**Evaluation of uptake kinetics during a wastewater diversion into nearshore coastal waters
in southern California.**

Raphael M. Kudela^{a*}, Meredith D. A. Howard^b, Kendra Hayashi^a, Carly Beck^b

^aUniversity of California, Santa Cruz, Ocean Science Department, 1156 High Street, Santa Cruz,
CA USA

^bSouthern California Coastal Water Research Project, Biogeochemistry Department, 3535
Harbor Blvd. Suite 110, Costa Mesa, CA, USA

*Corresponding Author: kudela@ucsc.edu, 831-459-3290

15

16 **Abstract**

17 The global eutrophication of coastal ecosystems from anthropogenic nutrients is one of the most
18 significant issues affecting changes to coastal oceans today. A three-week diversion of
19 wastewater effluent from the normal offshore discharge pipe (7 km offshore, 56 m depth) to a
20 shorter outfall located in 16 m water (2.2 km offshore) as part of the 2012 Orange County
21 Sanitation District Diversion provided an opportunity to evaluate the impacts of anthropogenic
22 nitrogen on phytoplankton community response. Nitrogen uptake kinetic parameters were used
23 to evaluate the short-term physiological response of the phytoplankton community to the
24 diverted wastewater and to determine if potential ammonium suppression of nitrate uptake was
25 observed. Despite expectations, there was a muted response to the diversion in terms of biomass
26 accumulation and ambient nutrients remained low. At ambient nitrogen concentrations,
27 calculated uptake rates strongly favored ammonium. During the diversion based on the kinetic
28 parameters determined during short-term experiments, the phytoplankton community was using
29 all three N substrates at low concentrations, and had the capacity to use urea, then ammonium,
30 and then nitrate at high concentrations. Ammonium suppression of nitrate uptake was evident
31 throughout the experiment, with increasing suppression through time. Despite this interaction,
32 there was evidence for simultaneous utilization of nitrate, ammonium, and urea during the
33 experiment. The general lack of phytoplankton response as evidenced by low biomass during the
34 diversion was therefore not obviously linked to changes in uptake rates, physiological capacity,
35 or ammonium suppression of nitrate uptake.

36

37

38

39 Key words: wastewater effluent, nitrogen, phytoplankton, nitrogen isotopes, ammonium

40 compounds, nitrogen uptake

41

42

43

44 **1. Introduction**

45 The contributions of anthropogenic nitrogen loads to the eutrophication of coastal
46 systems has been well documented (see reviews, Howarth 2008; Paerl and Piehler 2008) and is
47 considered one of the most globally important human-accelerated changes to coastal oceans
48 (Howarth and Marino 2006; Scavia and Bricker 2006). Anthropogenic nutrient inputs have been
49 linked to increased primary production and algal blooms (Lapointe et al. 2004; Beman et al.
50 2005), and are considered the most significant factor contributing to the increased frequency of
51 harmful algal blooms (HABs) (Anderson et al. 2002; Hallegraeff 2004; Glibert et al. 2005;
52 Heisler et al. 2008). Most coastal eutrophication studies have focused on nitrogen (N), since it is
53 the primary macronutrient that limits the growth of phytoplankton in coastal waters (Ryther and
54 Dunstan 1971; Eppley et al. 1979). The form of N is also important in the stimulation of some
55 algal species responsible for HABs (Glibert et al. 2006), including in California (Kudela et al.
56 2010). Upwelling dominated systems have generally been perceived to be less affected by
57 anthropogenic nutrients due to the sheer magnitude of natural (upwelled) nutrients as well as the
58 highly dynamic conditions making these systems potentially more resilient. However, a growing
59 number of studies have suggested that our perception of the resilience of these systems may be
60 flawed (c.f. Capone and Hutchins 2013). The large quantities of anthropogenic nutrient sources
61 in the Southern California Bight (SCB), mainly from wastewater treatment plants and
62 agricultural activities, have sparked a series of studies focused on the impacts and effects of
63 anthropogenic inputs on coastal ecosystems. Anthropogenic N sources, mainly as wastewater
64 effluent, were shown to provide an equivalent contribution of N when compared to natural
65 (upwelled) sources, thus essentially doubling the N loading to nearshore coastal waters in the

66 urbanized areas of the SCB (Howard et al. 2014). This highlights not only the magnitude of N in
67 the coastal environment, but also implies potentially altered composition of N forms as well,
68 since wastewater is typically comprised of ammonium and upwelling is dominated by nitrate
69 (Howard et al. 2014).

70 A historic analysis of satellite imagery documented chronic algal bloom hotspots co-
71 located with major anthropogenic sources of nutrients and determined algal blooms have
72 increased in geographic extent in the SCB beyond what could be supported by increased
73 upwelling (Nezlin et al. 2012). Consistent with increased anthropogenic loading, temporal trends
74 in dissolved oxygen concentrations in the SCB show that the rate of decline is four times higher
75 in the nearshore where anthropogenic nutrient discharge is substantial, compared with offshore
76 locations (Booth et al. 2014). Additional studies, focused on more refined spatial scales, have
77 documented the stimulatory effects of terrestrial and wastewater effluent discharges, resulting in
78 increased phytoplankton biomass and productivity as well as altered community composition
79 (Corcoran et al. 2010; Reifel et al. 2013). The inhibitory impacts of wastewater effluent,
80 specifically due to ammonium inhibition of nitrate uptake by phytoplankton, have also been
81 linked to decreased primary production and significantly altered phytoplankton community
82 composition (c.f. Dortch 1990; Dugdale et al. 2012; Glibert et al. 2015).

83 The Orange County Sanitation District (OCSD) conducted a planned diversion of treated
84 wastewater effluent from the primary outfall pipe located 7 km offshore (56 m water depth) off
85 Huntington Beach, California to a short outfall pipe, located only 2.2 km offshore in 16 m of
86 water, in order to inspect and rehabilitate the primary outfall pipe. This planned diversion of
87 treated wastewater effluent discharge into the shallow nearshore environment provided what
88 should have been an ideal opportunity to evaluate the impacts of anthropogenic N on

phytoplankton. The diversion of wastewater had the potential to impact both the quantity of N biologically available, as well as the form of N, both of which can affect phytoplankton uptake rates of N, community composition, growth and biomass.

The goals of this study were to use N uptake kinetics as a short-term metric of physiological capacity, to evaluate the response of phytoplankton to the diverted wastewater, to determine if ammonium suppression of nitrate uptake was observed, and to document any changes in N uptake rates before, during and after the effluent diversion. The experimental design assumed that elevated ammonium concentrations would be evident at station 2203 near the outfall, and that a strong biological response to the wastewater diversion would be observed, based on previous studies (Reifel et al. 2013). The lack of high levels of ammonium and the lack of biological response (Caron et al. this issue, Kudela et al., this issue) resulted in adjustment of the experimental design midway through the experiment, and introduced methodological issues that complicated interpretation of the results. Nonetheless, the data presented here provides useful information about the physiological status and response to nutrient enrichment by the ambient phytoplankton community. Specifically, these data can be used to address two questions: first, is there evidence for physiological inhibition of the phytoplankton assemblage that could explain the modest biological response observed, and second, is there evidence for a physiological response to the availability of anthropogenic nutrients?

2. Materials and Methods

2.1 Study Area

The Orange County Sanitation District (OCSd) discharges treated wastewater effluent through an ocean outfall that terminates 7 km offshore of Huntington Beach in 56 m water depth

at the shelf break (Figure 1). There is also a secondary, shorter outfall, located 2.2 km offshore at a depth of 16m, for which only emergency discharges are permitted under the National Pollutant Discharge Elimination System (NPDES). In order to inspect, assess and rehabilitate the 7 km outfall pipe, OCSD diverted wastewater to the short, nearshore outfall from 11 September 2012 until 3 October 2012. There were 6 cruises from 6 September 2012 through 17 October 2012 during which CTD measurements, ambient nutrient concentrations and biomass measurements were collected in the vicinity of both outfalls, capturing the pre-diversion, diversion, and post-diversion periods. While we focus on station 2203, data for all stations are provided as context for the environmental conditions during the study.

2.2 *Kinetics methods and experimental procedures*

Whole water was collected from station 2203 (Figure 1; maximum depth 33 m) for all experiment dates to determine the N uptake kinetics of three N substrates (nitrate, ammonium and urea) and to evaluate ammonium suppression of nitrate uptake. The overall chlorophyll *a* (chl *a*) concentrations throughout the study area were low (see Kudela et al., this issue and Caron et al. this issue), therefore, experiment water was collected from the chlorophyll maximum in order to maximize the concentration of algal biomass in the incubation experiments (Figure 2). Experiments were conducted during 4 different timepoints: prior to the start of the diversion on 6 September 2012 (experiment water collected from 15 m depth), during the diversion on 20 September 2012 (experiment water collected from 7 m depth), hours after the diversion ended on 3 October 2012 (experiment water collect from 12 m depth), and 2 weeks after the diversion on 17 October 2012 (experiment water collected from 15 m depth). The sampling depth was

consistently in the upper part of the chlorophyll maximum, with subsurface photosynthetic available radiation (PAR) of 100-200 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$.

Samples were collected on cruises aboard the R/V *Yellowfin* in September and the M/V *Nerissa* in October from twelve-liter and five-liter (respectively) PVC Niskin bottles mounted on an instrumented rosette. Seawater was collected in 20-liter acid-cleaned polycarbonate (Nalgene) carboys and kept in dark coolers during transportation back to the laboratory. In the laboratory, water was dispensed into acid-cleaned 250 ml polycarbonate bottles and discrete samples of chl *a* and nutrients were collected, all within 24 hours of collection. Nutrients and chl *a* samples were also collected directly from the sample bottles (see Caron et al. this issue), and were not significantly different from the values obtained from the kinetics experiments (Table 1). For 6 September 2012 the nutrients were lost during storage, and nutrient concentrations from 5 m depth were substituted (both 5 m and 15 m depths were above the pycnocline). The uptake kinetics incubation bottles were inoculated with either ^{15}N -ammonium chloride (99 atom%; Cambridge Isotopes), ^{15}N -sodium nitrate (98 atom%), or ^{15}N -urea (98 atom%) at 12 substrate concentrations ranging from 0-38 $\mu\text{M N}$ to duplicate sample bottles. To avoid confusion, all N values are reported as $\mu\text{M N}$, accounting for the molar difference in N between urea versus nitrate and ammonium. The total number of samples used for curve-fitting is noted in Table 3, accounting for some samples lost during processing or analysis. The ammonium suppression experiments were inoculated with 12 substrate concentrations of ammonium chloride ranging from 0-38 $\mu\text{M N}$ and 10 $\mu\text{M }^{15}\text{N}$ -sodium nitrate to duplicate sample bottles.

All bottles were incubated in a laboratory incubator at ambient temperature (16-19°C) under 65-80 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ irradiance using standard cool-white fluorescence illumination. The incubator irradiance was lower than ambient mid-day values at the time of collection (200,

175, 500, and 180 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ respectively) and was also lower than half-saturation values (except for 17 October) based on Pulsed Amplitude Modulation Electron Transport Rate measurements (E_k ; $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$; see Kudela et al. this issue), which were 479, 619, 371, and 49 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ for each date respectively. Incubations were maintained for 1 hour and filtered onto precombusted GF/F filters (<100 mm Hg), frozen immediately, and subsequently dried at 50° C. Samples were analyzed for total particulate N and isotopic enrichment using a Finnigan Delta XP isotope ratio mass spectrometer at the University of California, Santa Cruz, using acetanilide as the reference standard.

Nitrogen specific uptake rates were calculated as described by Dugdale and Wilkerson (1986) from accumulation of ^{15}N into the particulate material at the end of the incubation and were not corrected for the effects of isotopic dilution. Ammonium uptake rates are therefore underestimates of *in situ* rates. For determination of kinetic constants, raw data was first inspected visually for biphasic, multiphasic, or linear behavior. For biphasic/multiphasic kinetics, only data points corresponding to the first phase were selected to compute affinity (α) and the maximum uptake rate (V_{max}), following Collos et al. (2007). In the case of a linear uptake relationship, the slope of the regression was considered comparable to the initial slope of a hyperbolic curve (e.g. Cochlan et al. 2008; Lee et al. 2015). In two cases, an inhibitory relationship was observed for nitrate uptake (in the absence of ammonium) and ammonium uptake; there is no obvious physiological explanation for suppression of nitrate uptake at moderate N concentration, and it was therefore assumed that this was an experimental artifact. Suppression of ammonium uptake has occasionally been reported and is likely the result of a more complex physiological response involving interaction of nutrient uptake with other cellular processes (Glibert et al. 2015).

An iterative non-linear least squares technique was used for the curve fitting (Kaleidagraph; Abelbeck Software) that utilizes the Levenberg-Marquardt algorithm (Press et al. 1992) to determine the half-saturation (K_s , $\mu\text{M N}$) and maximum uptake ($V_{\max}$, h^{-1}) parameters of a Michaelis-Menten curve for N kinetics, using the following equation:

$$V = \frac{V_{\max} \cdot S}{K_s + S} \quad (1)$$

Where $V_{\max}$ values were calculated as V_{chl} , equivalent to ρ divided by the chlorophyll concentration (Dickson and Wheeler, 1995) and S ($\mu\text{M N}$) is the initial substrate concentration, accounting for both ambient and added nutrients. For 6 September, the nutrient concentration from the closest available depth (30 m) was used; $0.06 \mu\text{M NO}_3$, $0.15 \mu\text{M NH}_4$, and $0.68 \mu\text{M N}$ -urea. For ammonium suppression experiments, ambient nitrate concentrations were not known at the time of incubation, so $10 \mu\text{M NO}_3$ was added. Ambient nitrate and ammonium concentrations were included in the calculated substrate concentrations. For comparison to previous publications we also provide $V_{\max}$ normalized to PN in Table 3, for comparison with historical estimates. The substrate affinity (α) was calculated as $\alpha = V_{\max}/K_s$ and determined from the initial slope of the Michaelis-Menten plot at sub-saturating concentrations ($<K_s$) or from the slope of the regression for those kinetics curves exhibiting a non-saturating, but not multiphasic, response (Healey, 1980). For standardization purposes and for comparison to the literature, a Michaelis-Menten equation was fit where possible, and non-standard kinetics fits are noted where appropriate in Table 3 and 3-5. For calculation of ammonium inhibition, the variation of the Michaelis-Menten equation proposed by Varela and Harrison (1999) was used:

$$\rho = \rho_{\max} \cdot \left[1 - \frac{I_{\max} \cdot [\text{NH}_4]}{K_i + [\text{NH}_4]} \right] \quad (2)$$

where ρ = the nitrate uptake rate ($\mu\text{M N h}^{-1}$), ρ_{max} is the maximum rate in the absence of ammonium, I_{max} is the maximum inhibition (ranging from 0 to 1), K_i is the concentration of ammonium where ρ is half-maximum, and $[NH_4]$ is the ambient ammonium concentration ($\mu\text{M N}$).

2.3 Analytical methods

Chl *a* samples were collected in duplicate, filtered onto Whatman GF/F filters, extracted in 7 mL of 90% acetone for 24h at -20°C and analyzed using a model 10AU fluorometer (Turner Designs, CA) using the acidification method (Parsons et al. 1984) with pure chlorophyll as the calibration standard. Dissolved inorganic nutrients were filtered through $0.45\ \mu\text{m}$ Whatman syringe filters into 50 mL low-density polyethylene tubes and stored frozen (-20°C) before analysis. Nitrate plus nitrite (hereafter referred to as nitrate) were analyzed with a LaChat Quick Chem 8000 Flow Injection Analysis system using standard colometric techniques (Smith and Bogren 2001). Ammonium samples were manually analyzed using the fluorometric method of Holmes et al. (1999) and urea samples were also analyzed manually using the diacetyl monoxime thiosemicarbazide technique (Goeyens et al. 1998) with a 10 cm pathlength cuvette. Particulate nitrogen (PN) and particulate organic carbon (POC) samples were filtered onto precombusted GF/F filters ($<100\ \text{mm Hg}$), frozen immediately, and subsequently dried at 50°C . These samples were acidified and analyzed at the Marine Sciences Institute Analytical Laboratory at the University of California, Santa Barbara on an Exeter Analytical Elemental Analyzer.

3. Results

3.1 Ambient nutrient concentrations

All three forms of N (nitrate, ammonium and urea) were consistently low throughout the study period. The ambient nutrient concentrations collected simultaneously with experiment water are provided in Table 1. Nitrate was below 0.5 $\mu\text{M N}$, ammonium below 0.25 $\mu\text{M N}$ and urea below 0.7 $\mu\text{M N}$. The chl *a* concentrations collected from the experiment water were low pre-diversion (1.0 $\mu\text{g L}^{-1}$), and increased to 3.5 $\mu\text{g L}^{-1}$ during the diversion, which is relatively low for the San Pedro area (Seubert et al. 2013; Seegers et al. 2015; Caron et al., this issue). The PN concentration remained relatively unchanged throughout the study period while POC doubled. The C:N ratio increased during the diversion to 8.1 from a pre-diversion and 2 weeks post-diversion value of 6.4.

Throughout the study period, diatoms dominated the phytoplankton community composition, as reported in Caron et al. (this issue). In the 6 September and 20 September experiments, community composition was analyzed from the experiment water and diatoms comprised >96% of the community composition (E. Seubert and D. Caron, unpublished data). The specific genera that were present in the water collected for the experiment conducted on 6 September 2012 included *Cylindrotheca* spp., *Guinardia* spp., *Pseudo-nitzschia* spp., (both size classes) and *Rhizosolenia* spp. Of those, *Rhizosolenia* spp. and *Pseudo-nitzschia* comprised 90% of the diatom assemblage (E. Seubert and D. Caron, unpublished data). The genera identified in the water collected for the experiment on 20 September 2012 included *Chaetoceros* spp., *Cylindrotheca* spp., *Lauderia* spp., *Leptocylindrus* spp., *Navicula* spp., *Pseudo-nitzschia* spp. (both size classes), *Rhizosolenia* spp., *Skeletonema* spp., and *Thalassiosira* spp. *Pseudo-nitzschia* comprised 60% of the diatom composition and was the dominant genera observed (D. Caron and E. Seubert, unpublished data). The floral composition was not analyzed in the experiment water

from the October experiments but Caron et al. (this issue) reports on overall assemblage throughout the study.

3.2 *N Uptake Kinetics*

The relative preference and affinity of different N substrates in low and high nutrient environments can be assessed using nutrient uptake kinetic parameters. At low ambient nutrient concentrations, ($S < K_s$), the initial slope (α) is a more robust indicator of affinity whereas at high ambient nutrient concentrations, the maximum uptake rate, V_{max} , can be used to assess preference (Healey, 1980). Phytoplankton uptake kinetics can take many different forms, with saturation-uptake (Michaelis-Menten) kinetics often referred to as “classic” kinetics. Following Collos et al. 1997, we refer to induced kinetics where the response is approximately linear, and biphasic kinetics where a plateau at lower substrate concentrations is observed followed by either a second saturation-uptake response or linear response to increasing substrate concentrations. We note that these observed responses are further complicated for experiments using natural assemblages rather than monospecific cultures, since the observed kinetics are some weighted function of the response of each individual species, each of which may exhibit classic, biphasic, or linear kinetics (e.g. Flynn 1999).

Based on the isotopic enrichment and concentration of ^{15}N -substrate added, and the atom % excess and PN at the termination of the experiments, an average (± 1 SD) 1.67-4.74 $\pm$ 2.71-6.68%, 1.64-6.61 $\pm$ 2.72-8.71%, and 0.80-3.82 $\pm$ 1.34-6.24% of the ^{15}N -substrate was incorporated for nitrate, ammonium, and urea uptake kinetics respectively. Substrate exhaustion was therefore not considered an issue, and was not an obvious source of error in defining the uptake-kinetics responses.

The pre-diversion experiment exhibited the full range of possible kinetics responses (Fig. 2, Table 3). For ammonium, reasonable saturation-uptake behavior was observed. Nitrate and urea results were unsaturated (biphasic or linear), and therefore, preclude calculation of $V_{\max}$ as described above using the full dataset. Nitrate and urea both exhibited saturation-uptake below approximately 6 $\mu\text{M N}$, with a second (biphasic) phase above that concentration. We therefore truncated the data between 6-10 $\mu\text{M N}$ for those N species, based on visual examination of the data, calculating the kinetics parameters using the reduced range, and also fit a linear response curve (induced kinetics) to the urea data (Figure 3, Table 3). $V_{\max}$ is reported as the highest measured value for urea uptake and α was determined from linear regression for the initial slope, while the truncated kinetics are provided for both nitrate and urea. Given the non-classic uptake response, these data from the Michaelis-Menten equation should be interpreted cautiously. K_s was generally comparable to or higher than ambient concentrations (see Table 1) suggesting that α is the most appropriate metric for comparison of N utilization; mean ambient concentrations were also lower than 6 $\mu\text{M N}$, suggesting that the truncated kinetics parameters generally represent uptake responses for pre-diversion waters when ambient N concentrations were low. Based on α , preference at low (ambient) concentrations was urea>nitrate>ammonium, while urea > ammonium >> nitrate using $V_{\max}$ values.

The experiment results during the diversion (20 September 2012) exhibited saturation-uptake responses for all three substrates (Figure 3, Table 3) but with some indication of suppression at elevated ammonium concentrations above ~12 $\mu\text{M N}$, and with considerable variance at the highest nitrate concentration. The α values were similar across substrates, but ambient concentrations were at (ammonium) or greater than (urea) K_s values. Ammonium and nitrate K_s values were comparable (0.58 and 0.33 $\mu\text{M N}$, respectively), while the K_s for urea was

considerably higher ($4.79 \mu\text{M N}$), and V_{max} values showed a strong gradient with urea > ammonium > nitrate. Taken together the results indicated that the community was likely utilizing all three substrates at low nutrient concentrations (α , urea $\approx$ ammonium $\approx$ nitrate), with comparable uptake for nitrate and ammonium at moderate (ambient) nutrient concentrations. The V_{max} results indicated a greater potential for urea uptake at high nutrient concentrations.

The first post-diversion experiment (3 October 2012) was conducted several hours after the end of the diversion and ammonium exhibited a classic Michaelis-Menten response (Figure 4, Table 3), with urea possibly exhibiting biphasic kinetics but with a statistically significant fit using a saturation-uptake response. The nitrate results were unsaturated, therefore, V_{max} and α are reported but K_s was not calculated. Ambient concentrations for all stations were similar to values during the diversion and K_s values for ammonium and urea were again similar to or greater than ambient concentrations. At low nutrient concentrations urea and nitrate exhibited similar affinity and lower affinity for ammonium (α , urea $\approx$ nitrate $\gg$ ammonium) while nitrate was preferred over urea and ammonium (V_{max} , nitrate > urea > ammonium) at high nutrient concentrations.

The final experiment conducted on 17 October 2012, 2 weeks after the diversion ended, exhibited the highest V_{max} and α results for ammonium throughout the study period (Figure 5, Table 3). While all three substrates could be fit to a saturation-uptake response, urea exhibited a biphasic response (increasingly linear) above $\sim 1 \mu\text{M N}$ and did not saturate at the highest nutrient addition, while nitrate exhibited a potential inhibitory response at intermediate (~ 5 - $20 \mu\text{M N}$) additions. With the increase in V_{max} for ammonium, at high concentrations V_{max} followed the pattern ammonium > urea > nitrate, while affinity was highest for urea (urea > nitrate > ammonium). At moderate concentrations, K_s values followed the same pattern as for α .

Using the ambient nutrient concentrations for station 2203 where the incubation water was collected, it is possible to calculate the relative utilization of nitrate, ammonium, and urea for each experiment from Table 4 using saturation-uptake kinetic parameters. The relative percent use of nitrate, ammonium, and urea are presented in Table 4. Given the caveat that a saturation-uptake response was used for (truncated) data and the majority of uptake kinetics responses exhibited biphasic or linear kinetics, the percentages provide an approximation of what N sources were being used by the phytoplankton assemblage before, during, and after the diversion. Before and after the diversion, ammonium dominated N-uptake. During the diversion (20 September) urea was dominant, while at the end of the diversion (3 October) nitrate strongly dominated. There was no consistent pattern across all time periods, other than utilization of all N-substrates.

3.3 *Ammonium Inhibition Experiments*

All of the experiments exhibited reasonable uptake-inhibition responses (using equation 2) throughout the study period, shown in Figure 6 and summarized in Table 5. Since ambient nitrate concentrations were not known at the time of the experiments, 10 μM NO_3 concentrations were used, complicating interpretation of the results given that ambient concentrations did not exceed 1 μM NO_3 , and inhibition parameters are therefore only directly relevant to ambient conditions if suppression of nitrate uptake by ammonium is not sensitive to nitrate concentration. The estimated concentration of ammonium required to completely suppress nitrate uptake (calculated as 9X K_i ; Cochlan and Bronk, 2003) was high relative to ambient concentrations (Table 2), and ranged from 3.8-32.4 μM N. The maximum uptake of nitrate at zero ammonium (ρ_{max}) was low throughout the study period and ranged from 0.001 to 0.03 μM N h^{-1} , but

generally consistent with the kinetics results. The K_I values ranged from 0.4 to 3.6 $\mu\text{M NH}_4 \text{ L}^{-1}$, higher than ambient ammonium concentrations at the time of experiment water collection (Table 1), but within the ambient concentration range observed in the pre- and during diversion timepoints for the overall study (Table 2).

4. Discussion

While it was anticipated that the diversion of treated wastewater effluent into the shallow, nearshore zone would provide a unique opportunity to evaluate how changes in anthropogenic N inputs affect nearshore coastal ecosystems, the realized conditions were more consistent with low ambient nutrient concentrations, despite the considerable discharge of effluent. As the primary limiting macronutrient in coastal waters, changes in N inputs or N forms can have significant effects on phytoplankton growth, community composition and biomass. The diversion of wastewater effluent into the nearshore had the potential to impact both the quantity and forms of N present on local spatial scales. The unanticipated lack of biological response during the experiment, the methodological issues such as mismatch between *in situ* irradiance and incubation conditions, the small number of kinetics experiments, and the lack of highly elevated ammonium concentrations resulted in a limited, but still meaningful, dataset. Specifically, these data can be used to address two questions: first, is there evidence for physiological inhibition of the phytoplankton assemblage that could explain the modest biological response observed, and second, is there evidence for a physiological response to the availability of anthropogenic nutrients?

4.1 Overall field observations of ambient nutrient concentrations and biological response

The results of a previous diversion event in Santa Monica Bay, California occurring from 28-30 November 2006 (Reifel et al. 2013) were used to estimate the phytoplankton response to the OCSD diversion as a persistent patchy bloom with predicted chl *a* concentrations up to 40-50 $\mu\text{g L}^{-1}$ (OCSD, 2011). This was based on the expected ambient concentration of 42 $\mu\text{M NH}_4 \text{ L}^{-1}$ after accounting for dilution, and the formation of a shallow plume with a thickness of 4-5 m. The resulting biological response was anticipated to be 4 times higher than the historical mean high value. Given the prevalence of diatoms and dinoflagellates in the region, it was expected that HAB organisms such as the diatom genus *Pseudo-nitzschia* or several dinoflagellate genera including *Alexandrium*, *Dinophysis*, and *Cochlodinium* could become a significant component of the bloom response.

Throughout the study period, the observed chl *a* concentrations during the OCSD diversion were low, $<5 \mu\text{g L}^{-1}$ (Caron et al., this issue) with some evidence of increased chl *a* towards the end of the diversion and in subsurface observations (Lucas and Kudela, this issue; Seegers et al., this issue), but overall, much lower than expected based on the predicted response documented in the Environmental Impact Report (OCSD, 2011). Grow-out experiments revealed the physiological capacity of the phytoplankton community to utilize the effluent for growth (Seubert et al., this issue).

As described in Kudela et al. (this issue), the Santa Monica Bay diversion was similar in terms of ambient physical conditions, pre-diversion nutrient concentrations, discharge depth, and time of year; primary differences were the diversion durations and the amount of chlorination. OCSD employed enhanced chlorination (approximately doubling the chlorine concentration to 5-6 $\mu\text{g L}^{-1}$) followed by dechlorination (neutralization with sodium bisulfite) of the effluent discharge in order to minimize the impact of discharge on microbial populations. This enhanced

chlorination process produced disinfection byproducts that strongly inhibited phytoplankton photophysiology and growth lasting for 24 hours and 3 days respectively (Kudela et al., this issue). It is unknown to what extent the disinfection byproducts influenced results of these reported kinetics experiments. Given the impact on photosynthesis, it is possible that nitrate uptake would be more sensitive than either ammonium or urea uptake, given the energetics associated with both uptake and assimilation (reviewed by Glibert et al. 2015).

Caron et al. (this issue) observed a strong response of bacterial biomass (by an order of magnitude) and suggested nutrient immobilization within the bacterial food web as an explanation for the low ambient dissolved N concentrations that were observed by both Caron et al. (this issue) and McLaughlin et al. (this issue). The ambient N concentrations described in Caron et al. and McLaughlin et al. (this issue) were well below the estimated concentrations of total inorganic N of up to 40 μM N in the EIR report based on expected plume dilution (OCS&D, 2011). While the plume dilution was estimated at 1:30 based on the diffuser design, Rogowski et al. (2014) estimated dilution of greater than 1:100 within 1 km of the outfall pipe using Lagrangian drifter data. The observed discrete nutrient data were consistent with this estimate (Caron et al., this issue), but note that Lucas and Kudela (this issue) present evidence suggesting that dilution was not uniform in space and time. McLaughlin et al. (this issue) measured nitrification rates throughout the study period and concluded that rapid oxidation of effluent ammonium proximal to the outfalls contributed significantly to the pool of ‘new’ nitrate. Additionally, the observed low $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ from suspended particulate organic matter suggests the “nitrified” effluent ammonium was incorporated into the biomass. Taken together, these results suggest that temporary suppression of phytoplankton, accompanied by rapid

bacterial transformation of N, resulted in both low autotrophic biomass and low ambient nutrient concentrations during the diversion.

The current study used N uptake kinetics and ammonium inhibition experiments as a metric to evaluate the potential and realized response of phytoplankton to the diverted wastewater so as to determine whether there were inhibitory effects to N uptake rates, which would ultimately affect growth and biomass. From these results, it appears that the phytoplankton were fully capable of utilizing nitrate, ammonium, and urea. Relative utilization of the three N species depends in part on ambient concentrations. At low N concentrations ($<K_s$), the α values suggest equivalent affinity for all three substrates (Table 3). At moderate concentrations ($\sim K_s$) nitrate and ammonium would be preferred relative to urea. At high concentrations, generally greater than observed during the experiment, there was physiological capacity, particularly post-diversion, to increase uptake for all three N species but especially ammonium. Based on the kinetics and average ambient concentrations, all three N species were dominant at varying times (Table 4) and regardless of timepoint there was physiological capacity to utilize both low and high ambient concentrations of all three N substrates.

4.2 Ammonium inhibition of nitrate uptake was not a driver of low biological response

The inhibitory effects of high ammonium concentrations on nitrate uptake have been documented in both laboratory and field studies (see reviews by Dortch, 1990; Mulholland and Lomas 2008; Collos and Harrison, 2014). Elevated ammonium concentrations in southern California have mainly been attributed to wastewater discharges (e.g. MacIsaac et al. 1979; Thomas and Carsola, 1980; Howard et al. 2014), making ammonium inhibition a particularly relevant aspect of focus for the OCSF wastewater diversion study.

The ammonium concentrations before the diversion began (prior to 11 September 2012) were the highest observed, but still relatively low (Table 2), and the range of ammonium concentrations decreased during and post-diversion (Tables 1 and 2), with an average of less than 0.65 $\mu\text{M N}$. The concentration of ammonium required to completely inhibit saturated nitrate uptake ($9 \times K_i$; Table 5) was 3.8 – 32.4 $\mu\text{M N}$, well above ambient concentrations observed during the diversion (Tables 1 and 2). Comparing ρ_{max} (Table 5) to ρ_{max} for the kinetics experiments (V_{max} from Table 3 multiplied by chlorophyll from Table 1), values are similar except for 3 October, where the maximum nitrate uptake estimated from the ammonium inhibition experiment was an order of magnitude lower (0.001 versus 0.023 $\mu\text{M N h}^{-1}$). That date also exhibited induced or biphasic kinetics for nitrate uptake, and 10 $\mu\text{M NO}_3$ was not saturating. The similarity between values from the two sets of experiments suggests that ammonium was not strongly suppressing nitrate uptake at ambient concentrations, but given that ammonium was always present at some baseline level and that I_{max} values were also less than 1 $\mu\text{M N}$, there was undoubtedly some suppression of nitrate uptake, even at those low ambient concentrations. Based on the comparable ranges of α and K_s for nitrate and ammonium, and the generally higher V_{max} values for ammonium (Table 3), the kinetics data suggest that equivalent or higher growth rates would be attained when utilizing a combination of both nitrate and ammonium. Thus while there was clear evidence for suppression of nitrate uptake by ammonium it was not likely the primary cause of the suppressed biological response observed during the experiment.

These results are consistent with other studies that performed uptake-inhibition experiments using similar methods. Cochlan and Bronk (2003) summarized results from the Southern Ocean as well as other field studies, and reported suppression of nitrate uptake at low to moderate ammonium concentrations, with an average I_{max} of 0.63 and K_i of 0.10 $\mu\text{M N}$, while

Kokkinakis and Wheeler (1987, as reported in Cochlan and Bronk 2003) reported an $I_{\max}$ of ~0.50 for Oregon upwelling waters, and Dugdale and MacIsaac (1971, as reported in Cochlan and Bronk 2003) reported $I_{\max} = \sim 0.80$ for Peru upwelling waters. L'Helguen et al. (2008) emphasized the importance of cell size, and reported reduced suppression of nitrate uptake for large-sized ($> 2\mu\text{m}$) cells, with $I_{\max} = 0.67$ and $K_I = 0.13 \mu\text{M N}$. Those authors emphasized that larger cell-sized phytoplankton assemblages, such as found in this study, were less likely to exhibit suppression compared to smaller cell-size populations in the open ocean, consistent with the summarized data for coastal versus open ocean waters presented in Cochlan and Bronk (2003).

Several caveats exist in comparing our results to these previous studies. First, we used saturating nitrate concentrations, and it is unknown whether the suppression response would be similar at ambient nitrate concentrations. Second, as summarized by Dortch (1990), results are dependent on phytoplankton species composition and nutritional history, while Reay et al. (1999) reported reduced affinity for nitrate but not ammonium at suboptimal temperatures. Third, in at least one study (Yin et al. 1998), suppression of nitrate uptake by ammonium was eliminated under severe light-limiting conditions. In that study $I_{\max}$ went from 0.30 in saturating light to 0.38 in moderate light-limitation, but dropped to zero with severe light-limitation. The authors suggested that this was an adaptive response to increasing cellular N-quotas with increasing light limitation. Given that our experiments were conducted at irradiance levels less than E_k , it is possible that the observed $I_{\max}$ values (0.29-0.83) were lower than would be observed under full irradiance.

Despite these caveats several similarities emerge between the OCSD diversion and previous studies. As observed by others, particularly in coastal waters, $I_{\max}$ never reached 1.0 in

this study. Our K_I values ranged from 0.42-3.61 $\mu\text{M N}$, higher than those reported by Cochlan and Bronk (2003) and L'Helguen et al. (2008), perhaps due to the experimental design which included low ambient irradiance and saturating nitrate concentrations. Despite evidence for simultaneous utilization of all three N species, suppression of nitrate uptake by ammonium increased with time as evidenced by increasing I_{max} and decreasing K_I values (Table 5), while the relative proportion of ammonium used increased to nearly the same percentage as the beginning of the experiment (Table 4), suggesting that the phytoplankton assemblage did respond to changes in the form and concentration of N present, albeit subtly.

4.3 Uptake kinetics and preference of N form

The determination of both the half-saturation constant, one indicator of affinity in low nutrient conditions, as well as V_{max} , an indicator of preference in high nutrient conditions, allows for the calculation of the slope (α), a more robust indicator of nutrient affinity at sub-saturating conditions ($<K_s$), relevant for the nutrient concentrations observed in this study (Healey, 1980). These parameters should not be confused with the relative preference index (RPI, McCarthy et al. 1977) which is not ecologically relevant (Dortch, 1990), and is more relevant as a measure of N sufficiency, rather than physiological preference (McCarthy, 1981).

The full range of possible kinetics responses were observed during this study, including saturation-uptake (Michaelis-Menten), biphasic, linear, and potentially, inhibitory kinetics. Of the 12 curves generated, ammonium most often followed saturation-uptake kinetics while nitrate and urea were more often biphasic, exhibiting a saturation-uptake response at low (less than $\sim 6 \mu\text{M N}$) concentrations. On 20 September and 17 October, ammonium and nitrate (respectively) appeared to exhibit inhibitory kinetics. At the physiologically low concentrations used during the

experiments this inhibitory response is unexpected. However, since the same tracer was used for all experiments and the inhibitory response was not consistently observed, there is no obvious explanation for the observed suppression of nitrate uptake. We have therefore chosen to fit those data to an uptake-saturation response (equation 1 above), but note the discrepancy. Suppression of ammonium at elevated concentrations has been observed (Glibert et al. 2015) but given the variability in uptake at high ammonium concentrations, we elected to fit a saturation-uptake curve to these data as well, since fitting an uptake-inhibition relationship would not change the initial slope and would have a negligible effect on $V_{\max}$ (not shown).

Biphasic uptake kinetics (operating at low and high nutrient concentrations) and non-saturating kinetics have been described as adaptations to pulsed high nutrient inputs, particularly in coastal assemblages (Lomas and Glibert 1999). Collos et al. (1997), Lomas and Glibert (1999), and Fan et al. (2003) considered the involvement of diffusion of nitrate at high extracellular nitrate concentrations, while Serra et al. (1978) proposed diffusive and mediated transfer. Regardless of the mechanism, as discussed by Flynn (1999), biphasic or linear kinetics cannot be explained by diffusion of nitrate because it is incompatible with our understanding of algal physiology. Collos et al. (2005) suggested that for nitrate specifically, multiphasic kinetics may be the norm rather than the exception in coastal waters, which would be consistent with the results of this study (but does not explain the apparent inhibition observed for 17 October). A recent review (Glibert et al. 2015) also advocates for interaction of diffusive transport, low- and high-affinity transporters, leading to biphasic or multiphasic and induced kinetics, but also emphasizes that uptake is ultimately modulated by other downstream cellular processes.

The variability exhibited from a small number of experiments is a reminder that nutrient uptake and assimilation, particularly for mixed natural assemblages, can be complex and does

not necessarily follow saturation-type enzyme kinetics (c.f. Glibert et al. 2013; Glibert et al. 2015). In these experiments the overall conclusions do not change significantly as a result of the mathematical formulation for the uptake response, at least at the ambient nutrient concentrations observed, but it is important to remember that uptake is only one facet of nutrient utilization.

In the pre-diversion experiments, there was a higher affinity for ammonium; however, during the diversion, there was no obvious preference amongst substrates and the phytoplankton assemblage was likely utilizing all three species of N, a change from pre-diversion results. This was surprising as the expectations prior to the study were that the phytoplankton community would adapt to prefer ammonium due to the large load of ammonium discharged from the outfall pipe into shallow coastal waters (~2,000 μM N during the diversion event, Caron et al. this issue). At the end of the diversion (3 October) nitrate uptake accounted for the largest percentage (Table 4), while two weeks later ammonium was again dominant.

The indication of biphasic and induced kinetics at elevated nutrient concentrations does suggest that, if effluent discharge were to result in substantially higher nutrient concentrations, there could be a more dramatic response to N form and concentration than observed. In particular, urea uptake was low at ambient concentrations but exhibited a strong linear response with little evidence of saturation, while both the V_{max} and I_{max} for ammonium increased while K_I decreased through the experiment.

The overall community composition was dominated by diatoms before and during the diversion (Caron et al. this issue), therefore, changes in N preference cannot be attributed to broad changes in community composition. The ambient N concentrations, especially ammonium, were relatively low throughout the study (Tables 1 and 2; and Caron et al. and McLaughlin et al. this issue), which likely contributed to the observed uptake kinetics. The low ambient N

concentrations can be attributed to immobilization of nutrients due to the large bacterial response (Caron et al. this issue), and the low ammonium concentrations are likely due to the high rates of nitrification (McLaughlin et al. this issue). Given those circumstances it is perhaps not surprising that all three N substrates were utilized and that there was not a dramatic shift towards preference for any one substrate based solely on the kinetics results.

The values for α , the most relevant parameter for the observed ambient nutrient concentrations during this study, are low compared with similar diatom-dominated assemblages on the US West Coast (MacIssac and Dugdale, 1969, Dortch and Postel, 1989, Dickson and Wheeler, 1995, Kudela and Peterson, 2009). The K_s values for nitrate and ammonium were also low compared with other natural assemblages, including both dinoflagellate- and diatom-dominated communities from upwelling dominated areas (Dickson and Wheeler, 1995; Kudela et al. 2008a; Kudela and Peterson, 2009), whereas the urea K_s values were similar to previous studies (Dortch and Postel 1989; Kudela and Cochlan 2000; Kudela et al. 2008a, 2008b).

Xu et al. (2012) and Yuan et al. (2012) reported N uptake rates in regions of Hong Kong coastal waters that are influenced by sewage as well as river discharge. The maximal uptake rates ranged from 0.05-0.08 $\mu\text{M N } \mu\text{g Chl a}^{-1} \text{ h}^{-1}$ (Xu et al., 2012) and 0.01-0.07 $\mu\text{M N } \mu\text{g Chl a}^{-1} \text{ h}^{-1}$ (Yuen et al., 2012). These rates were much higher than the pre- and during-diversion rates reported in the current study, but similar to the post-diversion rates. The rates at the sewage influenced station were comparatively lower than the other stations, $<10 \mu\text{M N } \mu\text{g Chl a}^{-1} \text{ h}^{-1}$ (Xu et al., 2012), which is closer to the rates measured in this study pre- and during diversion, but lower than post-diversion rates. Thus the kinetics results from this study are consistent with adaptation to low nutrient concentrations, with potential uptake rates that are comparable to other coastal regions.

In summary, there was no obvious physiological impairment related solely to N uptake kinetics that would explain the low observed biological response to the diversion. Ammonium suppression of nitrate uptake was not sufficient to account for the lack of biological response, and there was physiological capacity to respond to ambient N substrates. Kudela et al. (this issue) documented photophysiological inhibition from the disinfection byproducts and Caron et al. (this issue) observed the large bacterial response and potential competition for nutrients. Low concentrations of ammonium observed throughout the study were likely due to high nitrification rates (McLaughlin et al. this issue) and immobilization of nutrients due to bacterial response (Caron et al. this issue) rather than advection of the plume out of the study area (Lucas and Kudela, this issue).

There was a clear change in the N uptake kinetics during the diversion of wastewater into the nearshore shallow waters, but the phytoplankton community was likely utilizing all three substrates (nitrate, ammonium and urea) for growth. At ambient concentrations, receiving waters exhibited a strong preference for ammonium pre-diversion and post-diversion. This is consistent with simultaneous presence of all three N species and rapid biochemical transformations driven by the microbial assemblage, leading to presence and simultaneous uptake of multiple N forms during the diversion. The increase in $V_{\max}$ for ammonium following the diversion, in combination with decreasing $I_{\max}$ and K_I values, suggests that a more pronounced bloom response, such as documented in Santa Monica (Reifel et al. 2013), would likely occur if not for the unusual circumstances (suppressed photophysiology of the phytoplankton assemblage; rapid nitrification driven by heterotrophic bacteria) that occurred during the diversion. Our results serve as a reminder that the response of natural phytoplankton assemblages to human perturbations such as wastewater discharge are complex and difficult to predict (c.f. Anderson et

al., 2002; Hallegraeff 2004; Glibert et al. 2005; Howarth 2008; Paerl and Piehler 2008), and eutrophication does not always lead to massive algal blooms, even when the physiological capacity to utilize the nutrients exists.

Acknowledgements

We thank the Orange County Sanitation District for all of the support provided during the J112 project, especially for the efforts from George Robertson, Michael Mengel and Mike VonWinkleman. We also thank Abel Santana (Southern California Coastal Water Research Project) for creating the map. Earlier versions of the manuscript were greatly improved with the input from several anonymous reviewers. Funding for this study was provided by the National Science Foundation through RAPID award OCE1251573 (RMK), through the NOAA ECOHAB program through award NA11NOS4780030 (RMK and MDH), and through the Southern California Coastal Water Research Project Authority. This is ECOHAB publication # 820.

References

- Anderson, D. M., Glibert, P.M., Burkholder, J. M., 2002. Harmful algal blooms and eutrophication: Nutrient sources, composition, and consequences. *Estuaries* 25, 704-726.
- Beman, M., Arrigo, K., Matson, P., 2005. Agricultural runoff fuels large phytoplankton blooms in vulnerable areas of the ocean. *Nature* 434, 211-214.
- Booth, A. T., Sutula, M., Micheli, F., Weisberg, S. B., Bograd, S. J., Steele, A., Schoen, J., Crowder, L. B., 2014. Patterns and potential drivers of declining oxygen content along the southern California coast. *Limnol. Oceanogr.* 59, 1127-1138.
- Capone, D. G., Hutchins, D. A., 2013. Microbial biogeochemistry of coastal upwelling regimes in a changing ocean. *Nature Geoscience* 6, 711-717.
- Caron, D.A., Gellene, A.G., Smith, J., Seubert, E.L., Campbell, V. Sukhatme, G.S., Seegers, B., Jones, B.H., Howard, M.D.A., Kudela, R.M., Hayashi, K., Ryan, J., Birch, J., Demir-Hilton, E., Yamahara, K., Scholin, C., Mengel, M., Robertson, G., 2016. Response of the phytoplankton and bacterial communities during a wastewater effluent diversion into nearshore coastal waters. *Estuarine Coastal Shelf Science*, [doi:10.1016/j.ecss.2015.09.013](https://doi.org/10.1016/j.ecss.2015.09.013).
- Cochlan, W. P., Bronk, D. A., 2003. Effects of ammonium on nitrate utilization in the Ross Sea, Antarctica: Implication for *f*-ratio estimates. *Ant. Res. Ser.* 78, 159-178.
- Cochlan, W. P., Herndon, J., Kudela, R. M., 2008. Inorganic and organic nitrogen uptake by the toxigenic diatom *Pseudo-nitzschia australis* (Bacillariophyceae). *Harmful Algae* 8, 111-118.

624 Collos, Y., Vaquer, A., Bibent, B., Slawyk, G., Garcia, N., Souchu, P., 1997. Variability in
 625 nitrate uptake kinetics of phytoplankton communities in a Mediterranean coastal lagoon.
 626 Estuar., Coast. Shelf Sci. 44, 369-375.

627 Collos, Y., Vaquer, A., Souchu, P., 2005. Acclimation of nitrate uptake by phytoplankton to high
 628 substrate levels. J. Phycol. 41, 466–478.

629 Collos, Y., Vaquer, A., Laabir, M., Abadie, E., Laugier, T., Pastoureaud, A., Souchu, P. 2007.
 630 Contribution of several nitrogen sources to growth of *Alexandrium catenella* during blooms
 631 in Thau lagoon, Southern France. Harmful Algae 6, 781.

632 Collos, Y., Harrison, P. J., 2014. Acclimation and toxicity of high ammonium concentrations to
 633 unicellular algae. Mar. Poll. Bull. 80, 8-23.

634 Corcoran, A. A., Reifel, K. M., Jones, B. H., Shipe, R. F., 2010. Spatiotemporal development of
 635 physical, chemical, and biological characteristics of stormwater plumes in Santa Monica Bay,
 636 California (USA). J. Sea Res. 63, 129-142.

637 Dickson, M. L., Wheeler, P. A., 1995. Nitrate uptake rates in a coastal upwelling regime: a
 638 comparison of PN-specific, absolute, and Chl a-specific rates. Limnol. Oceanogr. 40, 533–
 639 543.

640 Dortch, Q., Postel, J., 1989. Biochemical indicators of N utilization by phytoplankton during
 641 upwelling off the Washington coast. Limnology and Oceanography 34, 758–773.

642 Dortch, Q., 1990. The interaction between ammonium and nitrate uptake in phytoplankton. Mar.
 643 Ecol. Prog. Ser. 61, 183-201.

644 Dugdale, R. C., MacIsaac, J. J., 1971. A computational model for the uptake of nitrate in the
 645 Peru upwelling region. *Investigacion Pesquera* 35, 299-308.

646 Dugdale, R. C., Wilkerson, F. P., 1986. The use of ^{15}N to measure nitrogen uptake in eutrophic
 647 oceans; experimental considerations. *Limnol. Oceanogr.* 31, 673-689.

648 Dugdale, R. C., Wilkerson, F. P., Parker, A. E., Marchi, A., Taberski, K., 2012. River flow and
 649 ammonium discharge determine spring phytoplankton blooms in an urbanized estuary.
 650 *Estuar. Coast. Shelf Sci.* 115, 187–199.

651 Eppley, R., Renger, E., Harrison, W., 1979. Nitrate and phytoplankton production in California
 652 coastal waters. *Limnol. Oceanogr.* 24, 483-494.

653 Fan, C., Glibert, P. M., Burkholder, J. M., 2003. Characterization of the affinity for nitrogen,
 654 uptake kinetics, and environmental relationships for *Prorocentrum minimum* in natural
 655 blooms and laboratory cultures. *Harmful Algae* 2, 283-299.

656 Flynn, K. J. 1999. Nitrate transport and ammonium-nitrate interactions at high nitrate
 657 concentrations and low temperature. *Mar. Ecol. Prog. Ser.* 187, 283-287.

658 Glibert, P., Seitzinger, S., Heil, C. A., Burkholder, J. M., Parrow, M. W., Codispoti, L. A., Kelly,
 659 V., 2005. The role of eutrophication in the global proliferation of harmful algal blooms.
 660 *Oceanography* 18, 198-209.

661 Glibert, P. M., Harrison, J., Heil, C., Seitzinger, S., 2006. Escalating worldwide use of urea-a
 662 global change contributing to coastal eutrophication. *Biogeochemistry* 77, 441-463.

663 Glibert, P. M., Kana, T. M., Brown, K. 2013. From limitation to excess: the consequences of
 664 substrate excess and stoichiometry for phytoplankton physiology, trophodynamics and
 665 biogeochemistry, and the implications for modeling. *J. Mar. Sys.* 125, 14-28.

666 Glibert, P. M., Wilkerson, F. P., Dugdale, R. C., Raven, J. A., Dupont, C. L., Leavitt, P. R.,
 667 Parker, A. E., Burkholder, J. M., Kana, T. M. 2015. Pluses and minuses of ammonium and
 668 nitrate uptake and assimilation by phytoplankton and implications for productivity and
 669 community composition, with emphasis on nitrogen-enriched conditions. *Limnol. Oceanogr.*
 670 DOI: 10.1002/lno.10203.

671 Goeyens, L., Kindermans, N., Abu Yusuf, M., Elskens, M., 1998. A room temperature procedure
 672 for the manual determination of urea in seawater. *Estuar. Coast. Shelf Sci.* 47, 415–418.

673 Hallegraeff, G. M., 2004. Harmful algal blooms: A global overview, p. 25–49. *In* G. M.
 674 Hallegraeff, D. M. Anderson, and A. D. Cembella [eds., *Manual on harmful marine*
 675 *microalgae*. UNESCO Publishing.

676 Healey, F. P., 1980. Slope of the Monod equation as an indicator of advantage in nutrient
 677 competition. *Microb. Ecol.* 5, 281-286.

678 Heisler, J., Glibert, P. M., Burkholder, J. M., Anderson, D. M., Cochlan, W., Dennison, W. C.,
 679 Dortch, Q., Gobler, C. J., Heil, C. A., Humphries, E., Lewitus, A., Magnien, R., Marshall, H.
 680 G., Sellner, K., Stockwell, D. A., Stoecker, D. K., Suddleson, M., 2008. Eutrophication and
 681 harmful algal blooms: A scientific consensus. *Harmful Algae* 8, 3-13.

682 Holmes, R. M., Aminot, A., Kerouel, R., Hooker, B. A., Peterson, B. J., 1999. A simple and
 683 precise method for measuring ammonium in marine and freshwater ecosystems. Canadian J.
 684 Fish. Aquat. Sci. 56, 1801–1808.

685 Howard M. D. A., Sutula, M., Caron, D. A., Chao, Y., Farrara, J. D., 2014. Anthropogenic
 686 nutrient sources rival natural sources on small scales in the coastal waters of the Southern
 687 California Bight. Limnol. Oceanogr. 99, 285-297.

688 Howarth, R. W. 2008. Coastal nitrogen pollution: A review of sources and trends globally and
 689 regionally. Harmful Algae 8, 14-20.

690 Howarth, R. W., Marino, R., 2006. Nitrogen as the limiting nutrient for eutrophication in coastal
 691 marine ecosystems: Evolving views over three decades. Limnol. Oceanogr. 51, 364-376.

692 Kokkinakis, S. A., Wheeler, P.A., 1987. Nitrogen uptake and phytoplankton growth in coastal
 693 upwelling regions, Limnol. Oceanogr. 32, 1112-112.

694 Kudela, R. M., and Cochlan, W.P., 2000. The kinetics of nitrogen and carbon uptake and the
 695 influence of irradiance for a natural population of *Lingulodinium polyedrum* (Pyrrophyta) off
 696 Southern California. Aquat. Microb. Ecol. 21, 31-47.

697 Kudela, R.M., Lane, J. Q. and Cochlan, W. P., 2008a. The potential role of anthropogenically
 698 derived nitrogen in the growth of harmful algae in California, USA. Harmful Algae 8, 103-
 699 110.

700 Kudela R.M., Ryan, J.P., Blakely, M.D., Lane, J.Q., Peterson, T.D., 2008b. Linking the
 701 physiology and ecology of *Cochlodinium* to better understand harmful algal bloom events: A

702 comparative approach. Harmful Algae 7, 278-292.

703 Kudela, R., Peterson, T., 2009. Influence of a buoyant river plume on phytoplankton nutrient
 704 dynamics: what controls standing stocks and productivity? J. Geophys. Res. 114, C00B11.
 705 doi: 10.1029/2008JC004913.

706 Kudela, R. M., Seeyave, S., Cochlan, W. P., 2010. The role of nutrients in regulation and
 707 promotion of harmful algal blooms in upwelling systems. Prog. Oceanogr. 85, 122-135.

708 Kudela, R.M., Lucas, A.J., Negrey, K.H., Howard, M. McLaughlin, K. Accepted. Death from
 709 below: Investigation of inhibitory factors in bloom development during a wastewater effluent
 710 diversion. Estuar. Coast. Shelf Sci. <http://dx.doi.org/10.1016/j.ecss.2015.07.021>

711 Lapointe, B. E., Barile, P. J. and Matzie, W. R., 2004. Anthropogenic nutrient enrichment of
 712 seagrass and coral reef communities in the lower Florida keys: Discrimination of local versus
 713 regional nitrogen sources. J. Exp. Mar. Biol. Ecol. 308, 23-58.

714 Lee, J., Parker, A. E., Wilkerson, F. P., Dugdale, R. C. 2015. Uptake and inhibition kinetics of
 715 nitrogen in *Microsystis aeruginosa*: Results from cultures and field assemblages collected in
 716 the San Francisco Bay Delta, CA. Harmful Algae 47, 126-140.

717 L'Helguen, S., Maguer, J-F., Caradec, J., 2008. Inhibition kinetics of nitrate uptake by
 718 ammonium in size-fractionated oceanic phytoplankton communities: implications for new
 719 production and *f*-ratio estimates. J. Plankton Res. 30, 1179-1188.

720 Lomas M.W., Glibert, P.M., 1999. Temperature regulation of nitrate uptake: a novel hypothesis
 721 about nitrate uptake and reduction in cool water diatoms. Limnol. Oceanogr. 44, 556-572.

722 Lucas, A. J., Kudela, R. M. 2016. The fine-scale vertical variability of a wastewater plume in
 723 stratified coastal waters. *Estuar. Coast. Shelf Sci.* [doi:10.1016/j.ecss.2015.08.010](https://doi.org/10.1016/j.ecss.2015.08.010).

724 MacIsaac, J. J., Dugdale, R. C., 1969. The kinetics of nitrate and ammonia uptake by natural
 725 populations of marine phytoplankton. *Deep-Sea Res.* 16, 45–57.

726 MacIsaac, J. J., Dugdale, R. C., Huntsman, S. W., Conway, H. L., 1979. The effect of sewage on
 727 uptake of inorganic nitrogen and carbon by natural populations of marine phytoplankton. *J.*
 728 *Mar. Res.* 37, 51-66.

729 McCarthy, J.J., 1981. The kinetics of nutrient utilization. In: Platt, T. (Ed.), *Physiological Bases*
 730 *of Phytoplankton Ecology*. *Can. Bull. Fish. Aquat. Sci.* 210, 211–233.

731 McCarthy, J., Taylor, W., Taft, J., 1977. Nitrogenous nutrition of the plankton in the Chesapeake
 732 Bay. 1. Nutrient availability and phytoplankton preferences. *Limnol. Oceanogr.* 22, 996–
 733 1011.

734 McLaughlin, K. Howard, M. D. A., Beck, C. D. A., Kudela, R. M., Mengel, M., Nezhlin, N. P.,
 735 Robertson, G. L. 2016. Tracking the fate of anthropogenic N from wastewater discharge into
 736 the coastal ocean, Southern California, USA. *Estuar. Coast. Shelf Sci.*
 737 [doi:10.1016/j.ecss.2016.05.013](https://doi.org/10.1016/j.ecss.2016.05.013).

738 Mulholland, M.R. Lomas, M.W., 2008. Nitrogen uptake and assimilation, p. 303-384. *In* D.
 739 Capone, D. Bronk, M. Mulholland, and E. Carpenter [eds., *Nitrogen in the marine*
 740 *environment*. Elsevier.

741 Nezlin, N. P., Sutula, M. A. Stumpf, R. P. Sengupta, A., 2012. Phytoplankton blooms detected
 742 by SeaWiFS along the central and southern California Coast. *J. Geophys. Res.* 117, C07004.
 743 OCSD 2011. Outfall Land Section and OOBs Piping Rehabilitation Draft Environmental Impact
 744 Report, Volume 2: Appendices. Available online:
 745 <http://www.ocsd.com/Home/ShowDocument?id=10785>. Accessed 3 October 2014.
 746 Paerl, H. W., Piehler, M. F., 2008. Nitrogen and marine eutrophication, p. 529-567. *In* D.
 747 Capone, D. Bronk, M. Mulholland, and E. Carpenter [eds., Nitrogen in the marine
 748 environment. Elsevier.
 749 Parsons, T. R., Maita, Y., Lalli, C. M., 1984. A Manual of Chemical and Biological Methods for
 750 Seawater Analysis. Pergamon Press, Oxford, 50 pp.
 751 Press, W., Teukolsky, S., Vetterling, A., Flannery, B., 1992. Numerical Recipes in C: The Art of
 752 Scientific Computing. Cambridge University Press.
 753 Reay, D.S., Nedwell, D.B., Priddle, J. and Ellis-Evans, J.C., 1999. Temperature dependence of
 754 inorganic nitrogen uptake: reduced affinity for nitrate at suboptimal temperatures in both
 755 algae and bacteria. *Appl. Env. Microbiol.* 65(6), 2577-2584.
 756 Reifel, K. M., Corcoran, A. A., Cash, C., Shipe, R., Jones, B. H., 2013. Effects of a surfacing
 757 effluent plume on a coastal phytoplankton community. *Cont. Shelf Res.* 60, 38-50.
 758 Rogowski, P., Terrill, E., Thomas, J., Rosenfeld, L., Largier, J. 2014. 2012 Orange County
 759 Sanitation District (OCSD) Outfall Diversion–Summary Report. Final Report prepared for
 760 the Orange County Sanitation District. March 25, 2014, 92 pp, plus appendices.

761 Ryther, J., and Dunstan, W., 1971. Nitrogen, phosphorus and eutrophication in the coastal
 762 marine environment. *Science* 171, 1008-1112.

763 Scavia, D., Bricker, S.B., 2006. Coastal eutrophication assessment in the United States.
 764 *Biogeochemistry* 79, 187-208.

765 Serra, J. L., Llama, M., Cadenas, E., 1978. Nitrate utilization by the diatom *Skeletonema*
 766 *costatum*. I. Kinetics of nitrate uptake. *Plant Phys.* 62, 987-990.

767 Seubert, E. L., Gellene, A. G., Howard, M. D. A., Connell, P., Ragan, M., Jones, B. H., Runyan,
 768 J., Caron, D. A., 2013. Seasonal and annual dynamics of harmful algae and algal toxins
 769 revealed through weekly monitoring at two coastal ocean sites off southern California, USA.
 770 *Env. Sci. Poll. Res.* 20, 6878-6895.

771 Seubert, E.L., Gellene, A.G., Campbell, V., Smith, J., Robertson, G., Caron, D.A. 2016.
 772 Phytoplankton Community Response to the Addition of Treated Sewage Effluent. *Est. Coast.*
 773 *Shelf Sci.*, accepted.

774 Seegers, B. N., Birch, J. M., Marin, R. Scholin, C. A., Caron, D. A., Seubert, E. L., Howard, M.
 775 D. A., Robertson, G. L., Jones, B. H., 2015. Subsurface seeding of surface harmful algal
 776 blooms observed through the integration of autonomous gliders, moored environmental
 777 sample processors, and satellite remote sensing in southern California. *Limnol. Oceanogr.* 60,
 778 754-764, DOI: 10.1002/lno.10082.

779 Seegers, B. N., Teel, E. N., Kudela, R., M., Caron, D. A., Jones, B. H. 2016. Glider and remote
 780 sensing perspective of the upper layer response to an extended shallow coastal diversion of
 781 municipal wastewater effluent. *Estuar. Coast. Shelf Sci.*, [doi:10.1016/j.ecss.2016.06.019](https://doi.org/10.1016/j.ecss.2016.06.019).

782 Smith, P., Bogren, K., 2001. Determination of nitrate and/or nitrite in brackish or seawater by
 783 flow injection analysis colorimeter: QuickChem Method 31-107- 04-1-E, Saline Methods of
 784 Analysis. Lachat Instruments, Milwaukee, WI, 12 pp.

785 Thomas, W. H., Carsola, A. J., 1980. Ammonium input to the sea via large sewage outfalls-part
 786 1: Tracing sewage in Southern California waters. Mar. Env. Res. 3, 277-289.

787 Varela, D. E., Harrison, P. J., 1999. Effect of ammonium on nitrate utilization by *Emiliana*
 788 *huxleyi*, a coccolithophore from the oceanic northeastern Pacific. Mar. Ecol. Prog. Ser. 186,
 789 67-74.

790 Xu, J., Glibert, P.M., Liu, H., Yin, K., Yuan, X., Chen, M., Harrison, P.J. 2012. Nitrogen sources
 791 and rates of phytoplankton uptake in different regions of Hong Kong waters in summer.
 792 Estuar. Coasts 35, 559-571.

793 Yin, K., Harrison, P. J. and Dortch, Q., 1998. Lack of ammonium inhibition of nitrate uptake for
 794 a diatom grown under low light conditions. J. Exp. Mar. Biol.Ecol. 228, 151–165.

795 Yuen, X., Glibert, P.M., Xu, J., Liu, H., Chen, M., Liu, H., Yin, K., Harrison, P.J. 2012.
 796 Inorganic and organic nitrogen uptake by phytoplankton and bacteria in Hong Kong waters.
 797 Estuar. Coasts 35, 325-334.

798
 799
 800

Tables

Table 1 Nutrient concentrations and other ancillary data collected simultaneously with experiment water from station 2203 (see Figure 1) on the 4 cruises listed in the table. Units are in parentheses. Average particulate nitrogen (PN) values were calculated from the kinetics experiments; the standard deviation [SD] of the individual samples for kinetics curves from each experiment is provided.

	6 September 2012 Pre-diversion	20 September 2012 During Diversión	3 October 2012 Post- diversion	17 October 2012 Post- diversion
Temperature (°C)	16.1	18.0	19.6	17.3
Salinity	33.38	33.36	33.55	33.40
Chlorophyll ($\mu\text{g L}^{-1}$)	1.02	3.54	0.4	0.97
NO ₃ ($\mu\text{M N}$)	0.06*	0.11	0.21	0.48
NH ₄ ($\mu\text{M N}$)	0.15*	0.17	0.01	0.22
Urea ($\mu\text{M N}$)	0.68*	0.66	0.06	0.22
PO ₄ ($\mu\text{M P}$)	0.47*	0.30	0.48	0.45
C:N Ratio (molar)	6.74	8.11	7.61	6.44
PN ($\mu\text{M N}$) [SD]	5.95 [0.96]	12.8 [1.34]	3.75 [1.51]	7.97 [1.55]

*Water from 5 m depth was used because the samples from incubation depth leaked during storage.

Table 2 Average ambient nutrients concentrations throughout the study period collected on weekly cruises at all stations shown in Figure 1. Units of measurements are in parenthesis and under the timepoint columns, the range of concentrations observed are in parenthesis.

	Pre-diversion	During Diversion	Post-diversion
Chl <i>a</i> ($\mu\text{g L}^{-1}$)	0.6 (0.2-1.3)	1.2 (0.05-5.1)	1.1 (0.2-4.8)
NO ₃ ($\mu\text{M N}$)	5.4 (0.1-19.9)	2.1 (0.01-14.4)	2.1 (0.2-13.9)
NH ₄ ($\mu\text{M N}$)	0.9 (0.1-4.0)	0.6 (0.07-1.1)	0.5 (b.d.-2.6)
Urea ($\mu\text{M N}$)	1.4 (0.4-5.0)	0.6 (0.2-1.8)	0.2 (0.02-1.6)
C:N	7.3 (4.3-9.9)	6.6 (5.9-7.5)	7.0 (4.1-12.4)
PN (μM)	1.1 (0.2-2.7)	2.4 (0.4-5.9)	2.0 (0.4-2.9)
POC (μM)	8.6 (0.9-23.5)	16.4 (2.6-44.8)	14.3 (2.3-25.5)

b.d. = below the limit of detection.

818
819
820
821
822
823
824
825
826
827
828
829
830
831

Table 3 The kinetic parameters for N uptake determined from natural assemblages, reported as $\mu\text{M N } \mu\text{g Chl}^{-1} \text{ h}^{-1}$ for V_{max} , $\mu\text{M N}$ for K_s , and V_{max}/K_s for α . For comparison with previous studies the average PN and corresponding chlorophyll values for each date are provided in Table 1. For curve fits exhibiting non-saturating or biphasic kinetics, a subset of substrate concentrations was used and the kinetic parameters are reported separately by date (italicized values). For induced (linear) kinetics, V_{max} is the highest observed value, K_s is not calculated, and α is the initial slope of the data. Values in parentheses are Standard Errors. The coefficient of determination (r^2) and number of samples (n), excluding zero-values, used in the curve fits are reported. Ammonium uptake rates were not corrected for isotope dilution and so are underestimates of *in situ* rates.

Survey Date	NO ₃				NH ₄				Urea			
	V _{max}	K _s	α	r ² (n)	V _{max}	K _s	α	r ² (n)	V _{max}	K _s	α	r ² (n)
6 Sept. 2012	0.012	--	5E ⁻⁴	0.80 (14)	0.018 (5E ⁻⁴)	0.55 (0.08)	0.03	0.97 (22)	0.02	-	0.001	0.59 (7)
<i>6 Sept. 2012</i>	<i>0.005</i> <i>(3E⁻⁴)</i>	<i>0.22</i> <i>(0.22)</i>	<i>0.02</i>	<i>0.97</i> <i>(14)</i>	-	-	-	-	<i>0.004</i> <i>(1E⁻⁴)</i>	<i>0.86</i> <i>(0.1)</i>	<i>0.005</i>	<i>0.96</i> <i>(7)</i>
20 Sept. 2012	0.008 (5E ⁻⁴)	0.33 (0.11)	0.025	0.87 (22)	0.016 (9E ⁻⁴)	0.58 (0.16)	0.02	0.91 (22)	0.062 (0.004)	4.79 (1.4)	0.03	0.90 (11)
3 Oct. 2012	0.058	-	0.002	-	0.025 (7E ⁻⁴)	0.33 (0.05)	0.07	0.96 (22)	0.053	-	0.001	0.88 (12)
<i>3 Oct. 2012</i>	<i>0.003</i>	<i>0.44</i>	<i>0.007</i>	<i>0.75</i> <i>(7)</i>	-	-	-	-	<i>0.01</i> <i>(0.001)</i>	<i>0.73</i> <i>(0.33)</i>	<i>0.01</i>	<i>0.96</i> <i>(14)</i>
17 Oct. 2012	0.016 (0.001)	0.26 (0.11)	0.06	0.83 (22)	0.034 (0.001)	0.23 (0.07)	0.15	0.76 (22)	0.021	-	0.012	0.88 (22)
<i>17 Oct. 2012</i>	-	-	-	-	-	-	-	-	<i>0.017</i> <i>(7E⁻⁴)</i>	<i>0.35</i> <i>(0.06)</i>	<i>0.048</i>	<i>0.98</i> <i>(14)</i>

Table 4 Calculated N uptake as a percentage of total N uptake for each experimental time point, using kinetics parameters from Table 3 and ambient nutrient concentrations from Table 1 (for 6 September the mean values from Table 2 were used). Ammonium uptake rates were not corrected for isotope dilution and so are underestimates of *in situ* rates.

Experiment Date	<i>Percent Nitrate</i>	<i>Percent Ammonium</i>	<i>Percent Urea</i>
6 Sept. 2012	20.4	61.41	18.19
20 Sept. 2012	10.47	18.99	70.53
3 Oct. 2012	93.85	3.01	3.14
17 Oct. 2012	29.69	47.55	22.76

Table 5 Ammonium inhibition experiment results for each incubation experiment, conducted with 10 $\mu\text{M NO}_3 \text{ L}^{-1}$ concentrations. Parameters shown include the theoretical N uptake at zero ammonium concentration (ρ_{max}), maximal realized inhibition (I_{max}), the substrate (ammonium) concentration at which nitrate uptake is reduced to 50% of maximal value (K_I), the estimated concentration of ammonium required to completely inhibit nitrate uptake ($9 \times K_I$). Ammonium uptake rates were not corrected for isotope dilution and so are underestimates of *in situ* rates.

Experiment Date	ρ_{max} ($\mu\text{M N h}^{-1}$)	I_{max} (%)	K_I ($\mu\text{M N}$)	$9 \times K_I$ ($\mu\text{M N}$)	r^2
6 Sept. 2012	0.007	0.29	2.76	24.84	0.81
20 Sept. 2012	0.03	0.64	3.61	32.49	0.81
3 Oct. 2012	0.001	0.68	1.24	11.16	0.94
17 Oct. 2012	0.01	0.83	0.42	3.78	0.89

Figures

Figure 1 Map of study area. The two outfalls are indicated by the solid black lines, the primary outfall terminates at station 2205 and the secondary, shorter, outfall terminates at station 2202. All experiment water was collected at station 2203 and discrete samples (Table 2) were collected at all stations shown.

Figure 2 Vertical profiles of Temperature (solid line), Salinity (dashed line), Fluorescence converted to chlorophyll units (gray circles) and PAR (black diamonds) from Station 2203 for 6 September 2012 (A), 20 September 2012 (B), 3 October 2012 (C), and 17 October 2012 (D). The horizontal dashed line in each panel denotes the depth of water collection for kinetics experiments.

Figure 3 Chlorophyll-specific uptake rates ($V_{\max}$; $\mu\text{M N } \mu\text{g Chl}^{-1} \text{ h}^{-1}$) for nitrate (A), ammonium (B) and urea (C) for natural assemblages plotted versus substrate concentrations ($\mu\text{M N}$) calculated as ambient plus added N before the diversion began on 6 September 2012 (black circles) and during the diversion on 20 September 2012 (black squares). Grey shading indicates the subset of data used to fit kinetics curves when biphasic kinetics were observed. Ammonium uptake rates were not corrected for isotope dilution and so are underestimates of *in situ* rates.

Figure 4 Chlorophyll-specific uptake rates ($V_{\max}$; $\mu\text{M N } \mu\text{g Chl}^{-1} \text{ h}^{-1}$) (black circles) for nitrate (A), ammonium (B) and urea (C) plotted versus substrate concentrations ($\mu\text{M N}$) calculated as ambient plus added N for natural assemblages. Experiment water was collected on 3 October 2012 several hours after the diversion ended. Grey shading indicates the subset of data used to fit

kinetics curves when biphasic kinetics were observed. Ammonium uptake rates were not corrected for isotope dilution and so are underestimates of *in situ* rates.

Figure 5 Chlorophyll-specific uptake rates ($V_{\max}$; $\mu\text{M N } \mu\text{g Chl}^{-1} \text{ h}^{-1}$) (black circles) for nitrate (A), ammonium (B) and urea (C) plotted versus substrate concentrations ($\mu\text{M N}$) calculated as ambient plus added N for natural assemblages. Experiment water was collected on 17 October 2012, 2 weeks after the diversion ended. Grey shading indicates the subset of data used to fit kinetics curves when biphasic kinetics were observed. Ammonium uptake rates were not corrected for isotope dilution and so are underestimates of *in situ* rates.

Figure 6 Ammonium inhibition results for experiments conducted on 6 September 2012 (A) and 20 September 2012 (B), 3 October 2012 (C) and 17 October 2012 (D). Substrate concentrations included ambient and added substrate, comprised of $10 \mu\text{M } ^{15}\text{N-NO}_3$ and varying concentrations of $^{14}\text{N-NH}_4$. Ammonium uptake rates were not corrected for isotope dilution and so are underestimates of *in situ* rates.