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Death from below: Investigation of inhibitory factors in bloom development during a wastewater effluent diversion

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ABSTRACT

Eutrophication of coastal waters is an urgent and globally increasing problem. A significant source of nutrients to Southern California coastal waters is direct discharge of secondarily treated wastewater effluent from regional Publicly Owned Treatment Works. The planned diversion of treated wastewater from the Orange County Sanitation District's main (5-mile) pipe to a shallow 1-mile pipe off Huntington Beach, CA in autumn 2012 provided an unprecedented opportunity to monitor the response of the coastal phytoplankton community to a major anthropogenic loading event. Despite the continuous release of approximately 11.07×10^6 m^3 of effluent containing 1743 μM ammonium, there was virtually no detectable change in phytoplankton biomass, in striking contrast to the harmful algal bloom dominated community that quickly developed in response to a comparable diversion in Santa Monica Bay in 2006. Field and laboratory studies demonstrate that disinfection byproducts associated with enhanced dechlorination were present in the discharged water, and that these compounds had a strong inhibitory impact on phytoplankton photophysiology and growth, lasting 24 h for photosynthetic performance and at least 3 d for growth, assessed as change in chlorophyll. Thus, the perhaps fortuitous unintended consequence of enhanced chlorination was the production of inhibitory compounds that suppressed the potential phytoplankton response over a large swath of the continental shelf during the diversion.

1. Introduction

Eutrophication of coastal waters is a well-recognized problem (c.f. Howarth, 2008), perhaps the single greatest problem facing the majority of surface waters today (Smith and Schindler, 2009). Eutrophication has been directly or indirectly linked to increasing frequency of harmful algal blooms (Anderson et al., 2002; Heisler et al., 2008) and coastal hypoxia (Diaz and Rosenberg, 2008). Wastewater discharge is also associated with contamination of coastal waters from metals, pharmaceuticals, and pathogenic bacteria (c.f. Islam and Tanaka, 2004). To combat this, Publicly Owned Treatment Works (POTWs) in large metropolitan regions, such as Southern California, have implemented aggressive regulatory and management practices to minimize potential impacts in the last few decades (Lyon and Sutula, 2011). While significant reductions have been made, Howard et al. (2014) showed that nitrogen loading from POTW effluent discharge in Southern California greatly exceeds riverine and atmospheric contributions, and can be on par with coastal upwelling.

The Orange County Sanitation District (OCSD) is one of four large POTWs that discharges secondarily treated effluent into the coastal ocean in the greater Los Angeles basin. OCSD serves a population of more than 2.6 million residents; OCSD collects, treats, and disposes of sewage from two plants, discharged in the coastal ocean near Huntington Beach, CA. A 120-inch outfall pipe extends 5 miles from the shoreline and delivers approximately 138 million gallons d^{-1} (528×10^6 L d^{-1}) of secondarily treated effluent to a depth of 55–60 m.

In autumn (11 September to 3 October) 2012, OCSD diverted approximately 528×10^6 L d^{-1} of secondarily-treated effluent from their primary outfall pipe to a nearshore (1 mile) 78-inch outfall pipe (Fig. 1) as part of Project J-112: Ocean Outfall Land Section and Ocean Outfall Booster Pump Station Piping Rehabilitation Project.

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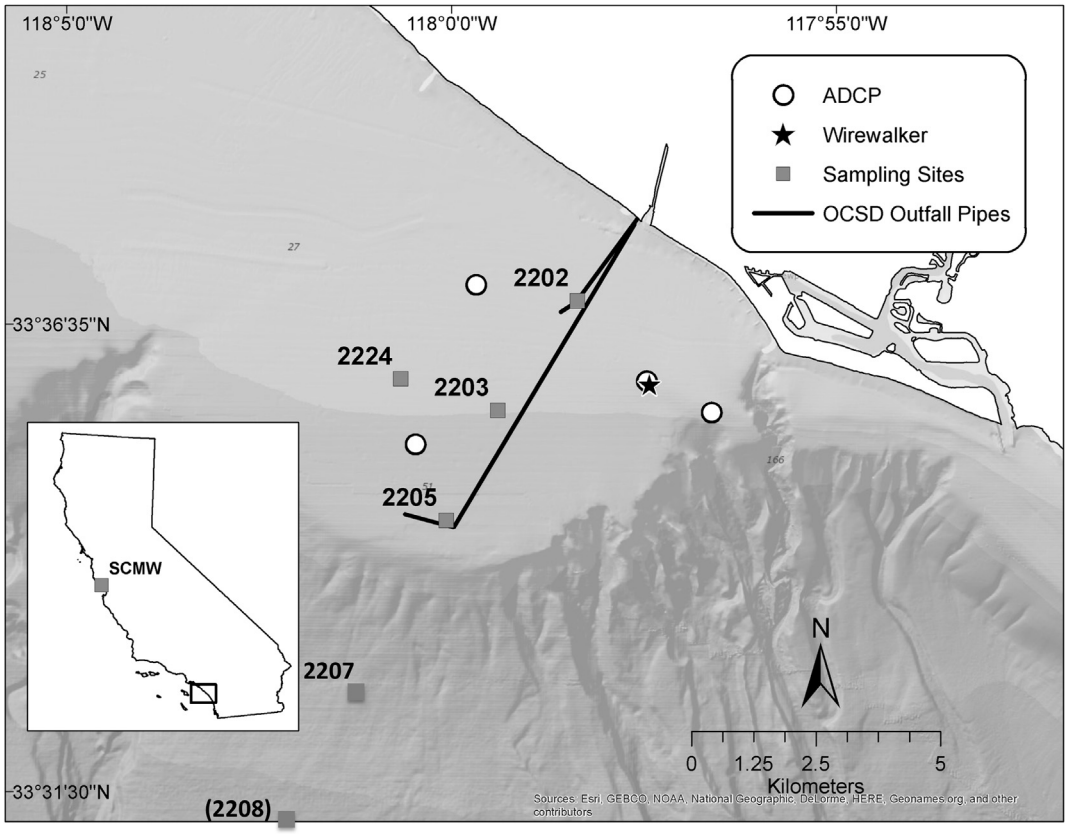

Fig. 1. A map of the study region showing the 1-mile (78-inch) and 5-mile (120-inch) outfall pipes and major stations with shaded bathymetry in the background. Both pipes discharge on the continental shelf, at approximately 16 and 56 m water depth. The shipboard transects shown in Fig. 3 proceeded perpendicular to the coast from Station 2202 to 2208. Note that Station 2208 is located slightly further offshore along the transect line, and was moved onto the map for visualization purposes.

No discharges of this magnitude have been conducted in decades. The most recent comparable example is from November 2006, when the City of Los Angeles diverted the flow from the Hyperion Treatment Plant, its oldest and largest facility, from an outfall five miles from the shoreline to a one-mile outfall. The diversion lasted three days and approximately 800 million gallons of secondary-treated wastewater were discharged off the coast of Santa Monica (Reifel et al., 2013). The biological response was rapid, resulting in large blooms of dinoflagellates, particularly the strong vertical migrators *Akashiwo sanguinea*, and *Cochlodinium* spp., with the vertical migrator *Lingulodinium polyedrum* present in multiple water types including plume, old plume, and stormwater. All of these dinoflagellates show strong growth responses to ammonium (Kudela et al., 2010). Typical ammonium concentrations in the OCSD outflow are 27 mg L^{-1}; given an initial dilution of 180:1 (the minimum diffusion estimated as part of OCSD's National Pollution Discharge Elimination System, NPDES, permit), this represents a continuous supply of ~8 μM ammonium to coastal waters.

Under normal conditions the OCSD effluent remains offshore and at depth with occasional advection to shallower and/or nearshore waters, associated with episodic forcing such as storm events (Boehm et al., 2002; Uchiyama et al., 2014). The OCSD diversion was expected to create a buoyant surface plume that would spread over much of the coastal region given the shallower discharge closer to shore. Model simulations suggest that the plume would penetrate the surface and be confined to the nearshore over the shelf (Uchiyama et al., 2014). The Environmental Impact Report, prior to the diversion, estimated the plume would contain up to 42 μM ammonium, generating a bloom of 40–50 mg m^{-3} chlorophyll (OCSD, 2011). To mitigate the potential threat of fecal indicator bacteria and other pathogens (Noble and Xu, 2004), OCSD employed enhanced chlorination followed by dechlorination of the discharge. In order to reduce the level of fecal indicator bacteria (FIB) in OCSD's final effluent and meet project goals for FIB concentrations at the final effluent sampling station, sodium hypochlorite was introduced with a dosage set point of 5–6 mg/L into selected wastewater streams, primarily at the trickling filter effluent, the activated sludge effluent and the Plant 1 effluent. Chlorine was neutralized prior to discharge by addition of sodium bisulfite. For comparison, under normal operations chlorine is added to 1.5–2.5 mg/L at the same three waste stream locations and neutralized with bisulfite prior to discharge from the 120-inch outfall pipe. Total, Fecal and Enterococci FIB remained below the 1000, 200, and 35 Most Probable Number targets, respectively, when sampled at the final effluent sampling station and prior to discharge.

Based on previous biological responses to both planned diversions (Reifel et al., 2013) and natural loading, due to stormwater runoff (Corcoran et al., 2010; Reifel et al., 2009), and recent estimates of phytoplankton productivity associated with effluent (Howard et al., 2014), a large bloom of phytoplankton, possibly including harmful algae, was expected. In contrast, this diversion resulted in minimal biological response by the plankton community. Here we present data showing that the phytoplankton community was fully capable of utilizing the effluent as a nutrient source, thereby enhancing growth and biomass, and that the

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"missing" bloom was likely due to the perhaps fortuitous production of disinfection byproduct compounds that had a deleterious effect on algal physiology.

2. Materials and methods

2.1. Overview of field sampling

Field sampling was conducted within the Southern California Bight in the vicinity of the Orange County Sanitation District (OCSD) outfall pipes (Fig. 1). OCSD monitored residual chloride in the effluent, flow to, and ammonium at the outfall. Additional discrete samples for nitrate + nitrite, ammonium, dissolved inorganic phosphorus, and silicic acid were sampled on 6 and 20 September and 17 October 2012. Chlorination of the effluent was achieved through addition of 5–6 mg L^{-1} sodium hypochlorite (used as a disinfecting agent) followed by dechlorination with an equivalent (molar) concentration of sodium bisulfite (the neutralizer/quencher). Total chlorine residual, or residual chloride, was determined using a digital colorimeter following method SM 4500-Cl_G (APHA, 1995). When chlorine as sodium hypochlorite is added to wastewater, the chlorine first reacts with organic material and metals. This is referred to as the chlorine demand. The remaining unreacted chlorine is the total chlorine. Residual chlorine (or chloride) is a term used to describe the concentration remaining of total chlorine as measured by method SM 4500-Cl_G, and is reported as the average of measurements over a 24 h period, as per OCSD NPDES permit requirements.

Monthly chlorophyll and sea surface temperature climatologies (2003–2013) and anomalies for the general region (34–35° N, 117.5–118.5° W) were obtained from MODIS Aqua at 4 km resolution from the Giovanni online data system, developed and maintained by the NASA GES DISC.

For this study, a subset of the full field data collected as part of the diversion experiment were used, focusing on an onshore-offshore transect following the diversion pipe for 6, 20 September 2012, 17 October 2012, and 6 November 2012 (Table 1). Underway conductivity (salinity), temperature, and variable fluorescence were measured from a flow-through system aboard the *R/V Yellowfin* and *M/V Nerissa* using surface (<2 m) seawater pumped through a cooler with a YSI 6600v2 CTD sonde and Turner Designs PhytoFlash sampling the flow-through water. The ship tracks followed a line perpendicular to the coast starting at Station 2202 and ending at Station 2208 (Fig. 1). These data were merged with GPS position at 5-min intervals. Vertical profiles of conductivity, temperature, and depth were collected from instrumented rosettes (SBE-911 CTD) aboard the research vessels, processed using standard oceanographic methods. For field experiments, water was collected into acid-cleaned polycarbonate carboys prior to dispensing into acid-cleaned HDPE bottles. To correct for an apparent downward drift in the YSI salinity with time, the YSI data record was adjusted by matching the salinities from the YSI (pumped) and SBE-911 (vertical profile) data at each station for the shallowest available depth from the SBE-911. A constant offset was applied to each cruise, with the offset determined (for each cruise) using the matched salinity data.

Additional laboratory experiments were conducted with surface water collected from the Santa Cruz Municipal Wharf (SCMW; 36° 57.48′ N, 122° 1.02′ W) on 10 June 2013 and 25 March 2014 using a clean plastic bucket. For laboratory experiments, water was transported to the laboratory and experiments were initiated within 24 h of collection.

Water was processed in the field or laboratory within less than 1 h for total chlorophyll, dissolved nutrients (nitrate + nitrite (hereafter referred to as nitrate), dissolved inorganic phosphorus (DIP), silicic acid (Si), ammonium, and urea), and phytoplankton enumeration following standard procedures (cf. Lane et al., 2009).

2.2. Moorings

A combination of moored assets collected observations of the currents, temperature and salinity structure, and the distribution of chlorophyll *a* over the shelf in the vicinity of the outfall. Four bottom-mounted Acoustic Doppler Current Profilers (ADCPs) were deployed for the duration of the experiment. The vertical and temporal resolution of the ADCPs were 1 m and 6 min, respectively. Concurrently, a trio of moored wave-powered profiling vehicles, the Wirewalker (Pinkel et al., 2011), collected profiles of temperature, salinity, chlorophyll fluorescence every ~5 min. ADCP velocity data were used to calculate progressive vector diagrams across the shelf for the 48 h bracketing each sampling event described above. Time series of temperature, salinity, and chlorophyll fluorescence from the Wirewalkers were used to investigate the structure of the plume.

2.3. Grazer-dilution experiments

Water collected in the vicinity of the OCSD outfall pipe on 6 September, 20 September, and 17 October 2012 was amended with f/20 nutrients (Guillard, 1975) with 10 μM ammonium as the nitrogen source. The amended whole water was then filtered through an acid-cleaned Supor 0.2 μm capsule filter. Water was dispensed into 1 L polycarbonate bottles using 100, 20, and 10% whole water. Bottles were incubated with neutral density screening at 50%

Table 1
Locations and environmental conditions for reported sampling sites. The first column indicates the experiment type; Grazer Dilution (field experiment), Amendment (field experiment), Laboratory.

Experiment	Date	Station	Depth (m)	Lat. (° N)	Lon. (° W)	Temp. (° C)	Sal.	NH_4 (μM)	Urea (μM)	NO_3 (μM)	DIP (μM)	Si (μM)	Chl (mg m^{-3})
Grazer Dilut.	6-Sep-2012	2203	15	33.595	117.989	15.37	33.39	0.05	0.68	0.06	0.62	4.36	1.02
Grazer Dilut.	20-Sep-2012	2203	7.5	33.595	117.989	18.74	33.37	0.17	0.33	0.11	0.30	0.19	2.79
Grazer Dilut.	17-Oct-2012	2203	15	33.595	117.989	17.23	33.40	0.22	0.11	0.48	0.45	2.22	1.59
Amendment	6-Sep-2012	2202	5	33.615	117.972	19.10	33.40	0.0	0.60	1.39	0.42	3.46	0.93
Amendment	6-Sep-2012	2203	5	33.595	117.989	19.38	33.45	0.61	2.20	4.24	0.47	1.73	0.28
Amendment	6-Sep-2012	2205	5	33.575	118.000	19.17	33.37	0.0	0.37	0.28	0.47	4.95	1.02
Amendment	20-Sep-2012	2202	5	33.595	117.989	17.68	33.38	0.29	0.26	0.10	0.38	1.18	1.19
Amendment	20-Sep-2012	2203	7.5	33.595	117.989	18.74	33.37	0.17	0.33	0.11	0.29	0.19	2.79
Amendment	20-Sep-2012	2205	8	33.595	117.989	19.10	33.36	0.47	0.19	0.13	0.28	1.18	2.09
Amendment	17-Oct-2012	2203	15	33.595	117.989	17.23	33.40	0.22	0.11	0.48	0.44	2.21	1.59
Amendment	17-Oct-2012	2205	34	33.595	117.989	14.43	33.35	0.40	0.06	1.53	0.69	3.50	0.51
Amendment	6-Nov-2012	2224	24	33.600	118.010	15.08	33.40	2.30	–	1.36	0.16	3.40	4.75
Laboratory	10-Jun-2013	SCMW	0	36.958	122.017	12.9	33.00	1.88	5.11	3.31	1.02	9.62	26.67
Laboratory	25-Mar-2014	SCMW	0	36.958	122.017	13.4	33.10	0.98	4.41	4.77	0.73	11.06	5.54

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ambient light levels at ambient surface water temperature in the harbor at the Southern California Marine Institute in San Pedro, CA. Initial and final chlorophyll samples, collected in triplicate, were used to calculate phytoplankton growth and microzooplankton grazing using a "modified 3-point method" as described by Gallegos (1989) and Worden and Binder (2003). Briefly, the data are fit using linear regression, with the slope providing the grazer mortality term and the intercept providing the phytoplankton growth rate in the absence of grazer mortality.

2.4. Nutrient amendment field experiments

Water was collected from 5 to 35 m depth (the chlorophyll maximum) at a series of stations (2202, 2203, 2205; Fig. 1) along the outfall pipe on 6 September, 20 September, and 17 October 2012. Water was dispensed into 0.25 L polycarbonate bottles and incubated as the grazer-dilution experiments, with sampling of chlorophyll on the initial day and 24 h later. Various amendments were conducted; here we focus on the control (no amendment), ammonium (10 μM ammonium), dissolved inorganic phosphorus (DIP; 10 μM), and f/20 nutrients with ammonium (10 μM) as the nitrogen source. Chlorophyll data were used to estimate net phytoplankton growth rates by difference between the initial and 24-h time point using log-transformed chlorophyll for all experiments.

2.5. Variable fluorescence

In addition to the PhytoFlash data collected from the underway system, discrete samples were analyzed using a Heinz-Walz WATER-PAM. Both instruments were blanked with 0.2 μm filtered seawater. Discrete samples were dark-adapted for 30 min, and then the gain was adjusted on the WATER-PAM. Subsequent samples used the same gain setting, with typically triplicate aliquots of the dark-adapted water analyzed. The initial reading provided variable fluorescence (Fv/Fm, or yield). Subsequent measurements of the same aliquot of water were then subjected to increasing ambient irradiance to calculate the relative Electron Transport Rate (rETR) response curve (Kudela et al., 2008). A more detailed discussion of the methodology and terminology is available in Kromkamp and Forster (2003). Results from the rETR measurements were used to estimate maximum rETR (unitless), E_k (μmol photons m^{-2} s^{-1}) and α (initial slope of rETR versus irradiance) using a hyperbolic tangent function (Jassby and Platt, 1976).

2.6. Effluent laboratory experiments

Raw effluent, sodium hypochlorite (Olin Chlor Alkali Products) and sodium bisulfite (Jones Chemical Inc.) were obtained from the Orange County Sanitation District. For phytoplankton amendments, three treatments were used: effluent, effluent with sodium hypochlorite, and effluent with sodium hypochlorite and sodium bisulfite. Treatments were designed to mimic the typical conditions used by OCSD during the enhanced disinfection employed for the diversion. Sodium hypochlorite was added at a concentration of 5.5 mg L^{-1} using a 12.5% solution (0.69 mg L^{-1} hypochlorite final concentration), and sodium bisulfite was added at a concentration of 4.3 mg L^{-1} using a 25% solution, sufficient to neutralize the hypochlorite. The effluent was held for at least 2 h after addition of hypochlorite before adding bisulfite, and the combined mixture was held for at least 15 min before using as an amendment with seawater samples. For some experiments the effluent plus hypochlorite was held longer to determine the impact of holding time. The treated effluent was then added to seawater at 3% concentration, with Milli-Q water used at the same percentage for non-effluent treatments. The same chemicals were used for separate additions (e.g. hypochlorite and bisulfite were added directly to seawater for some treatments).

The first of a series of laboratory experiments was conducted on 6 November 2012 at the Southern California Coastal Water Research Project Authority (SCCWRP) with seawater collected from 24 m depth (the chlorophyll maximum) at station 2224. There were 12 treatments conducted in triplicate 1 L bottles that included the following: control, sodium bisulfite, sodium hypochlorite, sodium bisulfite and sodium hypochlorite, effluent, effluent with sodium bisulfite, effluent with sodium hypochlorite, effluent with sodium hypochlorite and sodium bisulfite, and 4 effluent 'mimic' treatments, of f/20 nutrients with ammonium (10 μM) as the nitrogen source, with the same amendments as the effluent, sodium bisulfite, sodium hypochlorite, sodium bisulfite and sodium hypochlorite.

The treatments were incubated in an environmental chamber at 150 μmol photons m^{-2} s^{-1} irradiance (12:12 light:dark) at 15 °C and run for 96 h, sampled daily. Chlorophyll *a* samples were collected as described above but were analyzed using a model 10AU fluorometer (Turner Designs, CA) using the acidification method (Parsons et al., 1984). The average chlorophyll concentration was calculated for each treatment, each day, from triplicate bottle replicates and were used to estimate net phytoplankton growth rates from all 5 time points using log-transformed chlorophyll.

Laboratory experiments were conducted with SCMW water on 10 June 2013 and 25 March 2014 at University of California, Santa Cruz. Whole water was incubated in an environmental chamber (15 °C, ~100 μmol photons m^{-2} s^{-1}, 12:12 light:dark) with various treatments including a control, effluent, effluent plus hypochlorite, effluent plus hypochlorite and bisulfite, and f/20 nutrients. Samples from the laboratory experiment conducted on 25 March 2014 were collected for enumeration of heterotrophic bacteria by flow cytometry (Peacock and Kudela, 2012) at the initial time point, 24 and 48 h. All other treatments were sampled from 5 min to 48 h (depending on the experiment) for chlorophyll, variable fluorescence, and rETR curves.

3. Results

3.1. Environmental setting

In preparation for the planned diversion, the Orange County Sanitation District began enhanced disinfection procedures on 4 August 2012, and ended the enhanced disinfection on 2 October 2012. During this period a dosage set point of 5–6 mg L^{-1} chlorine was targeted, with neutralization to less than 1.0 mg L^{-1} prior to discharge. These measurements occur prior to the wastewater sludge being discharged through the diversion outfall pipe, and therefore prior to dilution (minimum of 180:1) within the receiving waters. Diversion to the 78-inch outfall pipe commenced 11 September 2012 and ended 2 October 2012, when discharge resumed at the 120-inch outfall pipe (Fig. 1; Table 2). For the period August–November, ammonium concentrations and discharge rates remained fairly constant, resulting in uniform load (Fig. 2).

Based on discrete nutrient samples collected on 6 and 20 September and 17 October, ammonium accounted for ~72% of the dissolved inorganic nitrogen, with an elevated N:P ratio of 116:1 (molar) within the plume. In contrast, chlorine residuals measured in the effluent were quite variable with maximum instantaneous residuals between 0 and 1.5 mg L^{-1} for several months, including during the diversion. OCSD reported exceedances for maximum daily residual chlorine on three separate occasions during the diversion, and reported that the running median was in exceedance for the entire period of the diversion, although that running median

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Table 2
Summary of relevant parameters and environmental conditions from the Hyperion Treatment Plant (HTP) and Orange County Sanitation District (OCSD) planned diversions. HTP data reported from Reifel et al. (2013) and NPDES reports available as public records.

	HTP	OCSD
Diversion Dates	28–30 November 2006	11 September–3 October 2012
Temperature (° C)	17–18	19.10–19.38
Salinity	33.3–33.45	33.37–33.45
Plume Nitrate (Effluent)[a], μM	4.1 (<1.4)	24.67 (680)
Plume DIP (Effluent)[a], μM	3.2 (97.5)	0.77 (20.8)
Plume Ammonium (Effluent)[a], μM	8.4 (2720)	58.1 (1743)
Pre-Diversion ambient Nitrate (μM)[b]	0.14–1.3	0.60
Pre-Diversion ambient DIP (μM)[b]	0.17–0.32	0.31
Pre-Diversion ambient Ammonium (μM)[b]	–	0.33
Discharge depth (m)	15	15
Total Discharge (m^3)	3.31×10^6	11.07×10^6
Initial Dilution (estimated)	11×	30×
Average Maximum Chlorine Load (kg d^{-1})	114.69	236.0

[a] Maximum reported value from the surface plume (HTP); Effluent values are from 24 h composite (HTP) and 3 d average (OCSD).
[b] As reported in McLaughlin et al. (this issue) for OCSD, based on the 6 September 2014 field survey.

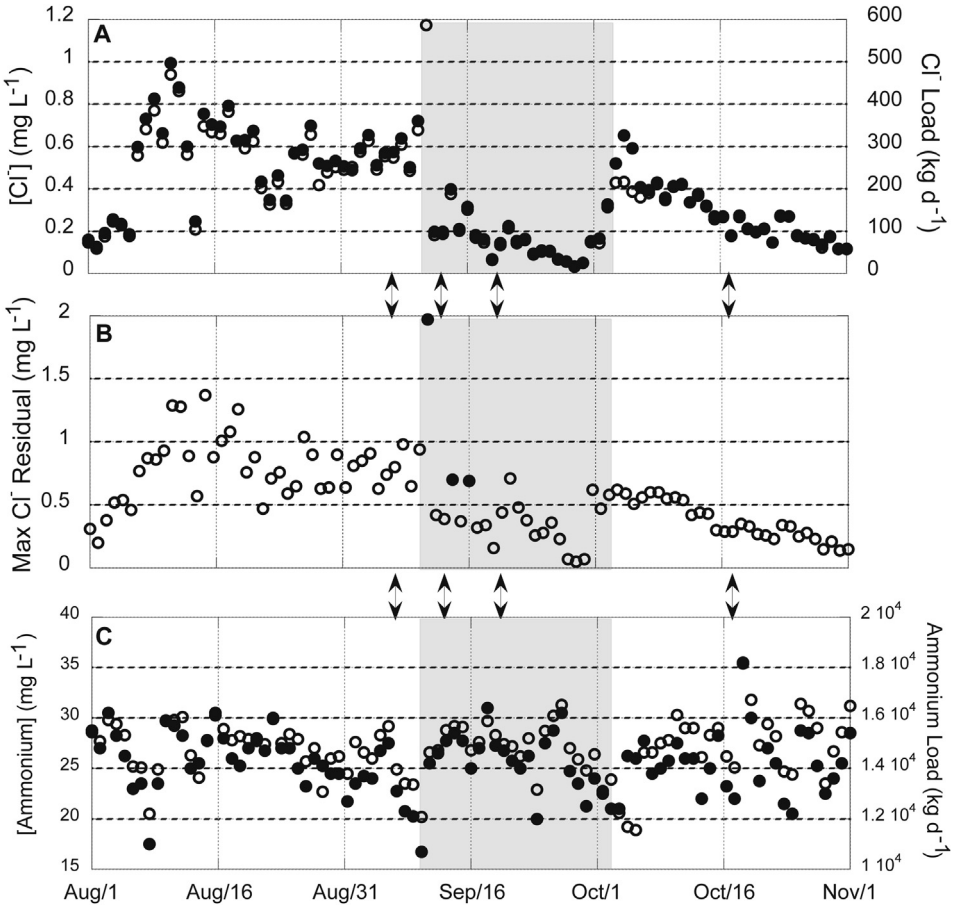

Fig. 2. Wastewater effluent concentrations of residual chloride concentration and load (A), maximum instantaneous chloride residual (B), and ammonium concentration and load (C) for 31 August to 30 November 2012. Grey shading indicates the diversion, and arrows indicate major field sampling dates depicted in Fig. 3. In panel B the solid symbols denote the three days when daily average chloride residuals exceeded permitted levels, while the entire diversion period was in exceedance for the 6-month running median. Enhanced disinfection began 31 July and ended 2 October 2012. The vertical and horizontal lines are provided for reference only.

is normally calculated on a six-month interval and the reported exceedance is therefore not directly related to regulatory guidelines during normal operation. This excess chloride was due, in part, to reduced dilution at the 78-inch pipe. Although chloride residuals are higher when effluent is released from the 120-inch pipe (non-diversion periods in Fig. 2) the greater dilution is assumed to result in lower concentrations in the immediate vicinity of the discharge pipe. The National Pollutant Discharge Elimination System (NPDES) Compliance permit program regulates point sources and OCSD is allowed maximum instantaneous chloride residuals of 10.86 mg L^{-1} and 2.22 mg L^{-1}, and daily maximums of 1.45 mg L^{-1} and 0.296 mg L^{-1}, from the 120-inch and the 78-inch pipes respectively (M. Von Winckelmann, pers. comm.).

Despite the relatively constant N-load, surface nutrient

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concentrations were generally low (McLaughlin et al. this issue; Table 1), but not completely depleted. Chlorophyll in surface waters was similarly low, never exceeding 5 mg m^{-3} in water collected for experiments (Table 1). Phytoplankton composition was relatively constant throughout the experiment with diatoms and dinoflagellates dominant and a background of other phytoplankton at an order of magnitude lower concentration, but with an increase in *Synechococcus* during the diversion (Caron et al. this issue). Consistent with the low ambient nutrients and phytoplankton abundance, the larger region (33–34° N, 117.5–118.5° W) exhibited positive SST anomalies (0.45–1.37 °C) and negative chlorophyll anomalies (0.26–0.56 mg m^{-3} chlorophyll) for August–November. SST anomalies were within one standard deviation (SD) of the climatology except for September (1.37 °C, 2 *SD* from climatology), while chlorophyll anomalies were within 1 *SD* with the exception of August 2012, which exceeded 1 SD.

As discussed in this issue (Seegers et al. this issue) the effluent plume was easily tracked from both the 120-inch and 78-inch outfall pipes using a combined signature of low salinity and elevated colored dissolved organic material (CDOM) absorbance or fluorescence. Temperature was a less consistent proxy, dependent on the prevailing physical conditions, but the plume was generally associated with cooler water and a surface thermal anomaly (Gierach et al. this issue). Underway data from the four dates when we collected the majority of the field samples exhibited a clear signature of lower salinity associated with surface manifestation of plume water (Figs. 3 and 4). This lower salinity water was also associated with lower variable fluorescence.

The surface expression of the plume was variable in space (based on salinity and Fv/Fm). While the discharge from the 120-inch pipe is expected to remain below the pycnocline, low salinity, low Fv/Fm waters were located further offshore along the transect on 6 September (before diversion) and again on 20 September (during the diversion), and centered over the 78-inch outfall pipe (shoreward) on 12 September during the diversion, but also on 17 October after the diversion was terminated. Prior to the diversion, salinity in the region was gradually declining with transient decreases in observed salinity from moorings and shore stations to ~33.1 (Farrara et al., this issue).

It is not clear why the underway mapping data exhibited much lower salinity (Fig. 3). Possibilities include an offset in the instrument calibration that was not corrected by comparison to the SBE-911 salinity, fresher water in the very near surface, or local freshwater sources in the region. Here we focus on the relative spatial patterns along the transects, and interpret the absolute salinity values from the underway mapping data with caution. Progressive vector diagrams from ADCP data collected at several moored sites (Lucas and Kudela, this issue) and WireWalker data demonstrated variable flow patterns (Fig. 4) but consistent retention for at least 48 h on the shelf within the vicinity of the outfall discharge pipes, suggesting that the spatial variability observed in Fig. 3 is consistent with small-scale mixing of plume waters discharged from the 78-inch outfall pipe during the diversion. We attribute the presence of low salinity, low yield waters on 17 October to residual plume waters from the 78-inch outfall pipe since there was no direct evidence for surface expression of wastewater from the deeper, 120-inch pipe (see also Seegers et al., this issue).

Variable fluorescence yield was consistently low in the plume (low salinity) waters, ranging from approximately 0.1–0.4, while outside the plume values were higher, ranging from 0.4 to 0.6. Higher values were associated with both more (20 September) and less biomass (17 October), demonstrating that standing stock and physiological "health" were not tightly correlated along the transects. The relationship between salinity and variable fluorescence for the data presented in Fig. 3 was tested using ordinary least squares regression. Pre-diversion, there was no relationship ($p = 0.63$). For the remaining three dates, there was a noisy but significant ($p < 0.01$) relationship between decreasing salinity and decreasing yield, with the relationship improving as the experiment progressed (6, 20 September and 17 October) as determined by increasing R^2 values of 0.2, 0.3, and 0.5, respectively. Variable fluorescence is quenched by solar irradiance, and the underway data were not corrected for this effect. Absolute values are therefore suppressed relative to dark-adapted values, but sky conditions were consistent for each cruise and there was no obvious correlation between fluorescence yield and time of day or solar irradiance, suggesting that the spatial patterns were associated primarily with water masses rather than light history.

3.2. Phytoplankton growth rates

The initial experimental design for the field program was based on the assumption that the diversion would result in rapid phytoplankton growth as was seen in a previous planned diversion (Reifel et al., 2013). The predicted conditions for the OCSD diversion (based on the 2006 diversion results) were a shallow plume with average ammonium concentrations of 42 μM within the plume and a biological response of up to 40–50 mg m^{-3} chlorophyll (OCSD, 2011). Experiments were therefore designed to assess loss processes (grazer-dilution experiments) and limiting nutrients (amendments), as well as to compare the response of phytoplankton communities at the offshore (120-inch) and nearshore, (78-inch) outfall pipes. The grazer-dilution data showed reasonable reproducibility among replicates (Table 3) but one of the three experiments did not produce easily interpreted results. Salinity and variable fluorescence (Fig. 3) indicated that Station 2203 was not strongly influenced by the plume (as expected since the discharge was at station 2205). Grazing rates were negative, and growth rates were very low but positive. Subsequent experiments on 20 September and 17 October exhibited relatively high growth (μ) and net growth (k) values consistent with the nutrient amendment experiments, given that the grazer-dilution experiments were conducted with f/20 nutrients and 10 μM ammonium as the N source.

Nutrient amendment field experiments conducted on the same days (but at multiple stations and varying depths) suggested that phytoplankton were N-limited with possible secondary limitation by some other nutrient (Table 4). The 6 September experiments showed a positive response to DIP at station 2203 but not at station 2202 or 2205. Additionally, there was a strong response to ammonium at the onshelf stations (2202 and 2203), but not at the offshore (2205) outfall station. Growth rates from other water samples at varying depths (amendments) compared to grazer-dilution data indicated faster growth in the deep chlorophyll maximum (DCM) for 6 September, with net growth rate of 0.53 d^{-1} in the control bottle compared to 0.12 d^{-1} from the grazer-dilution experiment. Comparison of the data from 20 September and 17 October provided variable net growth rates of 0.91 (μ, d^{-1}) and 0.56 (k, d^{-1}) versus −0.03 and 1.43 d^{-1} from the amendments (20 September and 17 October respectively), while nearby station 2202 provided rates of 0.84 and 1.04 d^{-1} for the f/20 amendments on 6 and 20 September respectively.

During the diversion (20 September 2012), there was no obvious difference in growth rates from the nutrient amendments between the short and long outfall pipe stations, despite significant changes in nitrification rates at the offshore location (McLaughlin et al. this issue). Both grazer-dilution and amendment bottles were incubated at 50% light and were collected from the same depth for the latter experiments. Some of the differences between experiments are undoubtedly due to experimental variability, but it is not clear

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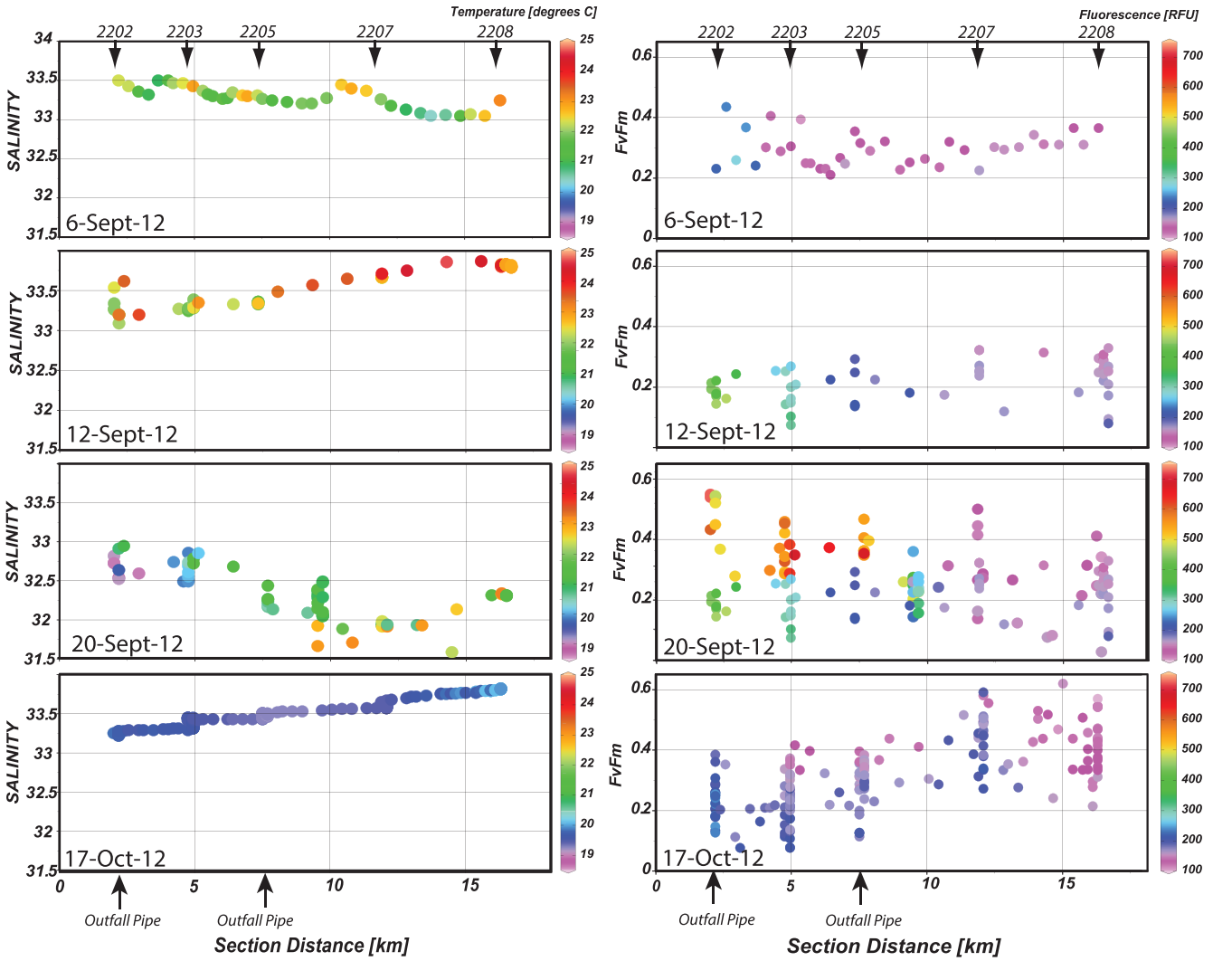

Fig. 3. Salinity and temperature (color shading) in the left panels, and, variable fluorescence and phytoplankton biomass as chlorophyll fluorescence (color shading) in the right panels for the four onshore-offshore transects used for the majority of field sampling. The 1-mile and 5-mile pipe locations are indicated along the bottom axis, and the major stations (see Fig. 1) are indicated along the top axis. Distance is relative to the shoreline. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

why the grazer-dilution experiment exhibited negative grazing for the 6 September experiment.

Growth rates in the presence of added N (as 10 μM ammonium or f/20 with 10 μM ammonium) generally increased, with values ranging from −0.02 to 1.36 d^{-1}. This corresponds to an average 2.5-fold increase in growth relative to the control bottles. Based on these results it was concluded that the phytoplankton assemblage should respond strongly to effluent discharge given the high loads of ammonium (Table 1, Fig. 2). Subsequent experiments focused on identifying potential inhibitors present in the effluent plume.

3.3. Effluent influences on phytoplankton

Preliminary experiments (not shown) and nutrient amendments demonstrated that phytoplankton growth rates, determined from change in chlorophyll, increased with the addition of effluent as expected. Subsequent experiments focused on treated effluent to assess the impact of chloride residuals. The field experiment from 6 November 2012 included treatments with varying combinations of effluent, hypochlorite, bisulfite, and f/20 nutrients (Fig. 5). The effluent plus hypochlorite treatment exhibited significantly decreased (paired t-test, $p < 0.05$) variable fluorescence compared to all other treatments (Fig. 5A). All treatments except effluent plus hypochlorite exhibited positive but insignificant (ANCOVA, $p > 0.05$) increased growth relative to the control (Fig. 5B). Consistent with the field amendments, the phytoplankton responded strongly to nutrient additions as either effluent or as f/20 nutrients. There was no significant response to the presence of hypochlorite alone or in combination with nutrients or bisulfite (two-tailed t-test, $p > 0.05$). In contrast to the other treatments, the hypochlorite plus effluent exhibited a strong and significant decrease in growth (ANCOVA, $p < 0.05$) compared to the effluent, effluent plus bisulfite, effluent plus bisulfite plus hypochlorite, and all nutrient additions, and a nearly significant ($p < 0.1$) decrease compared to the control, bisulfite, hypochlorite, and hypochlorite plus bisulfite treatments.

Variable fluorescence measurements taken at 24, 48, and 72 h show a similar strong negative response to effluent plus hypochlorite (Fig. 6A, the 24 h time point is shown) persisting for 72 h, while other treatments exhibited a gradual increase in variable fluorescence at 48 and 72 h. Estimated $rETR_{max}$ values (equivalent to P^B_{max} for photosynthesis versus irradiance curves) were not

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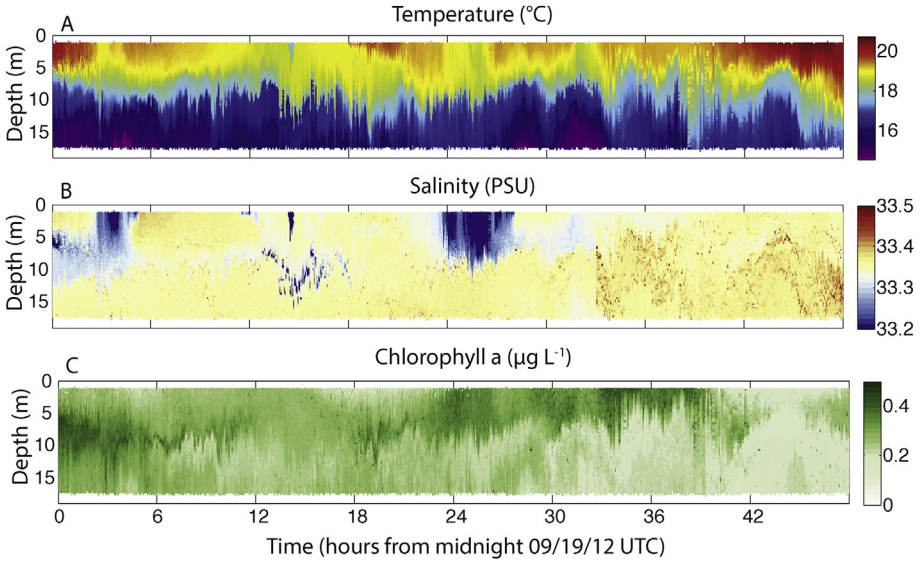

Fig. 4. 48 h of (A) temperature, (B) salinity, and (C) chlorophyll fluorescence data from a Wirewalker profiler deployed on the 18 m isobath 3 km south of the diversion outfall. Low salinity waters associated with the effluent plume regularly impacted the mooring locations. The effluent waters were distributed in the surface or at the pycnocline, and were not associated with elevated chlorophyll fluorescence anomalies. In general, chlorophyll was low in both local shelf waters and low salinity waters.

Table 3
Summary of growth and grazing rates from the grazer-dilution experiments. [*SE*] is the standard error of the linear regression, while growth and grazing are estimated from the slope and intercept of the regression.

	6 September 2012	20 September 2012	17 October 2012
Growth [*SE*] (μ, d^{-1})	0.12 [0.33]	1.19 [0.04]	0.73 [0.10]
Grazing [*SE*] (g, d^{-1})	−0.30 [0.17]	0.28 [0.05]	0.17 [0.07]
Net growth (k, d^{-1})	0.42	0.91	0.56
R^2	0.78	0.78	0.41

Table 4
Chlorophyll and estimated net growth rates for the 24-h nutrient amendment experiments. Stations refer to the locations depicted in Fig. 1. Growth was estimated from change in chlorophyll over the 24-h period. SD represents the standard deviation of replicate chlorophyll samples.

Date	Station	Treatment	Chlorophyll (mg m^{-3}) [SD]	Growth rate (μ, d^{-1})
6-Sep-2012	2202	Initial	0.93 [0.03]	
6-Sep-2012	2202	Control	0.68 [0.04]	−0.317
6-Sep-2012	2202	Ammonium	2.1 [0.26]	0.840
6-Sep-2012	2202	Phosphate	0.75 [0.04]	−0.218
6-Sep-2012	2203	Initial	0.28 [0.01]	
6-Sep-2012	2203	Control	0.48 [0.04]	0.533
6-Sep-2012	2203	Ammonium	0.85 [0.04]	1.11
6-Sep-2012	2203	Phosphate	0.68 [0.39]	0.880
6-Sep-2012	2205	Initial	0.23 [0.06]	
6-Sep-2012	2205	Control	0.24 [0.09]	0.046
6-Sep-2012	2205	Ammonium	0.24 [0.06]	0.039
6-Sep-2012	2205	Phosphate	0.17 [0.01]	−0.314
12-Sep-2012	2202	Initial	1.2 [0.23]	
12-Sep-2012	2202	Control	2.3 [0.79]	0.656
12-Sep-2012	2202	f/20	3.4 [0.96]	1.04
12-Sep-2012	2203	Initial	2.8 [0.81]	
12-Sep-2012	2203	Control	2.5 [0.89]	−0.100
12-Sep-2012	2203	f/20	2.7 [0.21]	−0.026
12-Sep-2012	2205	Initial	5.1 [0.51]	
12-Sep-2012	2205	Control	6.7 [0.71]	0.266
12-Sep-2012	2205	f/20	12 [1.01]	0.898
17-Oct-2012	2203	Initial	1.6 [0.75]	
17-Oct-2012	2203	Control	1.9 [0.65]	0.168
17-Oct-2012	2203	Ammonium	6.2 [0.73]	1.36
17-Oct-2012	2203	f/20	6.6 [1.3]	1.43
17-Oct-2012	2205	Initial	0.51 [0.45]	
17-Oct-2012	2205	Control	0.88 [0.14]	0.544
17-Oct-2012	2205	Ammonium	0.83 [0.31]	0.490
17-Oct-2012	2205	f/20	0.71 [0.37]	0.324

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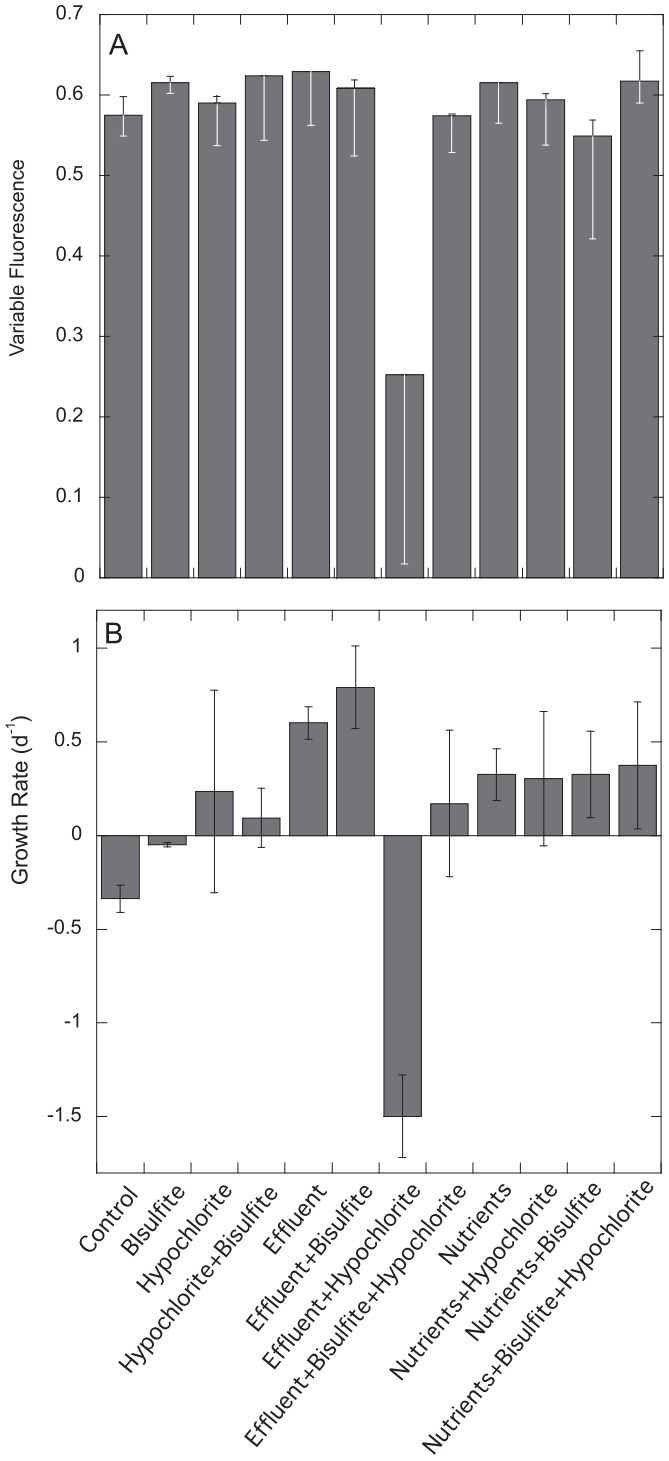

Fig. 5. Variable fluorescence at 24 h (A) and calculated growth rates (B) for the 6 November 2012 field experiment. Error bars in (A) indicate the minimum and maximum yield for 24, 48, and 72 h; error bars in (B) are based on the standard error of the linear regression slope. The hypochlorite + effluent treatment exhibited significantly lower variable fluorescence (t-test, $p < 0.05$) compared to all other treatments, while for growth the hypochlorite + effluent treatment was significantly or nearly significantly lower (ANCOVA, $p < 0.1$) for all treatments (see main text for details).

significantly different for all other treatments, but were reduced 30–70% at 24, 48, and 72 h (Fig. 6A). There was a corresponding decrease in α for the effluent plus hypochlorite treatment ranging from 24 to 75%, while other treatments were not significantly different.

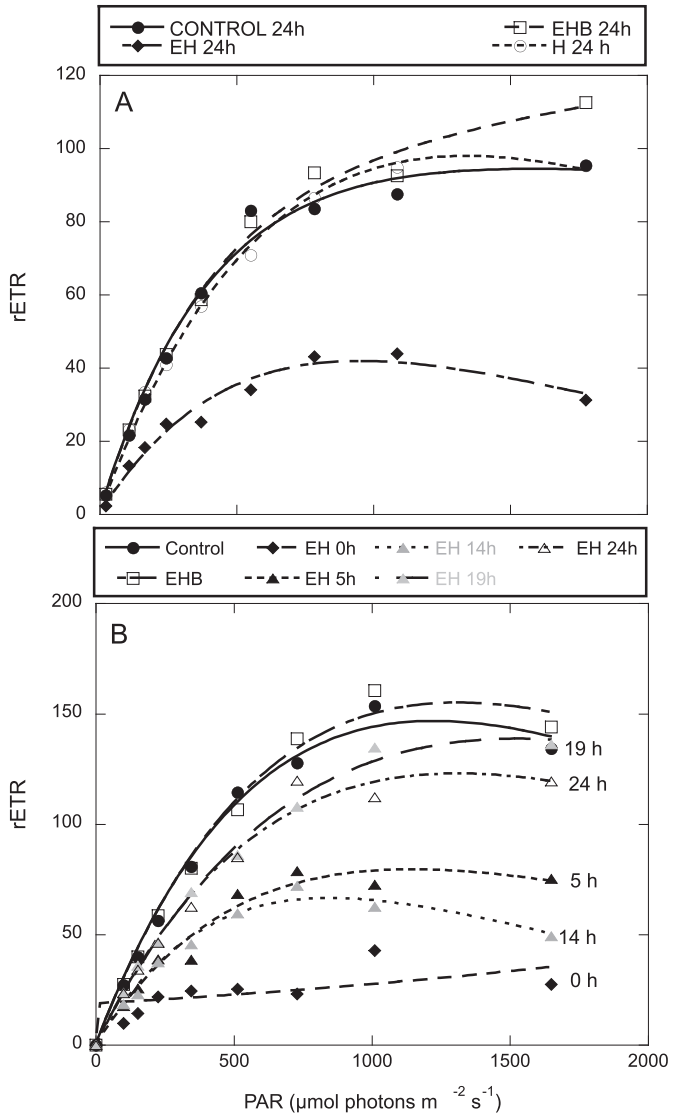

Fig. 6. rETR curves for (A) the 6 November 2012 field experiment and (B) the 10 June 2013 laboratory experiment. H = Hypochlorite; EH = Effluent + Hypochlorite; EHB = Effluent + Hypochlorite + Bisulfite. Panel A provides data from the 24 h time point; Panel B provides data from the time series, with curves annotated.

A series of experiments conducted with water from the Santa Cruz Municipal Wharf corroborated these initial findings. The 10 June 2013 experiment included finer temporal sampling to determine how quickly the negative photophysiological response to effluent plus hypochlorite occurs. There was a gradual recovery of photosynthetic competence as determined from rETR curves conducted for 24 h after exposure to hypochlorite and effluent (Fig. 6B). Separate analysis of variable fluorescence from the treated seawater exhibited a decrease after 4 h relative to the control (but had not decreased at 90 min) and continued to decrease over the first 24 h, followed by recovery at 48 h. Biomass (as extracted chlorophyll) decreased in the control, increased dramatically with the addition of effluent, hypochlorite, and bisulfite, and decreased at a more rapid rate than the control over the 48 h for the effluent plus hypochlorite treatment (Fig. 7). Thus physiological impairment of photosynthesis was alleviated between 24 and 48 h after exposure (faster for aged effluent plus hypochlorite; not shown), but biomass and corresponding growth rate did not show an equivalent recovery.

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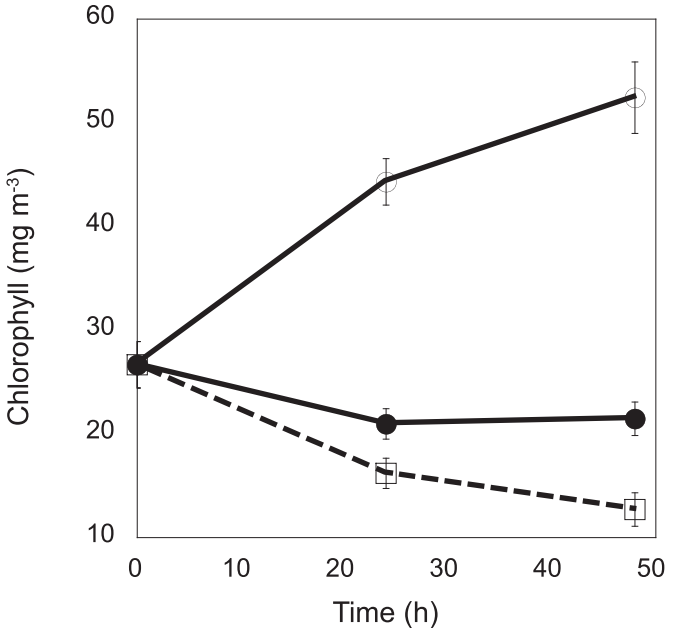

Fig. 7. Chlorophyll biomass at varying time points for the laboratory experiment conducted 10 June 2013. Filled circles are control treatments; open circles are effluent, hypochlorite, and bisulfite treatments; open squares are effluent and hypochlorite treatments. Error bars indicate 1 standard deviation of three replicates.

The latter experiment was repeated on 25 March 2014, but with the inclusion of flow cytometric analysis of heterotrophic bacteria. As with the previous experiment variable fluorescence began to decrease at 4 h, reached a minimum at 24 h, and recovered (but to a lesser extent) at 48 h (Fig. 8). Biomass increased in both the control and effluent plus hypochlorite plus bisulfite treatment, with higher overall biomass at 48 h with the addition of effluent (presumably a nutrient source). In contrast to the phytoplankton, there was no significant difference at 24 and 48 h in heterotrophic bacteria counts between the control and the amended samples (not shown), with or without bisulfite. As part of the last experiment, effluent plus hypochlorite was also mixed and allowed to stand at room temperature for varying amounts of time before being added to seawater. Holding the treated effluent for 5, 19, or 24 h resulted in an average 15% decrease in variable fluorescence relative to the effluent plus bisulfite treatment after 24 h exposure, versus a 69% decrease when using freshly mixed effluent and hypochlorite. Despite the apparent lack of a strong inhibition of photophysiology, biomass still decreased with aged effluent (Fig. 9). Corresponding growth rates increased from 0.43 d^{-1} (control) to 0.54 d^{-1} (effluent plus hypochlorite plus bisulfite), and ranged from −0.32 d^{-1} (fresh effluent plus hypochlorite) to 0.16 d^{-1} (held 19 h), averaging −0.17 (SD = 0.19) d^{-1} for seawater treated with effluent and hypochlorite.

4. Discussion

The planned diversion, by OCSD, of one of the largest point-source discharges in the greater Los Angeles region provided a unique opportunity to evaluate the impact of a large anthropogenic nutrient discharge to the coastal ocean. Following on the planned discharge from the Hyperion Treatment Plant (HTP), the largest POTW in the region, this also served as a comparative study (Table 2). HTP and OCSD are geographically adjacent and the receiving waters respond similarly to mesoscale forcing, but HTP, discharging into Santa Monica Bay, generally experiences more retentive (less advective) flow leading to less rapid dilution of

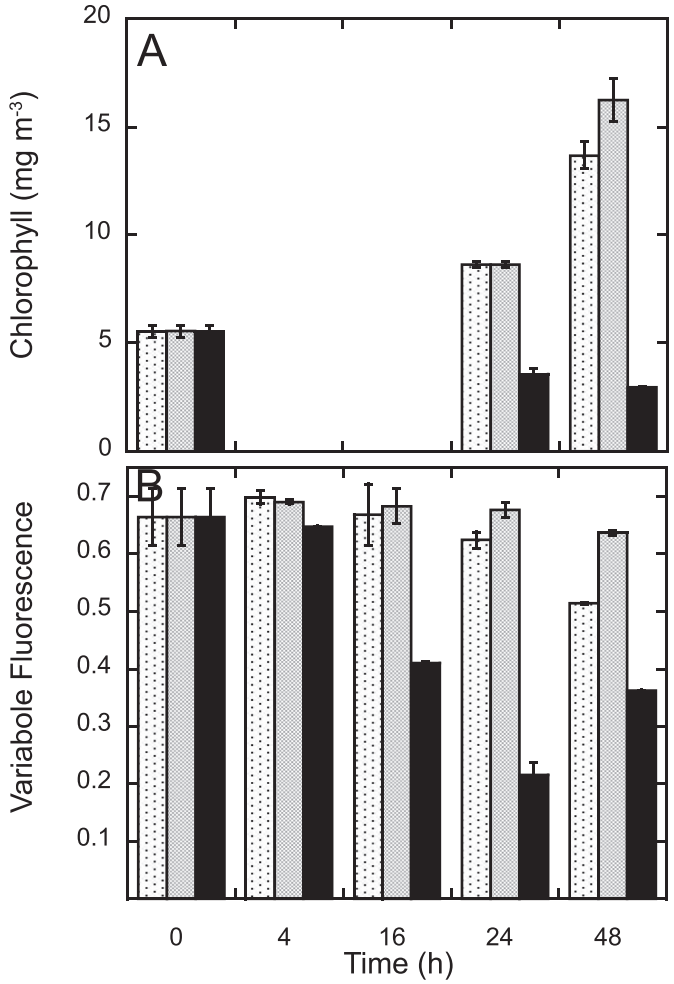

Fig. 8. Variable fluorescence (A) and Chlorophyll biomass (B) at varying time points for the laboratory experiment conducted 25 March 2014. Filled circles are control treatments; open circles are effluent, hypochlorite, and bisulfite treatments; open squares are effluent and hypochlorite treatments. Error bars indicate 1 standard deviation of three replicates (some error bars are within the graphical representation of the time point).

effluent (Uchiyama et al., 2014). Both diversions occurred towards the end of the year (28–30 November 2006 for HTP), but the HTP event only lasted for ~50 h and released 3.31 × 10^6 m^3 of effluent (Reifel et al., 2013). In contrast, the OCSD diversion released approximately 11.07 × 10^6 m^3 of effluent over 21 days. Given the similar time of year, roughly equivalent nutrient concentrations in receiving waters and in the plume (Reifel et al., 2013), as well as longer duration, which would presumably compensate for shorter retention times and more advective flow, an obvious question is why the HTP diversion resulted in a bloom dominated by harmful algae (Reifel et al., 2013) while virtually nothing happened during the OCSD diversion.

4.1. Phytoplankton growth potential

One possibility that would explain the lack of biological response relates to the potential inhibitory effect of high concentrations of ammonium. A series of papers (Dugdale et al., 2012, 2013; Parker et al., 2012) suggest that the Sacramento River and Northern San Francisco Estuary are directly regulated by this suppression of growth, particularly for diatoms, at similar ambient ammonium concentrations (4–10 μM) and that blooms do not

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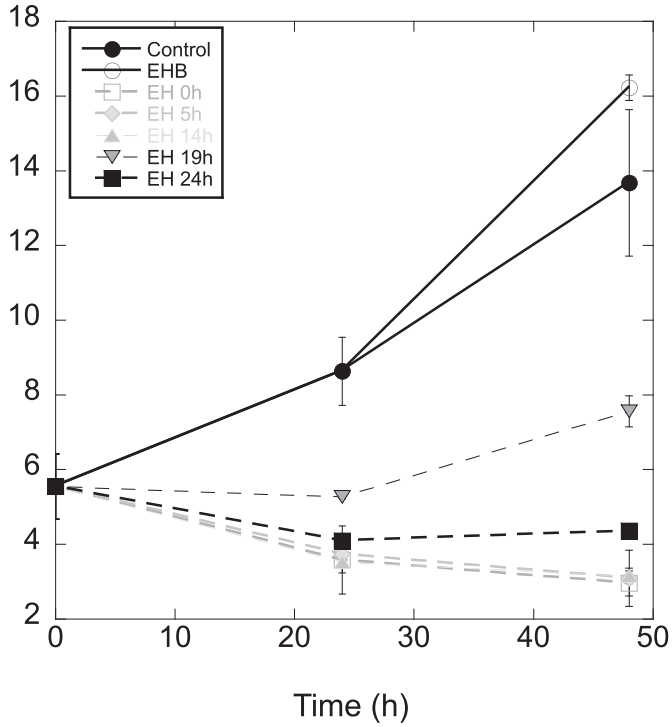

Fig. 9. Chlorophyll biomass at varying time points for the laboratory experiment conducted 25 March 2014. Filled circles are control treatments; open circles are the effluent, hypochlorite, and bisulfite treatment. The other symbols indicate increasing hold time (open square to filled square) with shading indicating increasing hold time. Error bars represent the standard deviation of replicate samples.

occur until ammonium is drawn down below a critical threshold allowing diatoms to resume growth, fueled by nitrate. There are two clear lines of evidence refuting this potential mechanism in the OCSD diversion, where receiving waters were dominated by diatoms. First, short-term nutrient kinetics experiments (Howard et al. this issue) show that ammonium inhibition of nitrate uptake is minimal. Second, 24 h amendment experiments conducted with reasonably high levels of ammonium (10 μM) resulted in rapid net growth rates (Table 4), often exceeding 1 d^{-1}.

Based on a commonly used growth–temperature relationship (Eppley, 1972) and an average temperature of 17.6 °C (Table 1), maximum growth rates should approximate 1.79 d^{-1}, suggesting that net growth rates in the presence of 10 μM ammonium over 24 h were at least 50% of maximum possible growth, not accounting for grazing, light limitation, or other factors that would lower the achieved maximum growth rate. These rates are consistent with, and higher than, growth rates from further offshore (Landry, 1995, 2009) and from the nearshore environment (Omand et al., 2012). Growth rates were similar with the addition of 10 μM ammonium and f/20 nutrients, or with just 10 μM ammonium, while addition of DIP resulted in variable (by station) response. At several stations growth was also positive for the control bottles (Table 4). Taken together, these results suggest that phytoplankton had the potential to respond positively to nutrients delivered with the effluent and were not negatively inhibited by high concentrations of ammonium. This is supported by the maximal growth rates on effluent or effluent + bisulfite seen in the multi-day amendment experiment conducted in November (Fig. 5).

A second possibility that could explain the lack of biological response to the effluent is that grazers were capable of modulating the phytoplankton response. Results from a limited set of experiments clearly refute that, with grazing rates much lower than growth rates (Table 3). If microzooplankton grazing were controlling the phytoplankton response we would expect suppression of net growth in the 24 h amendments and the multi-day grow-out (Table 4, Fig. 5). Supporting evidence for a lack of grazer control is the lack of response by grazers identified by microscopy (Caron et al. this issue).

4.2. Disinfection byproducts as inhibitors

Chlorine and residual chloride have long been recognized as potential inhibitors of marine phytoplankton photosynthesis (Eppley et al., 1976 and references therein), but early studies suggested that their use in wastewater treatment was not impairing phytoplankton photosynthesis, due to the rapid dilution in seawater (SCCWRP, 1973; Thomas et al., 1974). In laboratory studies, Eppley et al. (1976) reported that the inhibitory effects of chlorine were enhanced in the presence of 30 μM ammonium, reaching 50% inhibition over 24 h with 0.01 mg L^{-1} chlorine (as hypochlorite). Additional experiments showed that "aged" water was still inhibitory even when residual chloride was not detectable, but filtered, aged water was not inhibitory so long as residual chloride was again not detectable. The authors concluded that chlorine (and equilibrium formation of hypobromous acid and hypobromite in seawater) reacted with ammonium and organic matter to form a "bewildering array of products (Jolley, 1973)" (cited from Eppley et al., 1976).

Subsequent work confirmed the formation of bromine oxidants and suggested that chloride residuals are a poor proxy for dose-organism response in the marine environment, because other unknown and potentially inhibitory compounds were being produced (Goldman et al., 1979). Today it is recognized that chlorination of natural waters forms a suite of compounds, with trihalomethanes and haloacetic acids the most prevalent (Hua and Reckhow, 2007), including many newly detected brominated compounds in saline sewage effluents (Ding et al., 2013). Agus et al. (2009) noted that phytoplankton are particularly sensitive to haloacetic acids and that brominated compounds produced by chlorination of seawater would presumably be similar to chlorinated compounds. The US EPA has strict guidelines for nine of these disinfection byproducts (DBP) because of concerns for human health (USEPA, 2006) but the list of known DBP continues to grow (Krasner et al., 2006; Ding et al., 2013).

Since this early work, there has been more of a focus on inhibitory effects on marine phytoplankton resulting from chlorination of power plant cooling water (Abarnou and Miossec, 1992; Jenner et al., 1997; Choi et al., 2002; Poornima et al., 2006; Ma et al., 2011). Many of these DBP exhibit mutagenic and carcinogenic properties (Leenheer and Croué, 2003; Richardson et al., 2007) and there has been increasing interest in production and occurrence of DBP in desalination systems (Agus et al., 2009), wastewater treatment plant effluent (Krasner et al., 2009; Shahidul and Tanaka, 2004), drinking water (Richardson and Postigo, 2012), swimming pools (Zwiener et al., 2007; Weaver et al., 2009), ballast water treatment (Werschkun et al., 2012), and rivers (Chow et al., 2007; Kraus et al., 2010). Despite this interest and chemical characterization of DBP and precursor chemicals, relatively little emphasis has been placed on production and discharge of these compounds to coastal waters from wastewater treatment plants.

Coincident with the increasing interest in DBP, has been the routine use of chlorophyll fluorescence assays as a means of determining ecophysiological and toxicological responses to metals, herbicides, and other environmental contaminants (see review by Kumar et al., 2014) because it provides a rapid, non-invasive, and sensitive indication of cell "health". Variable fluorescence provides a general indicator of phytoplankton physiological

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status and ranges from 0 (senescent or dead) to ~0.7 (maximal value for phytoplankton); it can also be used to describe the photosynthesis-irradiance response (e.g. Kromkamp and Forster, 2003). It is a sensitive indicator of nutrient stress (e.g. Beardall et al., 2001) and is often used as an ecotoxicological assay for various metals, petrochemicals, and herbicides (Kumar et al., 2014; Ralph et al., 2007).

Of relevance to this study, Ma et al. (2011) examined the impact of residual chloride on the marine diatom *Phaeodactylum tricornutum* using a PAM fluorometer system to monitor changes in yield and rETR. They reported a sharp decrease in yield and rETR at chloride concentrations of 0.4 mg L^{-1}. In contrast, we saw no significant change in yield or rETR parameters (Fig. 6A) for the hypochlorite treatment from 6 November 2012 (nominally 0.69 mg L^{-1} but this did not account for the chloride demand from the ambient organic material in the seawater). Under typical discharge conditions, it is unlikely that residual chloride, despite the relatively high concentrations in the concentrated effluent (Fig. 2B), had any direct impact on the phytoplankton in receiving waters. However, when hypochlorite was mixed with effluent, there was a consistent decrease in both yield and rETR parameters (Figs. 5–8). This is consistent with the production of DBP with toxic or inhibitory effects on phytoplankton photosynthesis independent of residual chloride concentration.

Inhibition of yield occurred at least 4 h after addition of the effluent plus hypochlorite (Fig. 7), and the photophysiological response could be neutralized by addition of bisulfite or by holding the effluent plus hypochlorite for several hours. However, while the fluorescence response recovered, effluent plus hypochlorite continued to inhibit biomass accumulation (growth) when the effluent plus hypochlorite was held for up to 24 h before addition to seawater (Fig. 9). This is consistent with other studies that showed inhibition of chlorinated estuarine water after aging for 24 h (Eppley et al., 1976) and between 10 and 35 d (Sanders, 1984).

We did not directly measure DBP or residual chloride in receiving waters around the outfalls, but several lines of evidence suggest that inhibitory compounds were present long enough to impact the phytoplankton in the region. Surface mapping of variable fluorescence (Fig. 3) shows a consistent relationship with low yield associated with lower salinity water. Progressive vector diagrams (Lucas and Kudela, this issue) and modeling studies (Uchiyama et al., 2014) suggest that compounds released with the effluent would be retained on the shelf for several days. Addition of effluent or nitrogen as ammonium to seawater samples during amendment experiments exhibit positive growth, while the general region surrounding the outfalls were anomalously low in biomass and productivity during the study period. And finally, there was evidence for suppressed yield (Fig. 3) both before the diversion (but after initiation of enhanced chlorination), and after the diversion; the continued association between lower salinity waters and reduced yield suggests that either DBP were still being produced after the diversion, or that the inhibitory compounds were still present 15 days after the effluent was diverted back to the 5-mile pipe.

While limited, the results from the 25 March 2014 laboratory study suggest that the inhibition of phytoplankton in the presence of DBP was not seen for the heterotrophic bacteria community. At 24 and 48 h there was no significant decline in bacterial abundance; counts were actually enhanced at 24 h relative to the control and effluent, hypochlorite, and bisulfite treatments. This may partially explain the apparent paradox of the 2012 diversion, with large quantities of ammonium released and retained over the coastal shelf but with no phytoplankton bloom. If the heterotrophic bacteria were less impacted, then biological utilization and biogeochemical conversion could conceivably account for the "missing" nitrogen (McLaughlin et al., Caron et al., this issue).

4.3. Ecological and management implications

Returning to the comparison between the HTP and OCSD diversions, we propose that the most consistent explanation for the dramatically different biological response is the formation of DBP at higher than normal rates due to enhanced chlorination, combined with the surface expression and retention of the plume at the shallower coastal site. This leads to the conclusion that production of DBP may have mitigated any phytoplankton response to the diversion, including a potential harmful algal bloom, seen in the HTP study (Reifel et al., 2013), but increased the risk of exposure to toxic, mutagenic, and carcinogenic compounds. OCSD implemented enhanced chlorination to mitigate potential impacts of fecal indicator bacteria and associated pathogens. While this was successful, with FIB counts remaining below State of California water contract standards during the diversion (Rogowski et al., 2014), it clearly had an unintended impact on biomass and productivity of receiving waters. As has been recommended elsewhere (e.g. Kumar et al. 2014), the use of chlorophyll fluorescence may provide a rapid and sensitive monitoring tool to assess the impact of the potentially large suite of disinfection byproducts released from wastewater treatment plants from the chlorination process on the phytoplankton assemblage. Management of future planned diversions as well as normal operations for wastewater treatment plants should take into account the potential impact of DBP on receiving waters, especially because monitoring of residual chloride likely provides a poor indication of impacts on the phytoplankton community.

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