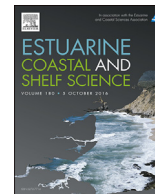




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## Evaluation of uptake kinetics during a wastewater diversion into nearshore coastal waters in southern California

Raphael M. Kudela<sup>a,\*</sup>, Meredith D.A. Howard<sup>b</sup>, Kendra Hayashi<sup>a</sup>, Carly Beck<sup>b</sup>

<sup>a</sup> University of California, Santa Cruz, Ocean Science Department, 1156 High Street, Santa Cruz, CA, USA

<sup>b</sup> Southern California Coastal Water Research Project, Biogeochemistry Department, 3535 Harbor Blvd. Suite 110, Costa Mesa, CA, USA

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## ABSTRACT

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

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### 1. Introduction

The contributions of anthropogenic nitrogen loads to the eutrophication of coastal systems has been well documented (see reviews, [Howarth, 2008](#); [Paerl and Piehler, 2008](#)) and is considered one of the most globally important human-accelerated changes to coastal oceans ([Howarth and Marino, 2006](#); [Scavia and Bricker, 2006](#)). Anthropogenic nutrient inputs have been linked to increased primary production and algal blooms ([Lapointe et al., 2004](#); [Beman et al., 2005](#)), and are considered the most significant factor contributing to the increased frequency of harmful algal blooms (HABs) ([Anderson et al., 2002](#); [Hallegraeff, 2004](#); [Glibert et al., 2005](#); [Heisler et al., 2008](#)). Most coastal eutrophication studies have focused on nitrogen (N), since it is the primary macronutrient that limits the growth of phytoplankton in coastal

waters ([Ryther and Dunstan, 1971](#); [Eppley et al., 1979](#)). The form of N is also important in the stimulation of some algal species responsible for HABs ([Glibert et al., 2006](#)), including in California ([Kudela et al., 2010](#)). Upwelling dominated systems have generally been perceived to be less affected by anthropogenic nutrients due to the sheer magnitude of natural (upwelled) nutrients as well as the highly dynamic conditions making these systems potentially more resilient. However, a growing number of studies have suggested that our perception of the resilience of these systems may be flawed (c.f. [Capone and Hutchins, 2013](#)). The large quantities of anthropogenic nutrient sources in the Southern California Bight (SCB), mainly from wastewater treatment plants and agricultural activities, have sparked a series of studies focused on the impacts and effects of anthropogenic inputs on coastal ecosystems. Anthropogenic N sources, mainly as wastewater effluent, were shown to provide an equivalent contribution of N when compared to natural (upwelled) sources, thus essentially doubling the N loading to nearshore coastal waters in the urbanized areas of the SCB ([Howard et al., 2014](#)). This highlights not only the magnitude of

\* Corresponding author.

E-mail address: [kudela@ucsc.edu](mailto:kudela@ucsc.edu) (R.M. Kudela).

N in the coastal environment, but also implies potentially altered composition of N forms as well, since wastewater is typically comprised of ammonium and upwelling is dominated by nitrate (Howard et al., 2014).

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

The Orange County Sanitation District (OCS D) conducted a planned diversion of treated wastewater effluent from the primary outfall pipe located 7 km offshore (56 m water depth) off Huntington Beach, California to a short outfall pipe, located only 2.2 km offshore in 16 m of water, in order to inspect and rehabilitate the primary outfall pipe. This planned diversion of treated wastewater effluent discharge into the shallow nearshore environment provided what 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., in this issue, Kudela et al., in 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 (OCS D) 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 (Fig. 1). There is also a secondary, shorter outfall, located 2.2 km offshore at a depth of 16 m, 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, OCS D 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 (Fig. 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., in this issue and Caron et al., in this issue), therefore, experiment water was collected from the chlorophyll maximum in order to maximize the concentration of algal biomass in the incubation experiments (Fig. 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 h of collection. Nutrients and chl *a* samples were also collected directly from the sample bottles (see Caron et al., in 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  $^{15}\text{N}$ -ammonium chloride (99 atom%; Cambridge Isotopes),  $^{15}\text{N}$ -sodium nitrate (98 atom%), or  $^{15}\text{N}$ -urea (98 atom%) at 12 substrate concentrations ranging from 0 to 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 to 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

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