheopigments to Chl *a* ratio and temperature (Fig. 19). These results indicate phytoplankton cell death and/or the entrainment of substantial quantities of sediment which are typically dominated by pheopigments relative to Chl *a*.

Phytoplankton physiology

The mean (± S.D.) photochemical yield (*Fv/Fm*) measured by variable fluorescence was higher at the intake site (Station 1) 0.45 ± 0.07 than the mean (± S.D.) photochemical yield at the surge tank (Station 2) 0.27 ± 0.15, indicating high stress in the phytoplankton at Station 2 (Figs. 20 and 21). The mean photochemical yield at Station 2

was significantly lower ($p \leq 0.001$) than all other stations. The discharge site (Station 3) had a higher average yield than Station 1 (mean ± S.D. = 0.49 ± 0.09) though not significantly different ($p \geq 0.971$) from the other bay samples (Station 4; mean ± S.D. = 0.52 ± 0.07 and Station 5; mean ± S.D. = 0.52 ± 0.08) (Figs. 20 and 21). Stations 4 and 5 did not differ significantly ($p = 0.907$). There was a strong inverse relationship ($r^2 = -0.8813$) between photochemical yield (*Fv/Fm*) and temperature (Fig. 22). The results indicate that the physiology of the phytoplankton was negatively affected by thermal stress and/or entrainment in the MLPP and could easily be measured at the surge tank (Station 2).

Laboratory heat experiments

To verify that heat affects phytoplankton, cultures and natural samples were exposed to temperatures between 15 and 50 °C. The results indicate that samples exposed to temperatures between 13 °C and 30 °C had normal yield values ranging between 0.4 and 0.7. As temperature increased above 30 °C photochemical yield decreased (Fig. 23). To determine if the cells recovered from the heat treatment, the samples were stored for 24 hours at 25 °C in the dark. After the 24 hour recovery period the photochemical yield was measured again and normalized to the control. The results indicate that phytoplankton recover from the heat treatments after exposure to temperatures between 15 and 35 °C (Fig. 24). Recovery is variable for samples exposed to 40 °C (5 – 95%) and recovery is low for samples exposed to 50 °C (>20%) (Fig. 24). As stated earlier MLPP is not permitted to discharge water with temp higher than ~27 °C

but the phytoplankton may be initially shocked with higher temperatures in the heat exchanger.

Community production and respiration

Oxygen exchange determined in light-minus-dark experiments provides unambiguous determination of net primary production specific to phytoplankton, while the corresponding dark oxygen depletion indicates respiration of the bulk planktonic community. On three sampling dates the chl-specific net production rate (μM O_2 μg Chl *a* L^{-1} d^{-1}) and dark respiration (μM O_2 d^{-1}) was determined using the Winkler titration method. Average net production was 7 times lower at the surge tank (Station 2; mean (± S.D.) = 0.36 ± 0.25 μM O_2 μg Chl *a* L^{-1} d^{-1}) than at the intake site (Station 1; mean (± S.D.) = 2.48 ± 0.44 μM O_2 μg Chl *a* L^{-1} d^{-1}) and, correspondingly, average respiration was 9 times higher at Station 2 (mean (± S.D.) = -97.94 ± 47.68 μM O_2 d^{-1}) than Station 1 (mean (± S.D.) = -11.07 ± 5.53 μM O_2 d^{-1}) (Figs. 25 and 26). The average net primary production for Station 2 was significantly lower than all other stations ($p \leq 0.0001$) and, average respiration at Station 2 was significantly higher than all other stations ($p \leq 0.0001$). The mean (± S.D.) net primary production at Station 3 was 2.59 ± 1.14 μM O_2 μg Chl *a* L^{-1} d^{-1}, and at Station 4 was 2.05 ± 1.27 μM O_2 μg Chl *a* L^{-1} d^{-1}. The mean (± S.D.) respiration at Station 3 was a -8.67 ± 3.92 μM O_2 d^{-1}, and at Station 4 was -10.91 ± 12.48 μM O_2 d^{-1}. Neither net primary production nor community respiration at Stations 1, 3, 4, and 5 were significantly different ($p \geq 0.129$). The significant increase in respiration at the surge chamber (Station 2) corroborates results of enhanced bacterial

growth while the significant decrease in net production at the surge chamber (Station 2) suggests negative affects on phytoplankton by thermal stress and/or entrainment in the MLPP.

DISCUSSION

Bacterial growth was enhanced as a result of passage through the MLPP cooling system, as evidenced from significant increases in bacterial colonies (Fig. 9), significant increases in the percentage of frequency of dividing cells (Fig. 12), bacterial productivity, determined by FDC, nearly doubled with every 10 °C increase in temperature (Fig. 13), and increased dark respiration in the surge chamber (Fig. 26). These results are supported by Miller et al. (1976) and Fox and Moyer (1973) who found that the numbers of bacteria increased in the discharge of power stations. Shiah and Ducklow (1994) also observed increased bacterial productivity with increasing temperature between 3 and 25 °C in incubation experiments. Bacteria biomass, however, was not a good indicator for assessing the impact of thermal stress and entrainment on bacteria, as would be expected given the short residence time of entrained water within the power plant. We can not reject the possibility that bacteria associated with wall growth within the power plant contributed to the signals detected at the surge chamber (Station 2). However, since significant increases in bacterial biomass at the surge chamber were not evident in bacterial direct counts, we must assume that the total contribution of such cells was relatively small.

The enhancement of bacterial growth due to thermal increase and entrainment in the power plant could pose health hazards to humans, fish, marine birds, and mammals in the Moss Landing area if the affected bacteria include pathogens. Several groups of human and vertebrate pathogenic bacteria can survive for long periods in surface waters; including *Escherichia coli, Vibrio cholerae, Salmonella* spp., and *Aeromonas* spp.

(Langford 1990). Gorden and Fliermans (1978) found that temperature, nutrients, and other factors associated with thermal effluent contributed to the increased survival and growth of *E. coli.* However, an abbreviated study of total coliforms and *Escherichia coli* concluded that the MLPP is not enhancing growth of coliforms (Wagner unpublished). There was no other attempt made to identify the bacteria in the effluent of the MLPP.

Phytoplankton were negatively impacted after passage through the MLPP cooling system as shown by significant reductions in photochemical yield (Fig. 21), increases in pheopigment/ Chl *a* ratios (Fig. 18), and decreases in phytoplankton net primary productivity in the surge chamber (Fig. 25). Suchanek (1975) also observed an increase in pheophytin as a result of thermal increase at a power plant in Long Island. Several previous studies have shown that primary productivity decreased 2-80 % in thermal effluent from power plants (Morgan and Stross 1965; Suchanek 1975; Bienfang and Johnson 1980; Miller et al. 1976; Chang and Rossman 1985; Servais and Billen 1989; Martinez-Arroyo et al. 1999; Guseva and Chebotina 2000); our results corroborate those findings. In this study we found roughly a five-fold decrease in chl-specific primary production determined from water immediately exiting the power plant at the surge tank. Phytoplankton biomass, as Chl *a*, was not a good indicator for assessing the impact of thermal stress and entrainment on phytoplankton because Chl *a* is present in both live and dead phytoplankton cells; this finding is supported by other studies which found no significant differences in phytoplankton biomass (Bienfang and Johnson 1980; Briand 1975; Chang and Rossman 1985; Martinez-Arroyo et al. 1999; Servais and Billen 1989; Suchaneck 1975).

Temperature increase may also affect the assemblage of phytoplankton by the elimination of desirable species with possible replacement by undesirable species (Laws 2000). Several studies have identified changes in phytoplankton assemblages by passage through the cooling system of power plants (Briand 1975; Rossman et al. 1980; Saravanane et al. 1998). Touliabah and Taylor (2004) found dominance of domoic acid producing *Pseudo-nitzschia pseudodelicatissima* in thermal effluent. The assemblage of phytoplankton on the Monterey Bay coast varies seasonally and there was no attempt to identify the phytoplankton community in this study. However, harmful algal blooms including toxin producing *Pseudo-nitzschia* spp. and *Alexandrium catenella* are known to occur in the Monterey Bay. The toxins accumulate through filter feeding fish and shellfish and when ingested by marine mammals, birds, and humans, can cause neurological dysfunction and mortality. If the harmful algal species in the Monterey Bay are also heat tolerant then the MLPP may enhance the growth of these undesirable phytoplankters.

Flourescence changes, due to heat stress, may directly reflect structural changes within the thylakoid membrane and overall photosynthetic performance (Schreiber and Bilger 1987). Using PAM fluorometry Bilger et al. (1987) determined, that following heat treatment (<45 °C), there was a decrease in variable fluorescence (*Fv/Fm*) of the leaves of the terrestrial plant *Arbutus unedo*. PAM fluorometry was used to assess physiological affects of heat stress on phytoplankton by once-through cooling in MLPP and laboratory manipulation of natural samples and phytoplankton cultures. Results of the laboratory manipulations confirm that temperatures above 35 °C have a negative

affect on phytoplankton (Figs. 23 and 24). The technique was rapid, sensitive, and density-independent; it clearly identified negative impacts of thermal stress on phytoplankton photochemical efficiency, *Fv/Fm*. The fact that PAM fluorometers can be plumbed for flow-through analysis (rather than discrete cuvet sampling) suggests this method may provide a cost-effective means for monitoring biological impacts of once-through cooling flow on a continuous basis.

The findings in this study of enhanced bacterial growth and negative impacts on phytoplankton are mainly based on results from surge chamber (Station 2) samples. It is important to acknowledge that the surge chamber is an artificial environment where water flow may be limited. However, this sampling site is useful because it allows us to investigate the effects of MLPP on bacteria and phytoplankton directly after heat stress and entrainment without the diluting effect of near shore waters at the discharge site. Future studies may choose to collect samples from a deeper source in the surge chamber as well as throughout the water column at the intake site to further understand the effects the once through cooling system of MLPP is having on marine micro-organisms.

In spite of the fact that differential enhancement and inhibition effects were clearly evident in the cooling water flow measured at the surge chamber, the corresponding impacts in Monterey Bay were much more difficult to detect. It appears that even at the high discharge rates that result in measurable surface temperature increases at the discharge site in Monterey Bay, the apparent biological effect is dissipated quickly within 100-500 m of the site by dilution in surrounding waters (Figs. 6, 9, 12, 15, 18, 21, 25, and 26). It is possible that bacteria and phytoplankton experienced

physiological recovery to the native biological state after reintroduction to normal ambient temperature. However, our results on negative rates of primary production and laboratory heat treatment experiments do not support this possibility since the experimental productivity incubations were conducted under normal cool temperatures for all samples and heat stressed phytoplankton did not recover after 24 hours at ambient temperature (Fig. 24).

In conclusion, bacterial enhancement and phytoplankton inhibition occur as a result of entrainment through the Moss Landing Power Plant. However, biological impacts after discharge and dilution in surrounding Monterey Bay waters were not perceptible. While the physical impact on the entire Monterey Bay may be localized, it is important to monitor impacts on lower trophic level organisms because marine food webs are supported primarily by marine microbiota. The effects of sublethal stress generally do not lead to spectacular events. It is possible that the greatest disruption to aquatic systems from power plant effluents may be caused by the continual exposure of organisms due to thermal shock (Laws 2000). Sublethal effects of pollutants can cause an alteration of the physiology of an organism or population which may become more vulnerable to further environmental change. In addition, impacts on local populations (i.e. increased or decreased productivity, immigration, and emigration) can influence the dynamics and structure of more distantly located populations (Raimondi and Reed 1996).

To bring the impacts of power plant entrainment and thermal increase into perspective let us consider a volume of 9.41×10^9 m^3 of coastal ocean (941,000 m long, 1000 m wide, and 10 m deep). The length is equivalent to the California coastline from

San Francisco Bay south to San Diego, which is where the 90% of California's coastal power plants are located. The MLPP is permitted to withdraw 4.56 x 10^6 m^3 of sea water per day. At this rate it would take it would take approximately 5.5 years to circulate 9.41 x 10^9 m^3 of coastal ocean water through the Moss Landing Power Plant. However, the 21 California coastal power plants that use once-through cooling are permitted to withdraw approximately 6.44 x 10^7 m^3 per day. At the California coast wide rate it would only take approximately 146 days to circulate 9.41 x 10^9 m^3 of coastal ocean water. This means the surface California coastal ocean from San Francisco to San Diego is entrained into coastal power plants 2.5 times every year. Unfortunately, the sea water used for cooling purposes contains an entire community of organisms. On average each cubic meter of coastal ocean water in Monterey Bay contains 1 x 10^{12} bacteria, 1 x 10^9 phytoplankton, 1 x 10^3 zooplankton, and 0.5 fish (York and Foster 2005). Considering the diversity and abundance of organisms contained in coastal ocean sea water; the impacts of entrainment and thermal increase by once-through cooling purposes may be considered substantial when considering the entire California coastal power plants impacts on bacteria, phytoplankton, zooplankton, fish and invertebrate larvae.

Many marine and terrestrial ecosystem changes have been attributed to global climate change (Valiela 2006). The temperature increase of sea water caused by once through cooling in power plants is far in excess in comparison to temperature increase of sea water caused by climate change. However, it is important to consider the effects of thermal increase by once through cooling in power plants on marine micro-organisms found in this study to consider the effects global climate change may have on marine

micro-organisms. In this study bacterial productivity, determined by FDC, nearly doubled with every 10 °C increase in temperature (Fig. 13). Increasing bacterial productivity in the world's oceans may disrupt the natural balance of the marine microbial loop, causing bacterial blooms and hypoxia, and may have impacts throughout the marine ecosystem. Global climate change may also affect the assemblage of phytoplankton, increasing the occurrence of harmful algal blooms. Changes in planktonic activities caused by changes in environmental conditions can confer significant impacts on functions of coastal ecosystems (Choi et al. 2002).

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Table 1. Sampling dates for bacterial colonies, bacteria biomass, bacteria frequency of dividing cells (FDC), phytoplankton biomass, phytoplankton degradation pigments, and phytoplankton photochemical yield.

Temperature Sampling Dates	Bacterial Colonies Sampling Dates	Bacteria Biomass Sampling Dates	Bacteria FDC Sampling Dates	Phytoplankton Biomass Sampling Dates	Phytoplankton Degradation Pigments Sampling Dates	Phytoplankton Photochemical Yield Sampling Dates
3/4/2004	3/4/2004	3/4/2004	3/4/2004			3/4/2004
3/11/2004	3/11/2004			3/11/2004	3/11/2004	3/11/2004
3/25/2004	3/25/2004	3/25/2004	3/25/2004	3/25/2004	3/25/2004	3/25/2004
3/30/2004	3/30/2004			3/30/2004	3/30/2004	3/30/2004
4/8/2004	4/8/2004	4/8/2004	4/8/2004	4/8/2004	4/8/2004	4/8/2004
5/6/2004	5/6/2004			5/6/2004	5/6/2004	5/6/2004
5/20/2004	5/20/2004			5/20/2004	5/20/2004	5/20/2004
6/3/2004	6/3/2004			6/3/2004	6/3/2004	6/3/2004
6/17/2004	6/17/2004					6/17/2004
6/23/2004	6/23/2004	6/23/2004	6/23/2004			6/23/2004
8/3/2004	8/3/2004			8/3/2004	8/3/2004	8/3/2004
8/18/2004	8/18/2004			8/18/2004	8/18/2004	8/18/2004
9/10/2004				9/10/2004	9/10/2004	9/10/2004
10/5/2004	10/5/2004	10/5/2004	10/5/2004	10/5/2004	10/5/2004	10/5/2004
10/29/2004				10/29/2004	10/29/2004	10/29/2004
11/16/2004	11/16/2004	11/16/2004	11/16/2004	11/16/2004	11/16/2004	11/16/2004
3/24/2005				3/24/2005	3/24/2005	3/24/2005
6/15/2005				6/15/2005	6/15/2005	6/15/2005
9/14/2005				9/14/2005	9/14/2005	9/14/2005
11/1/2005						11/1/2005

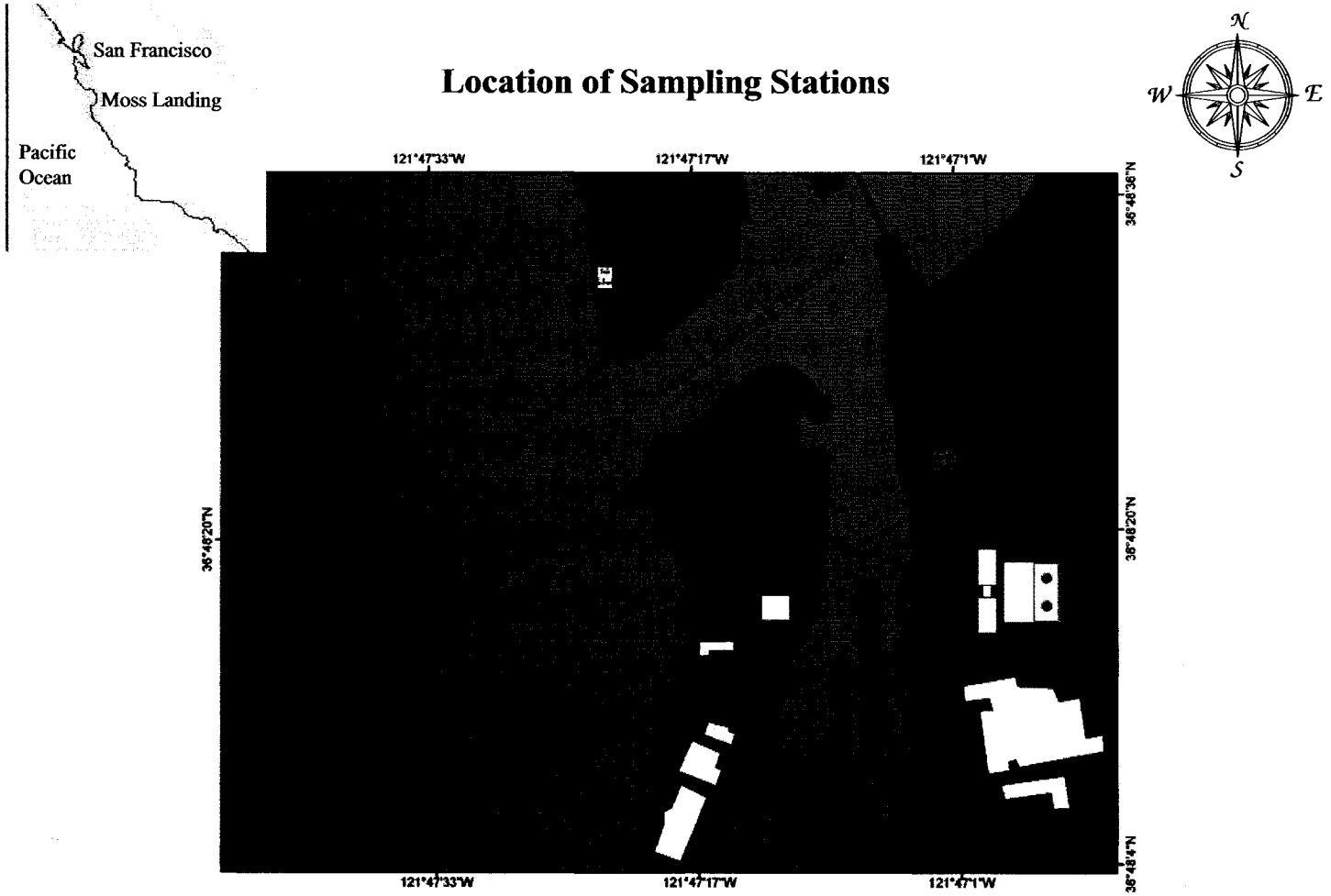

Figure 1. Location of sampling stations (1, 2, 3, 4 and 5) in Moss Landing Harbor, Moss Landing Power Plant, and Monterey Bay.

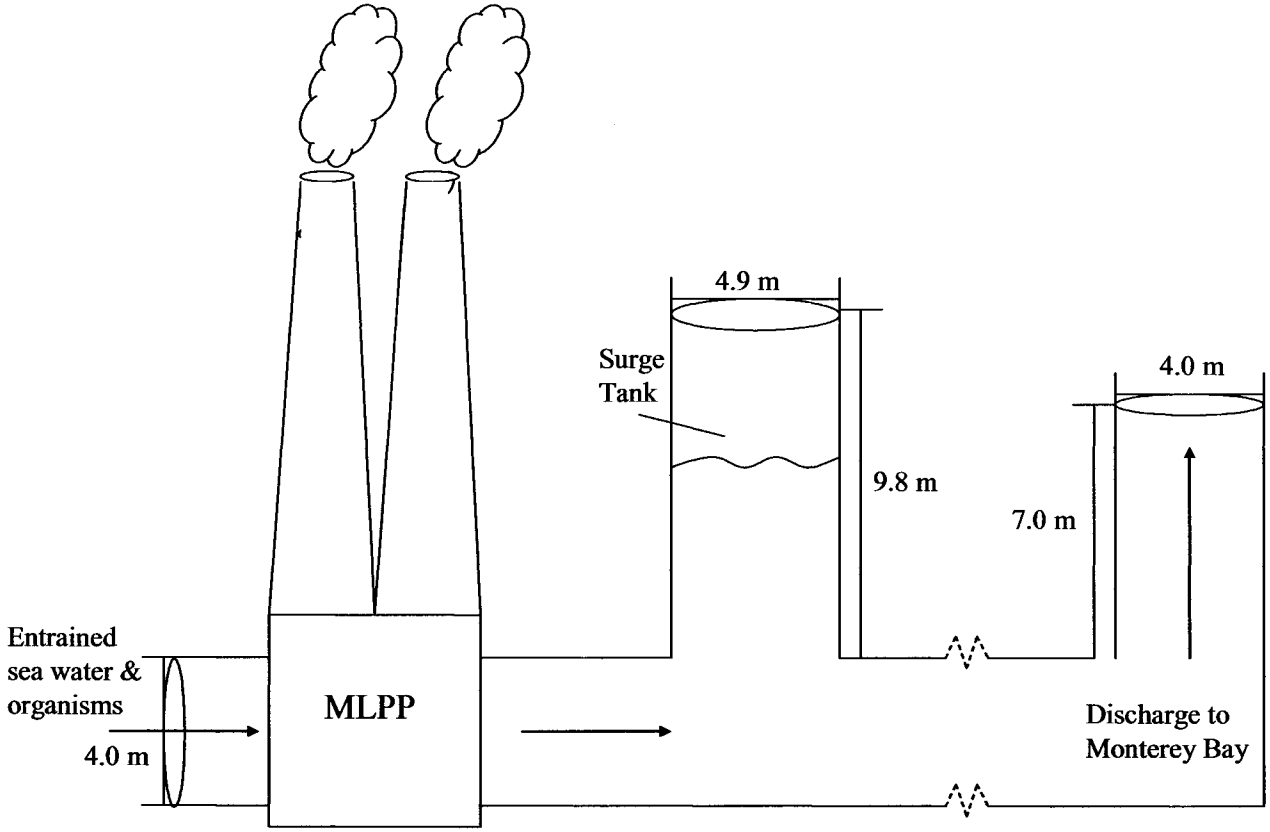

Figure 2. Diagram of Moss Landing Power Plant cooling system, Surge Tank (Station 2), and Discharge (image not to scale).

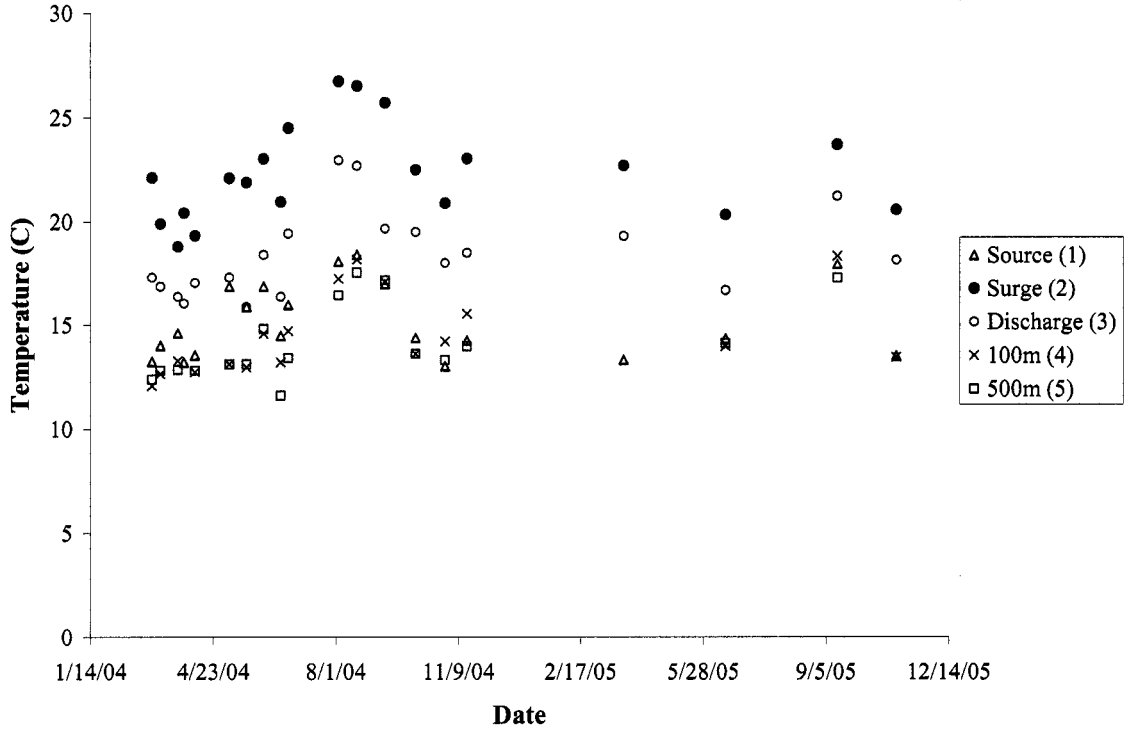

Figure 3. Temperature (C) for each sampling date between 3/4/04 – 11/1/05 at sampling stations (1 - 5).

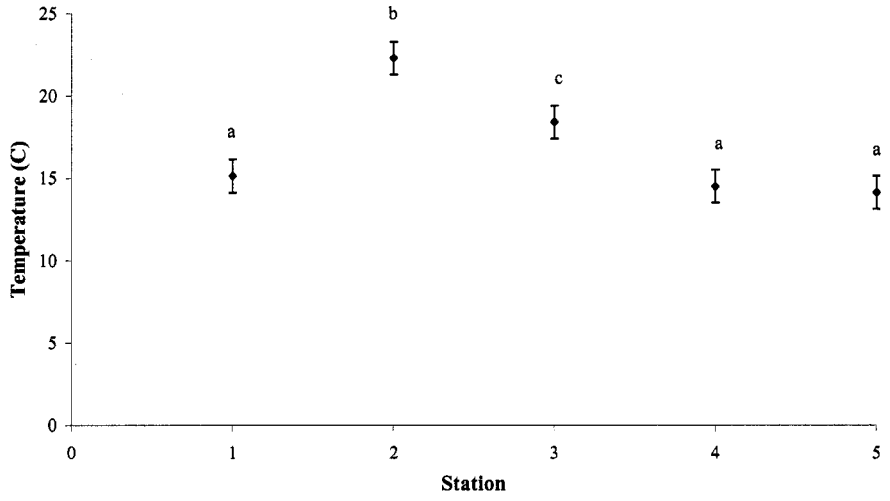

Figure 4. Mean temperature (C) ± SD for all sampling dates 3/4/05 – 11/1/05 (n = 20) at sampling stations (1 - 5) and results of an ANCOVA with Tukey's multiple comparisons test. Different letters above symbols indicate significant differences ($p \leq 0.05$).

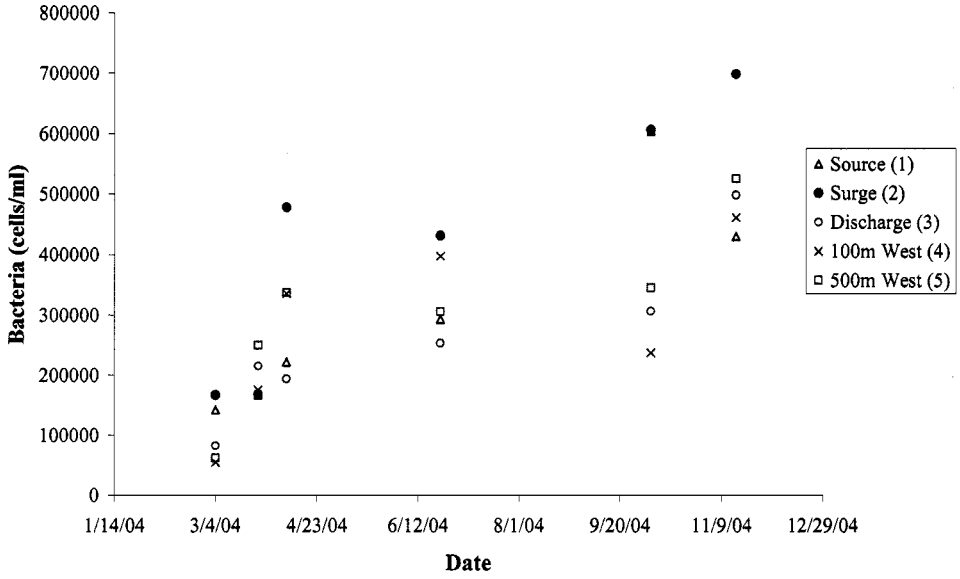

Figure 5. Bacteria (cells/mL) for each sampling date between 3/4/04 – 11/16/04 (n = 6) at sampling stations (1 - 5).

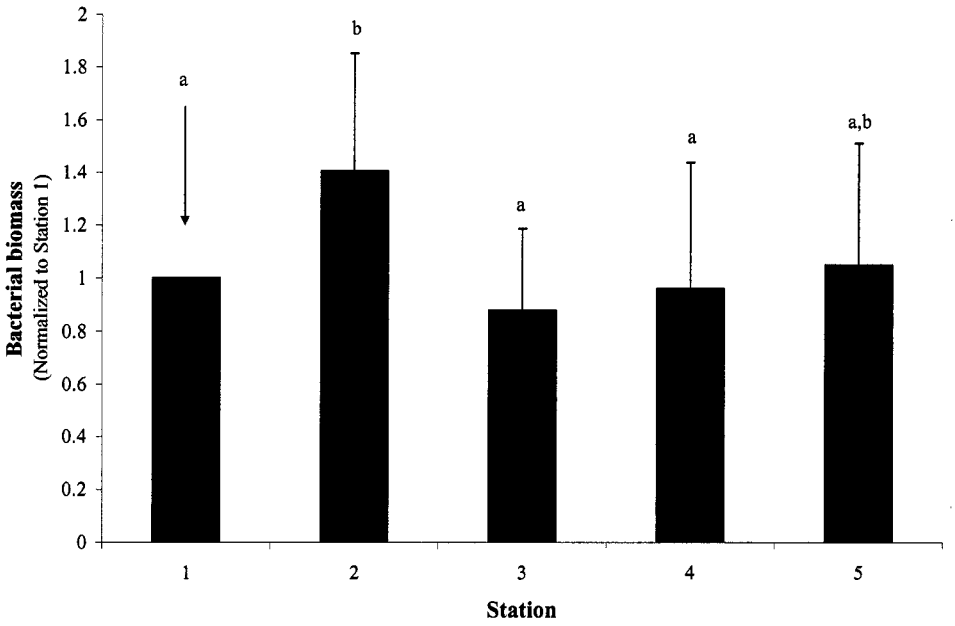

Figure 6. Bacterial biomass ± SD at sampling stations (1 - 5) for each sampling date between 3/4/04 – 11/16/04 (n = 6). All values normalized to 1.0 in reference to the intake site at Station 1 (identified by arrow) and results of an ANCOVA with Tukey's multiple comparisons test. Different letters above symbols indicate significant differences ($p \leq 0.05$).

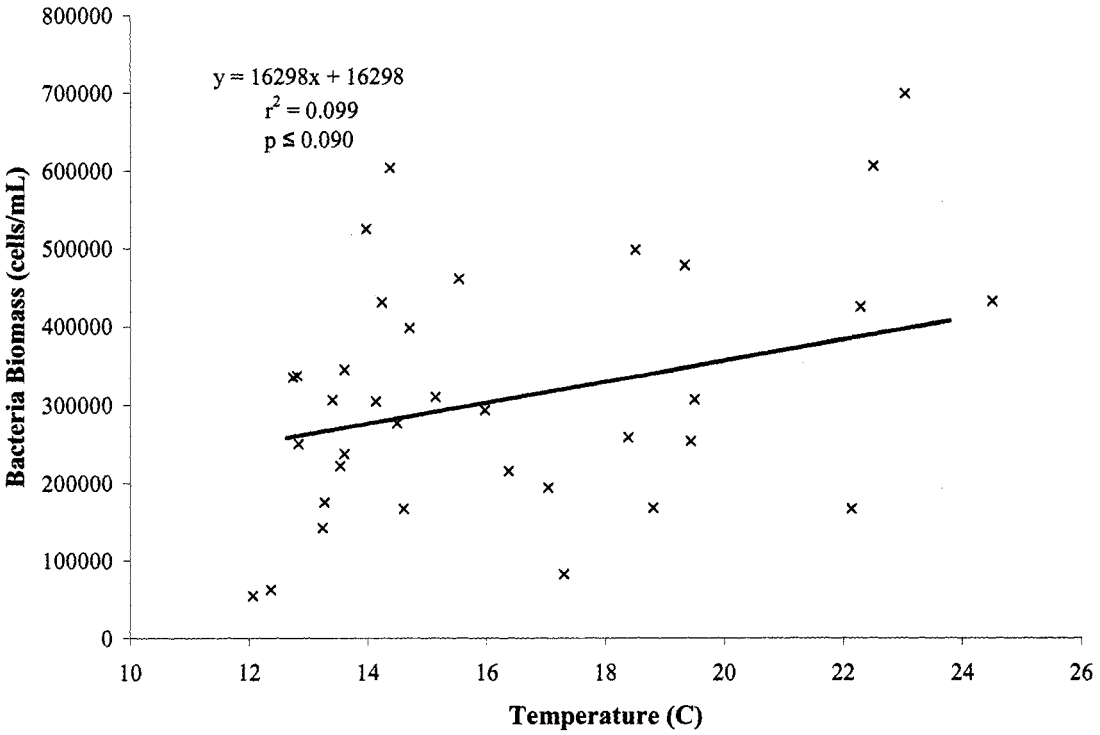

Figure 7. Relationship between bacteria biomass (cells/mL) and temperature. Linear regression equation is given and plotted (n = 6).

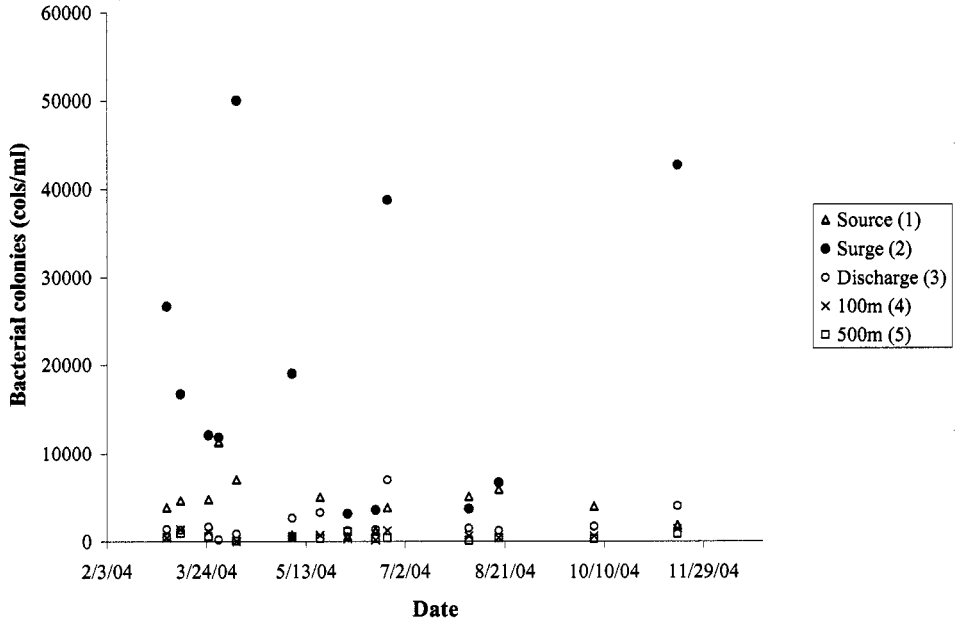

Figure 8. Bacteria colonies (cols/mL) for each sampling date between 3/4/04 – 11/16/04 (n = 12) at sampling stations (1 - 5).

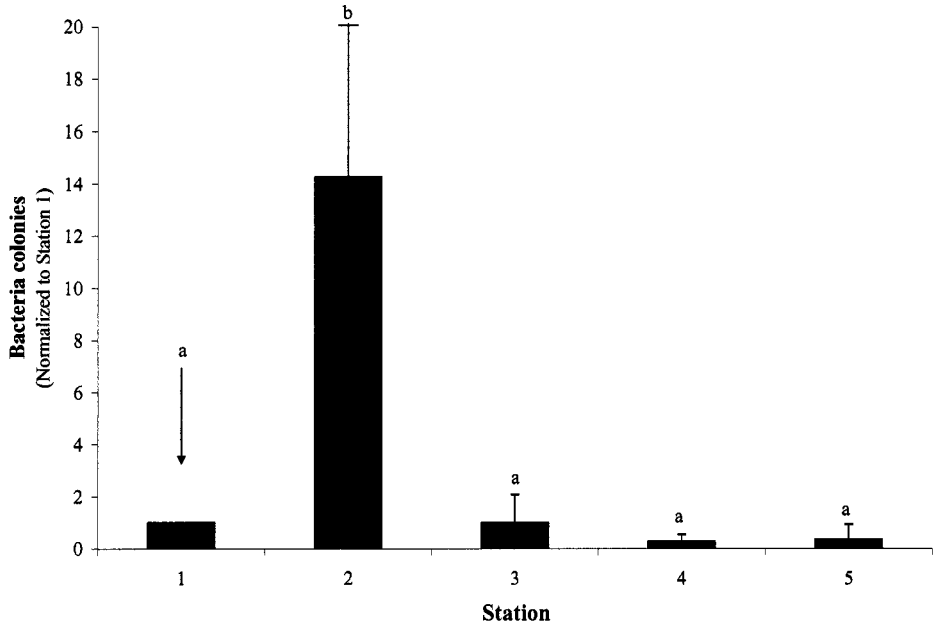

Figure 9. Bacteria colonies ± SD at sampling stations (1 - 5) 3/4/04 – 11/16/04 (n = 12). All values normalized to 1.0 in reference to the intake site at Station 1 (identified by arrow) and results of an ANCOVA with Tukey's multiple comparisons test. Different letters above symbols indicate significant differences ($p \leq 0.05$).

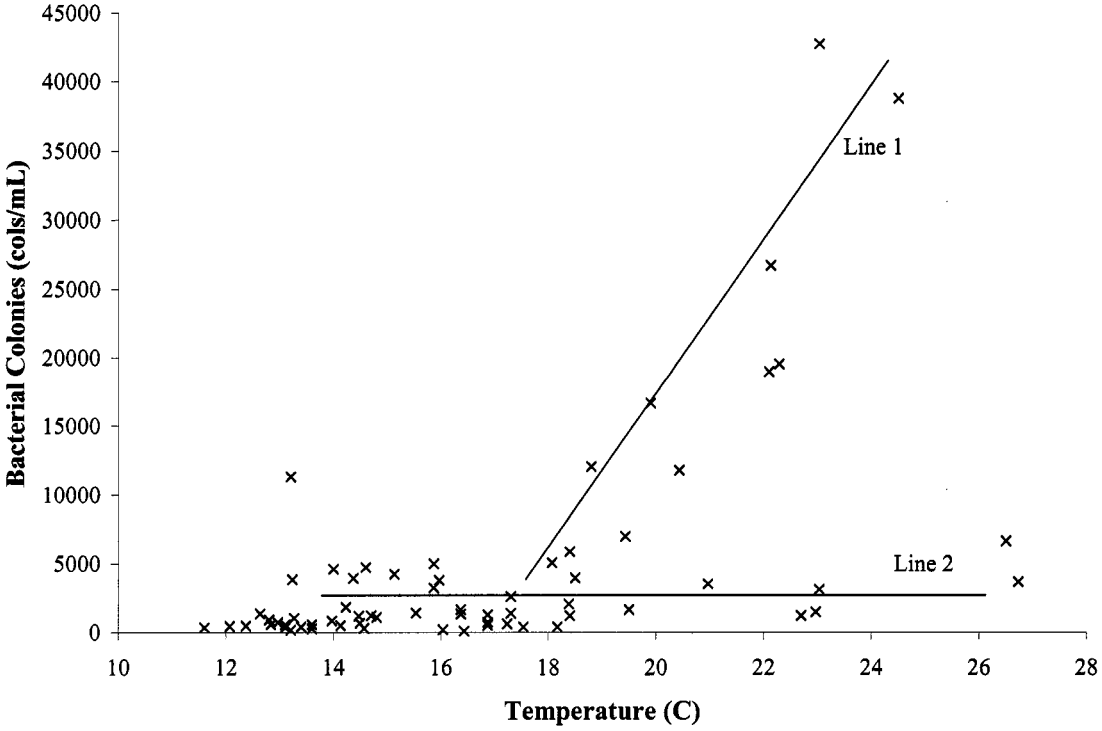

Figure 10. Relationships between bacterial colonies (cols/mL) and temperature (n = 12).

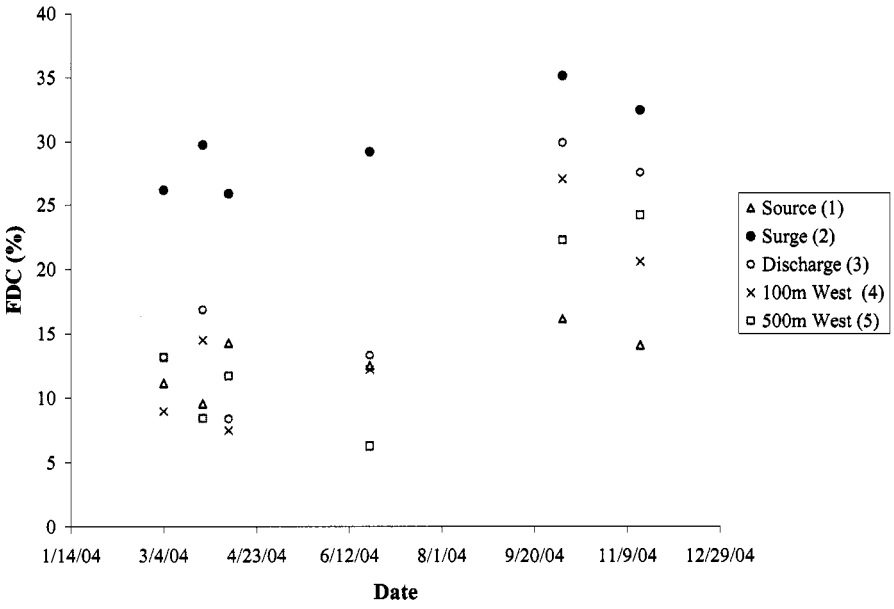

Figure 11. Frequency of dividing cells (FDC %) for each sampling date between 3/4/04 – 11/16/04 (n = 6) at sampling stations (1 - 5).

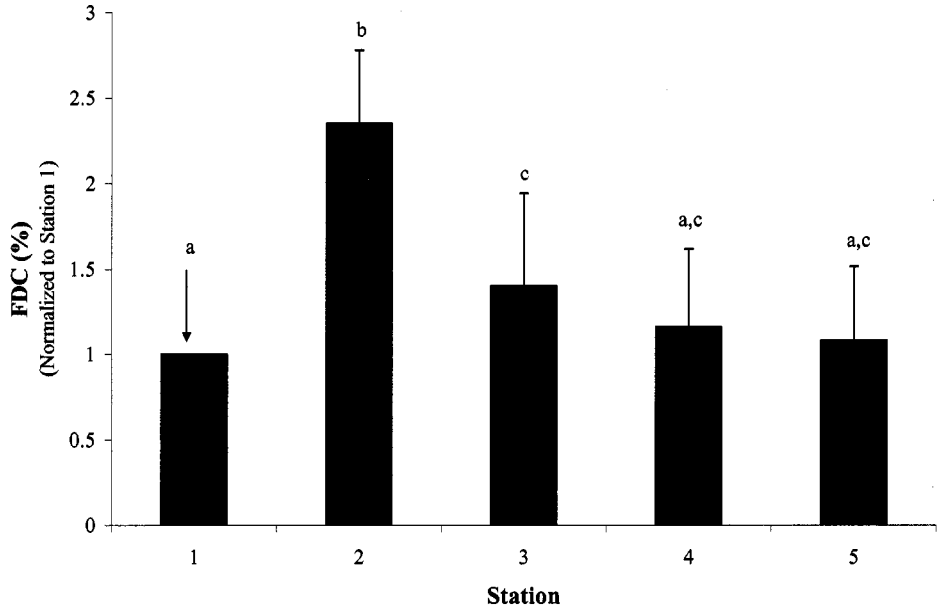

Figure 12. Frequency of dividing cells (FDC % ± SD) at sampling stations (1 - 5) 3/4/04 – 11/16/04 (n = 6). All values normalized to 1.0 in reference to the intake site at Station 1 (identified by arrow) and results of an ANCOVA with Tukey's multiple comparisons test. Different letters above symbols indicate significant differences ($p \leq 0.05$).

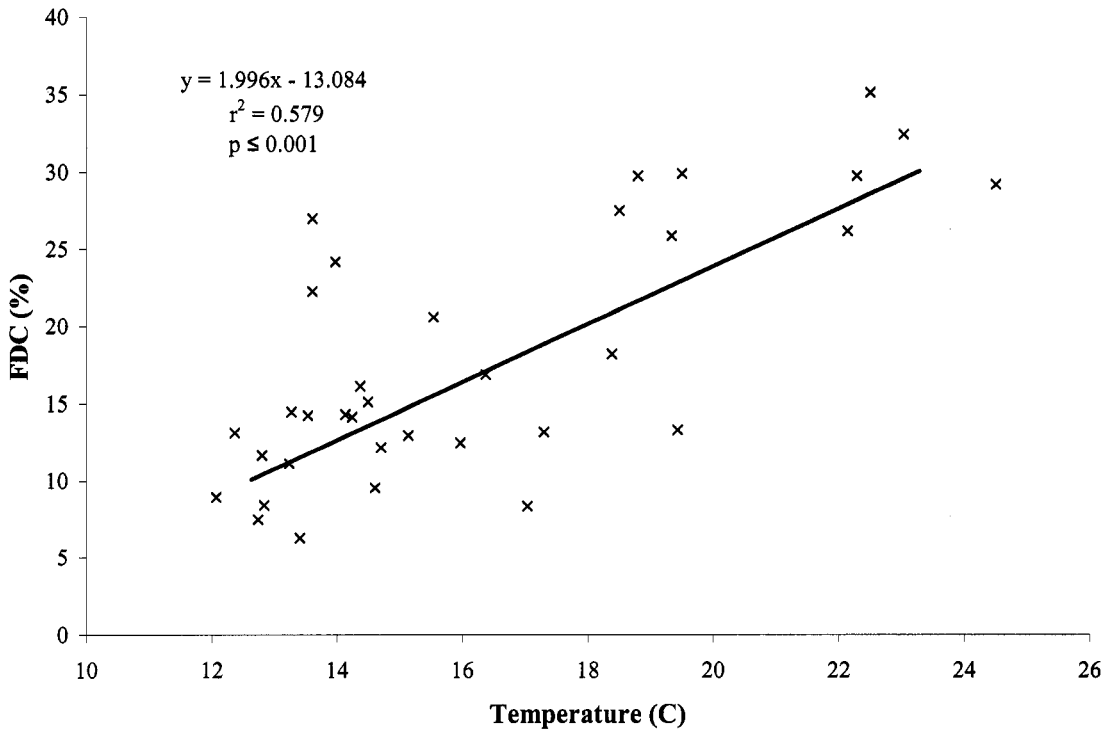

Figure 13. Relationship between frequency of dividing cells (FDC %) and temperature. Linear regression equation is given and plotted (n = 6).

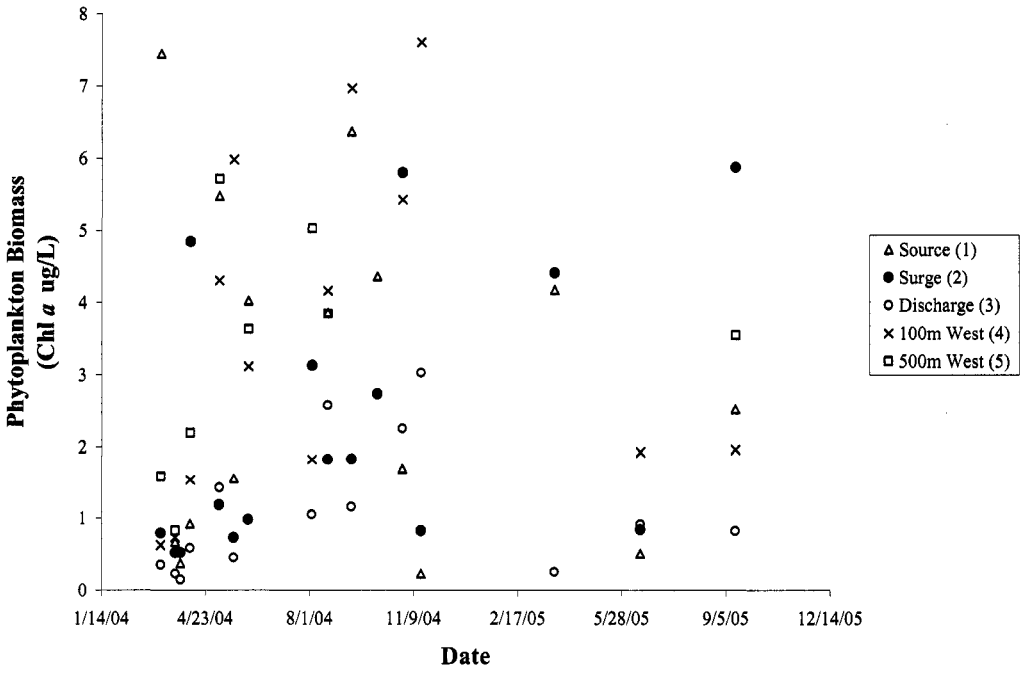

Figure 14. Phytoplankton biomass (Chl *a* µg/L) for each sampling date between 3/11/04 – 11/1/05 (n = 17) at sampling Stations (1 - 5).

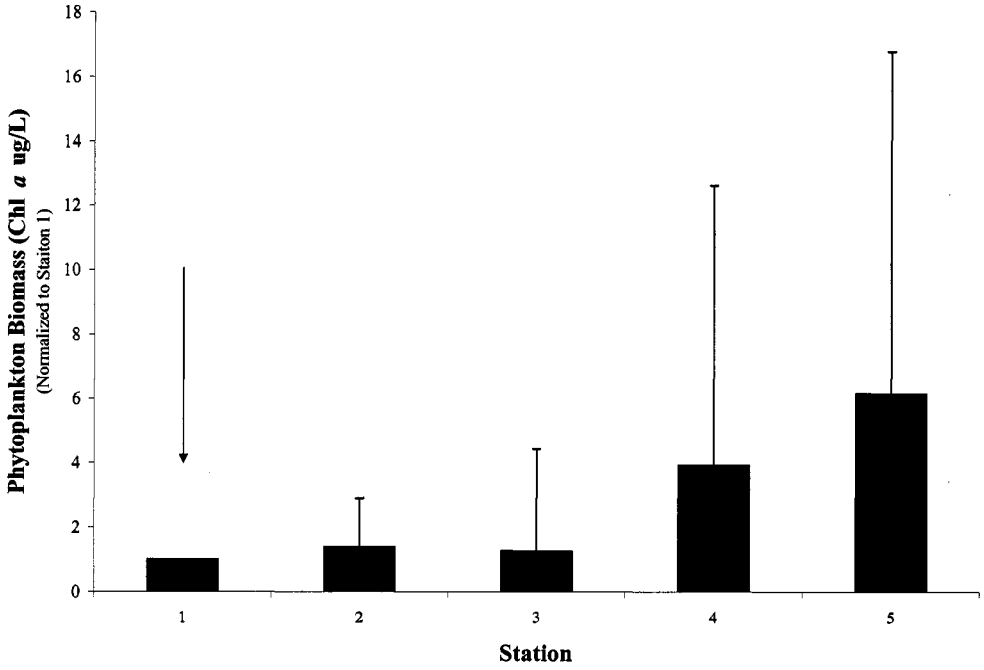

Figure 15. Phytoplankton biomass (Chl *a* µg/L) ± SD at sampling Stations (1 - 5) 3/11/04 – 11/1/05 (n = 17). All values normalized to 1.0 in reference to Station 1 (identified by arrow).

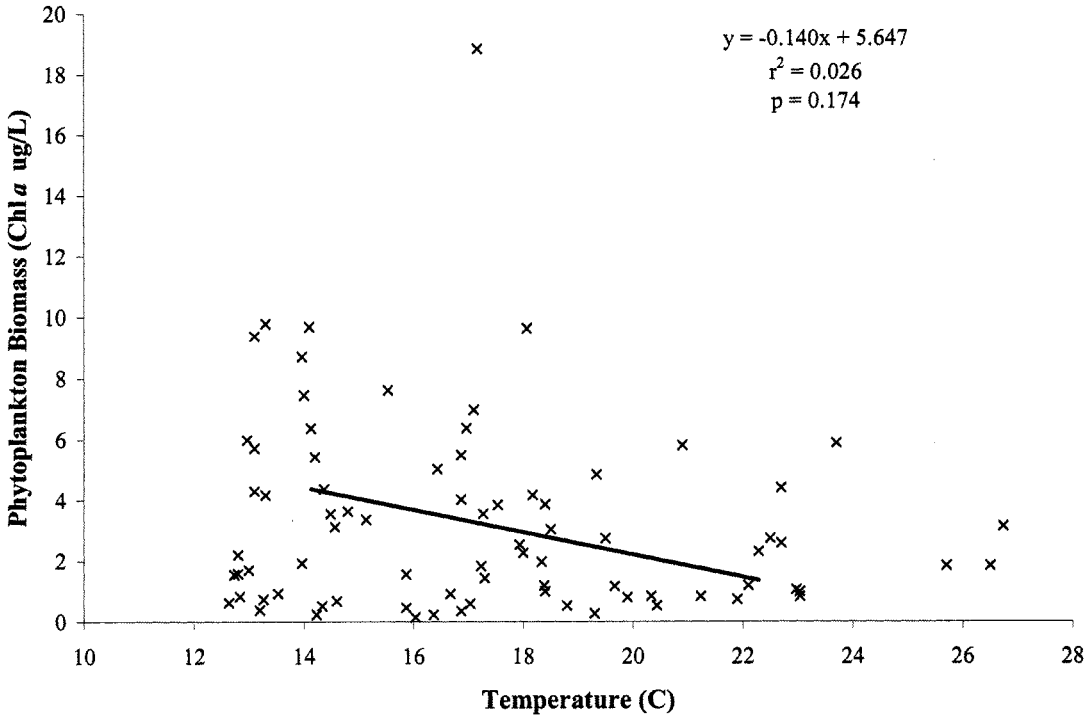

Figure 16. Relationship between phytoplankton biomass (Chl *a* μg/L) ± SD and temperature. Linear regression equation is given and plotted (n =17).

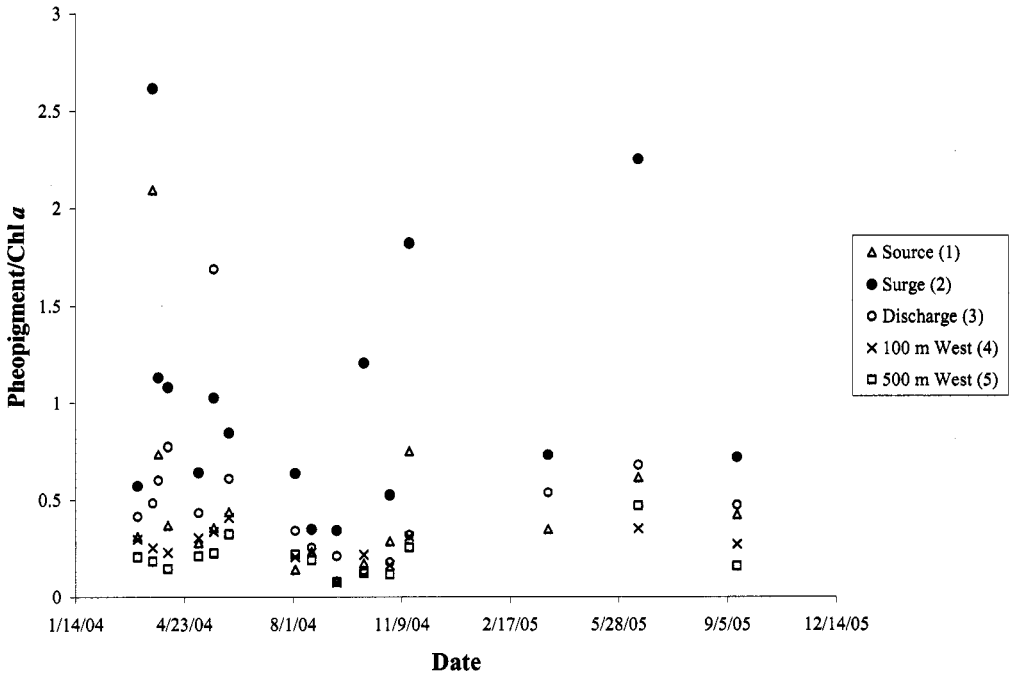

Figure 17. Degradation pigment ratio (Pheopigment/Chl *a*) for each sampling date between 3/11/04 – 11/1/05 (n = 17) at sampling Stations (1 - 5).

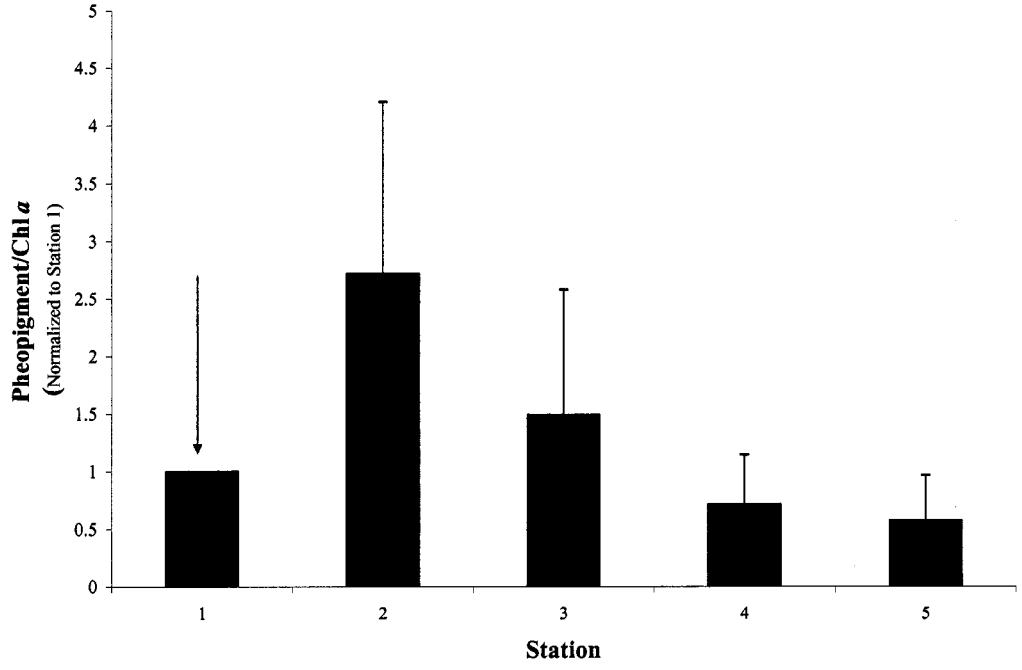

Figure 18. Degradation pigments (Pheopigment/Chl *a*) ± SD at sampling Stations (1 - 5) 3/11/04 – 11/1/05 (n = 17). All values normalized to 1.0 in reference to Station 1 (identified by arrow).

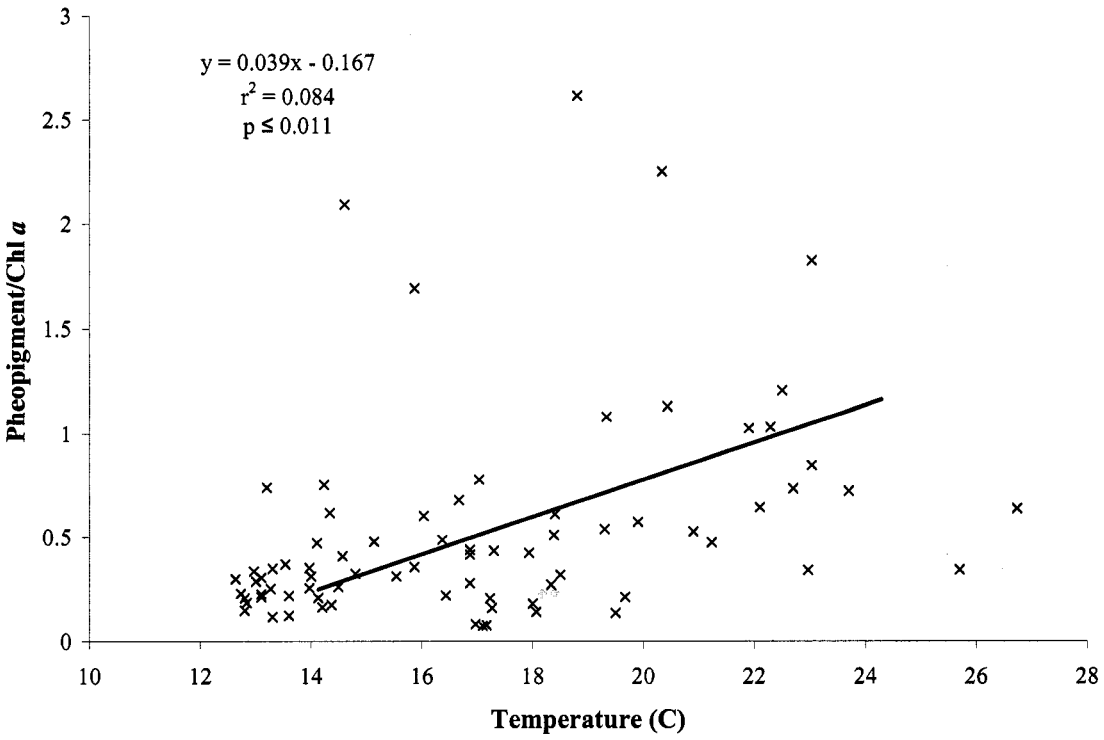

Figure 19. Relationship between degradation pigment ratio (Pheo/Chl *a*) ± SD and temperature. Linear regression equation is given and plotted (n =17).

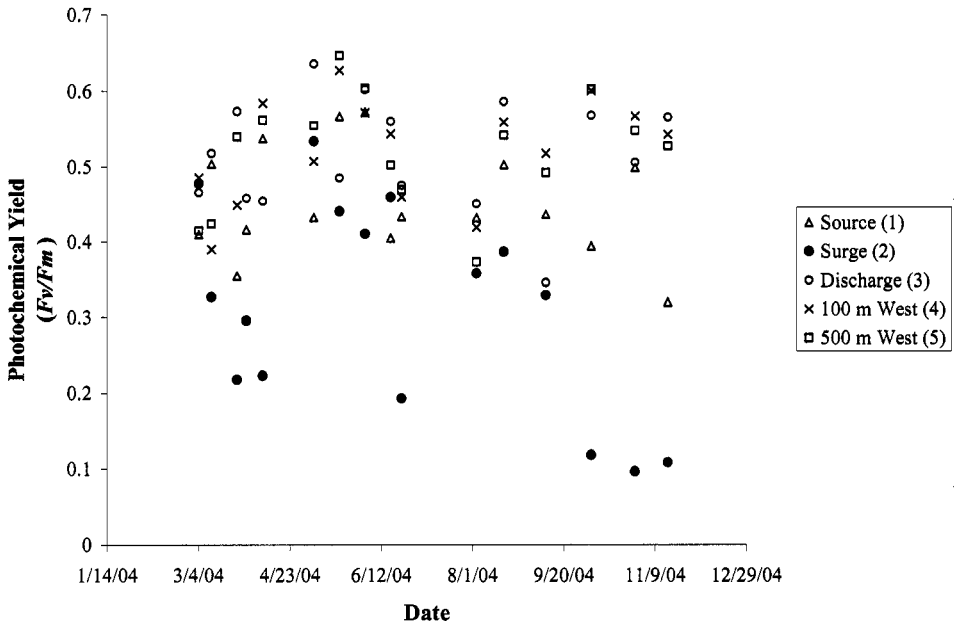

Figure 20. Photochemical yield (*Fv/Fm*) for each sampling date between 3/4/04 – 11/1/05 (n = 20) at sampling stations (1 – 5).

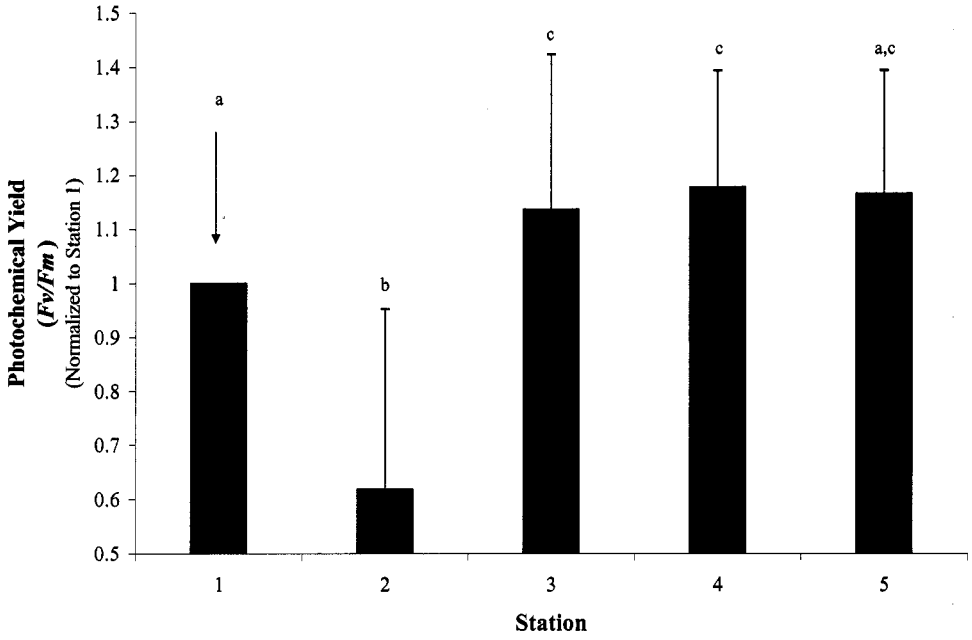

Figure 21. Photochemical yield (*Fv/Fm*) ± SD at sampling stations (1 - 5) 3/4/04 – 11/1/05 (n = 20). All values normalized to 1.0 in reference to the intake site at Station 1 (identified by arrow) and results of an ANCOVA with Tukey's multiple comparisons test. Different letters above symbols indicate significant differences ($p \leq 0.05$).

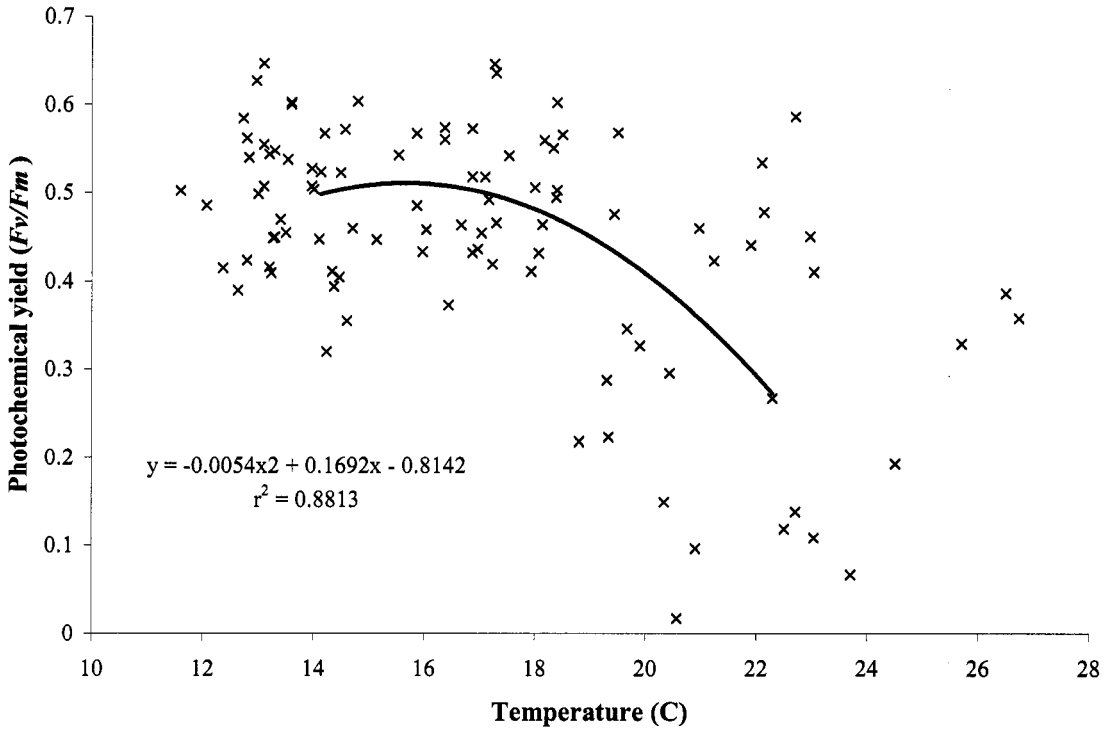

Figure 22. Relationship between photochemical yield (*Fv/Fm*) ± SD and temperature. Polynomial regression equation is given and plotted (n = 20).

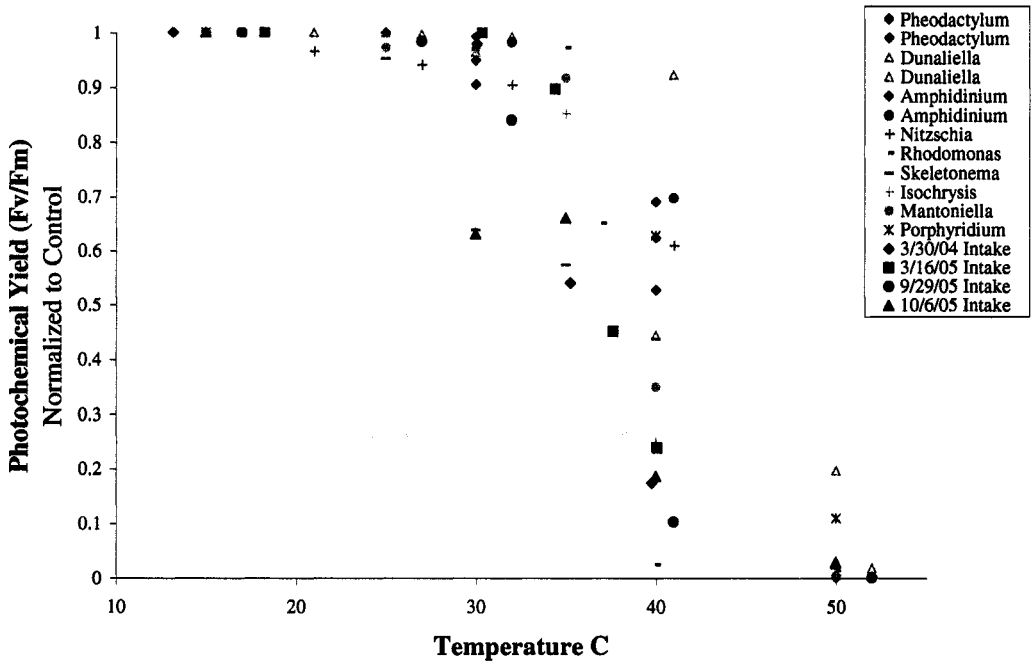

Figure 23. Photochemical yield (normalized to control) of nine phytoplankton cultures and four samples from the intake site (Station 1) exposed to temperature 15 – 50 °C.

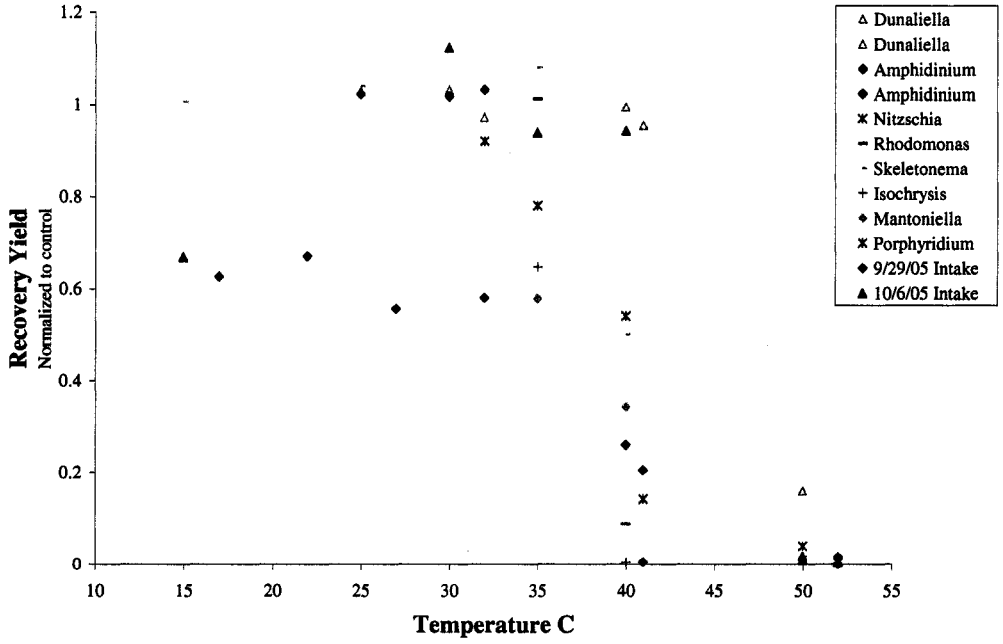

Figure 24. Photochemical yield (normalized to control) of eight phytoplankton cultures and two samples from the intake site (Station 1) exposed to temperature 15 – 50 °C after 24 hour recovery at room temperature in the dark.

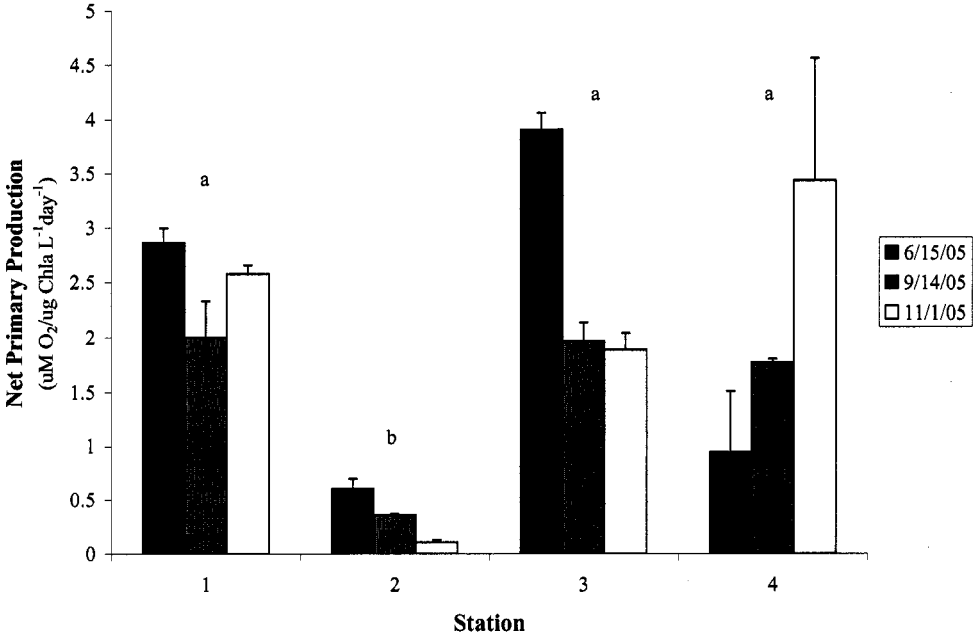

Figure 25. Average net primary production (μM O_2/μg Chl *a* L^{-1} day^{-1}) ± SD at sampling stations (1 - 4) for each sampling date between 6/15/05 – 11/1/05 (n = 3) and results of an ANCOVA with Tukey's multiple comparisons test. Different letters above groups indicate significant differences ($p \leq 0.05$).

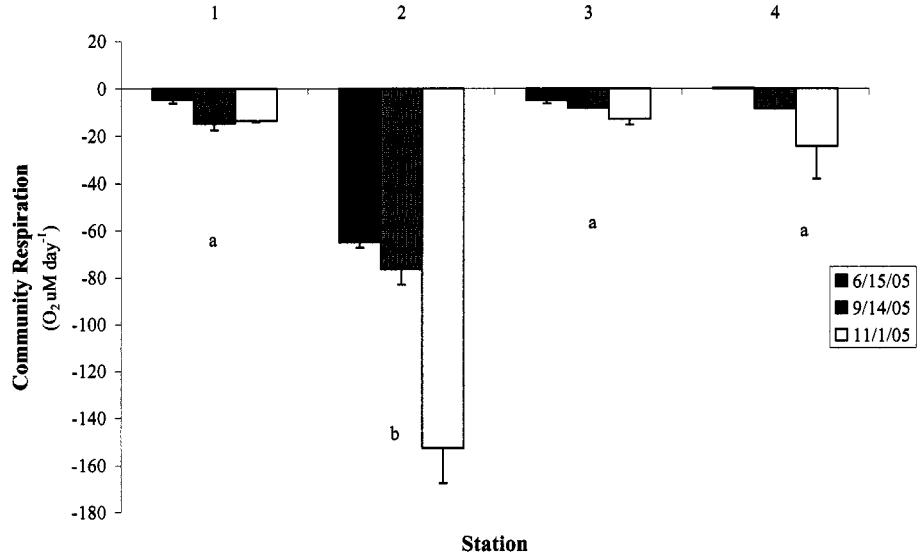

Figure 26. Average community respiration (O_2 μM/day) ± SD at sampling stations (1, 2, 3, 4) for each sampling date between 6/15/05 – 11/1/05 (n = 3) and results of an ANCOVA with Tukey's multiple comparisons test. Different letters above groups indicate significant differences ($p \leq 0.05$).