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Journal

Limnology and Oceanography, 59(3)

ISSN

0024-3590

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Publication Date

2014-05-01

DOI

10.4319/lo.2014.59.3.0660

Peer reviewed

Limnol. Oceanogr., 59(3), 2014, 660–673

doi:10.4319/lo.2014.59.3.0660

Evidence for active vertical migration by two dinoflagellates experiencing iron, nitrogen, and phosphorus limitation

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Abstract

Vertical migration and subsequent assimilation of iron (Fe) and nitrate at depth by the dinoflagellates *Akashiwo sanguinea* and *Prorocentrum micans* were investigated using laboratory vertically stratified water columns. Active migration was observed in four separate experiments with varying degrees of nitrogen (N), phosphorus, and Fe stress. Stable isotopes of Fe (^{57}Fe-$FeCl_3$) and N (^{15}N-$NaNO_3$) were assimilated from the bottom 0.4 m of the column during migration, with significant enrichment of particulate samples collected from the surface compared to the initial *A. sanguinea* culture or natural water from Monterey Bay containing *P. micans*. In all four experiments, there was significant drawdown of nitrate from the column, with depth-integrated drawdown of greater than 50 mmol m^{-2} in the *P. micans* experiment over 72 h. These experiments provide evidence for dinoflagellate assimilation of N and Fe at depth. Harmful algal blooms of vertically migrating dinoflagellates such as *A. sanguinea* and *P. micans* are often associated with macro- and micronutrient depletion in surface waters; assimilation of iron as well as macronutrients at depth could be an important ecological advantage in environments where light and nutrients are spatially separated.

Blooms of the dinoflagellate *Akashiwo sanguinea* are periodic and widespread along the West Coast of the United States (Horner et al. 1997; Ryan et al. 2010), the Black Sea (Gómez and Boicenco 2004), and Hong Kong (Lu and Hodgkiss 2004). Until recently, *A. sanguinea* was known as *Gymnodinium sanguineum* Hirasaka and is synonymous with *Gymnodinium splendens* Lebour and *Gymnodinium nelsonii* Martin (Daugbjerg et al. 2000). *A. sanguinea* is an autotrophic dinoflagellate classified as a harmful alga (Trainer et al. 2010) and has been linked to the mortality of abalone larvae (Botes et al. 2003) and coral bleaching (Litchman et al. 2002), although the exact mechanism for these deleterious effects is not clear. These blooms have also been coupled with seabird deaths by the production of large amounts of mycosporine-like amino acids released during bloom senescence (Jessup et al. 2009). This release, in combination with wind-driven turbulence, creates a surfactant-like foam that can interfere with the waterproofing capacity of seabird feathers, leading to death by hypothermia (Jessup et al. 2009; Du et al. 2011).

A. sanguinea blooms are initiated under various physical-chemical conditions (Matsubara et al. 2007; Ryan et al. 2010; Du et al. 2011), and significant bloom biomass often accumulates when the water column is vertically stratified. Vertical migration typically occurs under conditions with low surface nutrients (Ryan et al. 2010; Du et al. 2011) and is a common, competitive strategy employed by dinoflagellates (Smayda 2010) in a vertically stratified water column to acquire nutrients from depth (Eppley et al. 1968; Kudela et al. 2010). This has been documented for natural populations of *A. sanguinea* (Ryan et al. 2010; Du et al. 2011) and in laboratory experiments (Cullen and Horrigan 1981). Vertically migrating phytoplankton incorporate nitrate (Cullen and Horrigan 1981; Katano et al. 2011), ammonium (Hall and Pearl 2011), and P (Watanabe et al. 1988) from depth, providing a competitive benefit over nonmotile phytoplankton (Smayda 2010).

A second dinoflagellate, *Prorocentrum micans* Ehrenberg, is considered a nontoxic red-tide–forming algae. It is not directly associated with fish or invertebrate mortality, but prolonged blooms can discolor clams and oyster meat, causing economic damage for shellfish farmers who cannot sell tainted product (Horner et al. 1997), and the end of a large bloom of mixed dinoflagellates, including *P. micans*, in the Benguela upwelling system was responsible for a large die-off of multiple organisms due to anoxia (Pitcher et al. 1998). *P. micans* blooms are persistent, sometimes lasting up to 3 mo, and are routine along the West Coast of the United States (Horner et al. 1997), the Benguela upwelling system (Pitcher et al. 1998), and the Swedish coast (Hasle 1950; Olsson and Grénali 1991). Vertical migration of *P. micans* has been observed in situ (Olsson and Grénali 1991; Pitcher et al. 1998) and under laboratory settings (Kamykowski 1981), but like many dinoflagellates from upwelling systems, vertical migration of *P. micans* cannot be wholly predicted by phototaxis, as light intensity and the processes that influence irradiance (i.e., turbidity and vertical mixing) are variable (Smayda 2010). It is assumed that *P. micans*, along with the dinoflagellate *A. sanguinea*, is assimilating nitrogen (N) at depth to supplement nutritional needs.

Nutrient data suggest that Monterey Bay, California, not only is episodically nitrate limited but also can be seasonally Fe limited (Johnson et al. 1999; Bruland et al. 2001; McGaraghan and Kudela 2012), similar to other ecosystems that support dinoflagellate blooms. In Monterey Bay, the gradient of nitrate and Fe is typically very low in the presence of phytoplankton at the surface and increases to ~ 30 μmol L^{-1} of nitrate and 5–20 nmol L^{-1} of Fe (depending on the width of the continental shelf), corresponding to the bottom of the nitricline (Johnson et al. 1999; Bruland et al. 2001). During relaxation of upwelling, the vertical gradients

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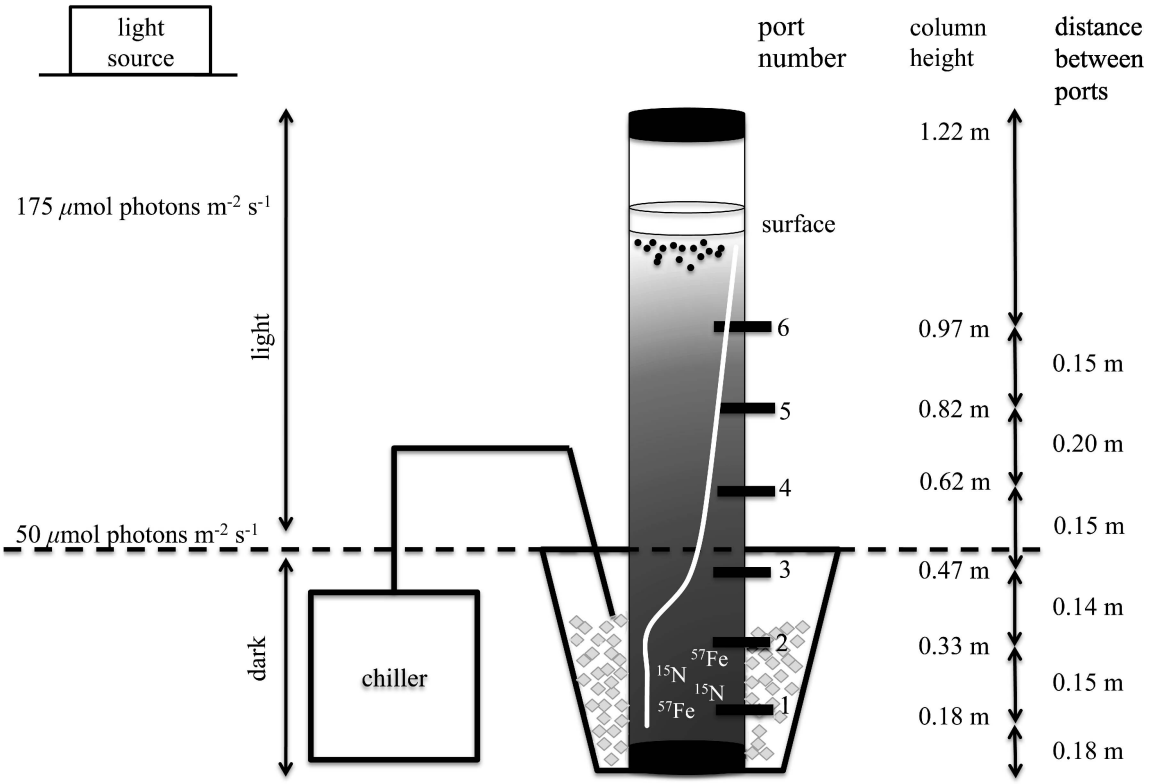

Fig. 1. Diagram of laboratory column used for all four experiments. The density profile is taken from observational data for T_0 of experiment 2. Density stratification was maintained either by the addition of ice (experiments 1, 3, and 4) or by a circulating water chiller (experiment 2). Stable isotopes were present in the bottom 5 liters of water. Sample ports are labeled with distances (m). The dotted line separates the light from the dark portion of the column.

of both nitrate and iron are intensified, leaving limited surface nutrients and a nutricline about 10–15 m below the surface (Ryan et al. 2010). These conditions often occur immediately following diatom blooms, when *A. sanguinea* and *P. micans* blooms are expected to occur (Horner et al. 1997; Matsubara et al. 2007; Du et al. 2011). Given the potential for limitation or colimitation of Fe during periods of nitrate and phosphorus (P) limitation, it would presumably be advantageous for vertically migrating phytoplankton to acquire both macro- and micronutrients at depth. Here we present the first evidence for two dinoflagellates (or any phytoplankton) actively migrating to acquire Fe from depth in stratified water columns under both nitrate-replete and nitrate-deficient conditions: *A. sanguinea* from laboratory cultures and a natural population of *P. micans* collected from Monterey Bay during a red tide event.

Methods

Experimental overview—Separate experiments were conducted to investigate vertical migration of two dinoflagellates, *A. sanguinea* and *P. micans*, to determine if N and Fe were being assimilated at depth and translocated to the surface as particulate biomass through vertical migration. A total of four experiments are presented. These were not set up to be true replicates but are stand-alone experiments employing different nutrient conditions. Experiments 1–3 were conducted with a monospecific culture of *A. sanguinea*, and experiment 4 employed water collected from a naturally occurring bloom of the dinoflagellate *P. micans*. For all four experiments, the bottom of the vertical column was enriched with ^{57}Fe, while each experiment had variations of nitrate (including ^{15}N-$NaNO_3$), P, and silicic acid.

Cultures—Nonaxenic cultures of *A. sanguinea* (MB1206) isolated from Monterey Bay were grown in 1 liter containers with sterile-filtered artificial seawater (ASW). ASW consisted of Instant Ocean basal salts at a salinity of 29–31. Cultures were maintained under 12 : 12 light : dark (LD) conditions using $\sim$ 100 μmol photons m^{-2} s^{-1} General Electric white-light fluorescent bulbs at a temperature of 16.5°C within an environmental chamber.

Experimental setup—The experimental system consists of a Plexiglas column 0.3 m diameter by 1.22 m height. The column was washed with 2 mol L^{-1} trace-metal–grade hydrochloric acid (HCl), rinsed with purified ($>$ 18 MΩ cm^{-1}) Milli-Q deionized water several times, then filled with pH 2 Milli-Q water until use. Six Luer-lock ports with attached silicone tubing were spaced 0.14–0.23 m apart, with port 1 at the bottom and port 6 at the surface (Fig. 1). Ports and tubing were cleaned with a series of acid baths (6 mol L^{-1} HCl for 48 h, then 3 mol L^{-1} nitric acid for 48 h) and rinsed and stored in pH 2 Milli-Q. For the experiments,

Table 1. Physical parameters, nutrient and Chl *a* concentrations for four experiments at T_0 for the bottom, middle, and top layers of the water column. All nutrients in the bottom layer were added before the middle and top layers were filled. Nutrients in the top layer were introduced with the addition of the culture (experiments 1–3) or natural water (experiment 4).

	Experiment 1 (surf. + N + P)			Experiment 2 (surf. − N − P)			Experiment 3 (bott. − N − P)			Experiment 4 (nat.)		
Parameters	Bott.	Mid.	Top	Bott.	Mid.	Top	Bott.	Mid.	Top	Bott.	Mid.	Top
Temperature	6	13	15	7	11	15	5	14	16	6	13	15
Salinity	32.0	31.5–29.5	29.0	34.5	34.5–29.5	29.0	33.5	33.5–30.0	30.0	32.5	32.5–30.0	30.0
Chl *a* (μg L^{-1})	0	0	62	0	0	128	0	0	64	0	0	213
Silicic acid (μmol L^{-1})	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6.5
Phosphorus (μmol L^{-1})	2.8	3.2	4.3	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.6
Nitrate (total) (μmol L^{-1})	23.8	1.3	58.0	25.8	1.8	3.4	1.1	1.7	1.5	88.1	2.1	2.3
NO_3	1.8	1.3	58.0	1.8	1.8	3.4	1.1	1.7	1.5	28.0	2.1	2.3
^{15}N-$NaNO_3$	22.0	0.0	0.0	24.0	0.0	0.0	0.0	0.0	0.0	60.1	0.0	0.0
Fe (total) (nmol L^{-1})	17.8	nd*	nd	26.9	nd	nd	nd	nd	nd	nd	nd	nd
Fe	7.8	nd	nd	6.9	nd	nd	nd	nd	nd	nd	nd	nd
^{57}Fe-$FeCl_3$	10.0	nd	nd	20.0	nd	nd	10.0	nd	nd	10.0	nd	nd
DFB (nmol L^{-1})	0.0	30.0	50.0	0.0	30.0	50.0	0.0	30.0	50.0	0.0	30.0	50.0

* nd, not determined.

the column was partitioned into three distinct density layers (modulating temperature and salinity; Fig. 1) by filling the column with ASW amended with nutrients as described in Table 1. Nutrients were added to the column in the bottom layer before the middle and surface layers were added. There were residual nutrients in the surface layer for each experiment, carried over from the addition of the culture or natural water (Table 1). The column was kept upright in an opaque plastic container filled with 5°C water covering the bottom 0.55 m (~ 8 liters; Fig. 1). The experiments were conducted in a temperature-regulated environmental chamber at 16°C on a 12 : 12 LD cycle. The bottom 0.55 m of the column was covered with black plastic to eliminate direct light. Lights were placed on a shelf about 0.3 m above and 1.0 m away from the top of the column, oriented perpendicular to the column. The gradient of light from the surface to the covered portion of the column ranged from 50 to 175 μmol photons m^{-2} s^{-1} with the highest light levels near the top of the chamber. Light levels were measured inside the column while it was filled with ASW (salinity = 29) without any phytoplankton added. Cells were added to the column by concentrating the *A. sanguinea* culture or *P. micans* bloom water to 250–500 mL, which was carefully poured over the back of a spoon to minimize vertical mixing.

Sampling—Replicate samples were not collected for all measurements due to water constraints but are noted in the results. All samples were collected through acid-cleaned silicone tubing attached to the ports or with an acid-cleaned seriological pipette from the surface and stored in either acid-cleaned syringes or acid-cleaned glass beakers.

Experiment 1 (surface nitrate and phosphorus replete: surf. +N +P): The purpose of this experiment was to determine if *A. sanguinea* would vertically migrate and assimilate nitrate and Fe from depth when nitrate and P are replete throughout the column but the surface layer and cells are limited for Fe. *A. sanguinea* MB1206 cultures were grown with L1 media from Provasoli-Guillard National Center for Culture of Marine Phytoplankton (CCMP; salinity = 29). Once stationary growth was reached (3 weeks), cultures were diluted every 5–7 d for 3 weeks (cultures were grown for a total of 6 weeks) by 1 : 1 dilution with ASW (no nutrient addition) for a total of four transfers. Thirty-six hours before the initiation of the vertical migration experiments, 50 nmol L^{-1} desferoximine-B (DFB, or desferral, an Fe chelator) was added to the cultures to bind any bioavailable Fe.

The column was filled with nutrient-deplete ASW using a peristaltic pump with acid-cleaned tubing at 5–20 mL min^{-1} to minimize mixing. There were three distinct layers set up with a density gradient, described here and in Fig. 1, with a summary in Table 1: bottom (0.5 m; salinity = 32), middle (0.5–0.7 m; salinity = 31.5–29.5), and surface (0.7–1.2 m; salinity = 29). The pycnocline was maintained by adding ice to the outside water bath every 2 h for the first 24 h and subsequently every 4–6 h, keeping a 7–9°C gradient between the surface and the bottom layer. The pycnocline was stable for 72 h as confirmed with periodic checks of salinity and temperature from the Luer-lock

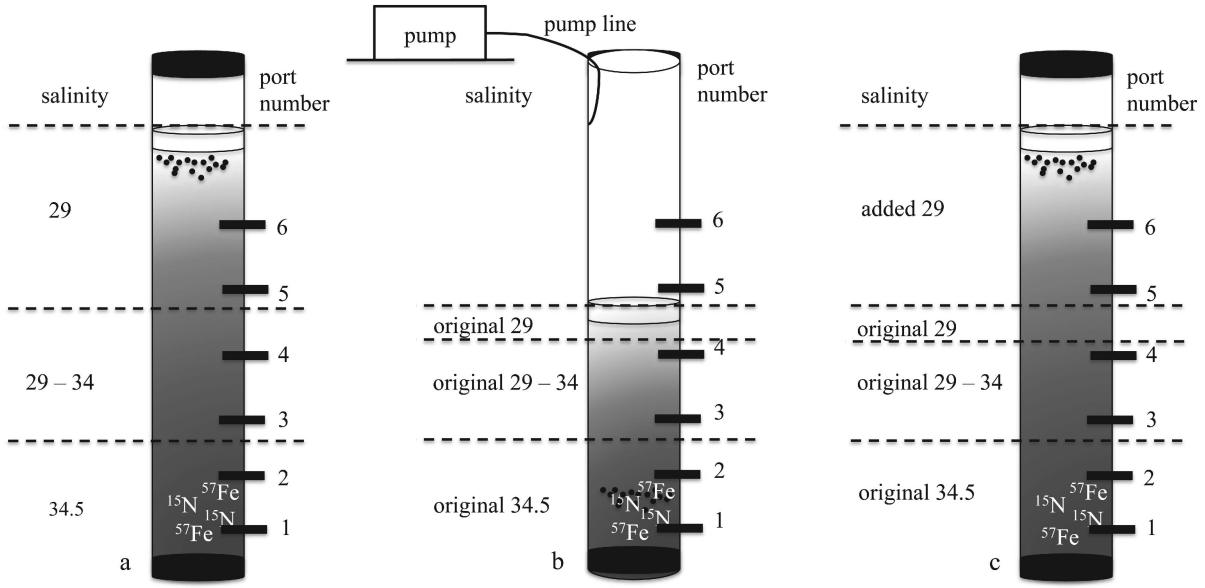

Fig. 2. Diagram of laboratory column used for experiment 2 (surf. − N − P). (a) describes the initial column setup with three distinct salinity layers, (b) describes the introduction of 29 salinity ASW at 72 h to the column to bring the water level back to the initial height, and (c) describes the column after the additional ASW has been added. Dotted lines differentiate between the separate salinity layers.

ports. Initial nutrient concentrations in each of the layers are described in Table 1.

Samples of 10–420 mL were collected in acid-cleaned syringes one-half hour before the experiment start (time zero [T_0]) from the surface, port 4 (middle), and port 1 (bottom; Fig. 1) as well as from the *A. sanguinea* culture. Chlorophyll *a* (Chl *a*) and inorganic nutrients (nitrate, P, and silicic acid) samples were collected hourly for the first 24 h and at 48 and 72 h from ports 1–5 and the surface. Samples were also collected hourly for the first 19 h from port 6, but from hours 20–72, the water level was below port 6. A Turner Designs Cyclops-7 fluorometer was used to measure raw fluorescence by holding it flat against the column and measuring the fluorescence at various points. Before the culture was added, initial fluorometer measurements were taken to establish a baseline. Total particulate Fe and N samples were taken at T_0 from the surface, port 1, and the *A. sanguinea* culture and again at 6, 12, 18, 24, 48, and 72 h from the surface and port 1. Dissolved Fe samples were collected from port 1 at T_0 and at 24 and 72 h. Temperature and salinity was measured periodically (about every 3–6 h) by taking a sample from each port and using a thermometer to determine the temperature and a refractometer to determine salinity.

Experiment 2 (surface nitrate and phosphorus deplete: surf. − N − P): The purpose of this experiment was to determine if *A. sanguinea* would vertically migrate to assimilate nitrate and Fe when the surface column and cells were nitrate, P, and Fe deplete. *A. sanguinea* MB1206 cultures were grown in 250 mL sterile containers with CCMP L1 media with nitrate, P, and Fe additions diluted to 1 : 100 standard L1 concentrations (all other nutrients kept the same; salinity = 29.5). Once in stationary growth, cultures were diluted 1 : 1 every 2–3 d with ASW and sampled daily for analysis of nitrate and P on a Lachat autoanalyzer until nitrate was < 2 μmol L^{-1}. Thirty-six hours before the initiation of the vertical migration experiments, 50 nmol L^{-1} DFB were added to the cultures.

The column was filled according to experiment 1, with three distinct layers (Fig. 1; Table 1): bottom (0.5 m; salinity = 34.5), middle (0.5–0.8 m; salinity = 34.5–29.5), and surface (0.7–1.2 m; salinity = 29; Table 1). The pycnocline was maintained with an external circulating chiller bath keeping a 6–8°C gradient between the surface and the bottom. The pycnocline remained stable under these conditions for 120 h. At 72 h, the column was replenished by peristaltic pump at 50 mL min^{-1} with 6 liters of nutrient-deplete ASW (salinity = 29; Fig. 2). Initial nutrient concentrations in each of the layers are described in Table 1.

Experiment 2 was sampled at T_0 as per experiment 1. Samples were collected with the same protocol as experiment 1 from ports 1–5 and the surface hourly for the first 24 h and again at hours 48, 72, 96, 102, and 120. Samples were also taken hourly for the first 13 h and for 72 and 96 h from port 6, but from hours 13–72 and 102–120, the water level was below port 6. At 72 h, 6 liters of nutrient-deplete ASW were added to the column (salinity = 29) to increase the water level; this is graphically depicted in Fig. 2. Dissolved Fe samples were collected from port 1 at T_0 and at 24 and 120 h. Temperature and salinity was measured periodically (about every 3–6 h) by taking a sample from each port and using a thermometer to determine the temperature and a refractometer to determine salinity.

Experiment 3 (surface and bottom nitrate- and phosphorus-deplete: bott. − N − P): The purpose of this experiment was to determine if *A. sanguinea* would vertically migrate to assimilate Fe in the absence of nitrate in the column. The cells were deplete for N, P, and Fe. *A. sanguinea* MB1206 cultures were grown with CCMP L1 media (salinity = 31). Once stationary growth was reached

(3 weeks), cultures were filtered onto 20 μm Nitex nylon mesh keeping a buffer of 20 mL of media so as not to dehydrate the cells and transferred into ASW (no nutrient addition) each day for 3 d. Twenty-four hours before the initiation of the vertical migration experiment, 50 nmol L^{-1} DFB were added to the cultures.

The column was filled according to experiment 1, with three distinct layers described here and summarized in Table 1 and Fig 1: bottom (0.5 m; salinity = 33.5), middle (0.5–0.7 m; salinity = 33.5–30.0), and surface (0.7–1.2 m; salinity = 30.0). The pycnocline was maintained by adding ice to the outside water bath every 2 h for the first 24 h and subsequently every 4–6 h, keeping a 7–9°C gradient between the surface and the bottom layer. The pycnocline was stable for 72 h as confirmed by salinity and temperature at the end of the experiment. Initial nutrient concentrations in each of the layers are described in Table 1. No enriched N (^{15}N-$NaNO_3$) was added to the bottom water of this experiment.

Experiment 3 was sampled at T_0 as per experiment 1. Chl *a* and inorganic nutrients were collected from ports 1–6 and the surface hourly for the first 13 h, then from ports 1–5 and the surface hourly until 17 h, and then every 2 h until hour 24 from ports 1–4 and the surface as the water level decreased. Total particulate N and Fe samples were taken from port 2 and the surface hourly for the first 17 hours, and then every 2 hours until 24 hours. The fluorometer was not used in this experiment, and dissolved Fe samples were not collected. Temperature and salinity were determined by a Yellow Springs Instrument Company (YSI) conductivity meter after the experiment was complete (so as not to disturb the water column).

Experiment 4 (natural bloom; nat.): The purpose of this experiment was to determine if a natural bloom of *P. micans* would vertically migrate to assimilate nitrate and Fe from depth. Water was collected from the surface using a clean plastic bucket from the Santa Cruz Municipal Wharf (36°57.48′N, 122°81.02′W) on 23 October 2010 during a large red tide event of the dinoflagellate *P. micans*. Other phytoplankton were present in rare numbers (< 1% relative abundance as determined by microscopy using a dissecting scope), but the bloom was dominated both numerically and as biomass by *P. micans*. The water was kept in a 20 liter plastic container at 16.5°C in an environmental chamber for 7 h before the experiment. Three hours before the initiation of the vertical migration experiment, 50 nmol L^{-1} DFB were added to 200 mL of the bloom water.

The column was filled according to experiment 1, with three distinct layers described here and in Table 1 and Fig. 1: bottom (0.5 m; salinity = 34.5), middle (0.5–0.7 m; salinity = 34.5–30.0), and surface (0.7–1.2 m; salinity = 30.0; Table 1). The pycnocline was maintained by adding ice to the outside water bath every 2 h for the first 24 h and subsequently every 4–6 h, keeping a 7–9°C gradient between the surface and the bottom layer. The pycnocline was stable for 24 h as confirmed with salinity and temperature at the end of the experiment. Initial nutrient concentrations in each of the layers are described in Table 1.

Experiment 4 was sampled at T_0 as per experiment 1. Chl *a* and inorganic nutrients were collected from ports 1–6 and the surface every 3 h for the first 18 h, then every 6 h from ports 1–5 and the surface until 72 h. Total particulate N and Fe samples were taken from port 1 and the surface every 6 h. The fluorometer was not used in this experiment, and dissolved Fe samples were not collected. Temperature and salinity were determined by a YSI conductivity meter after the experiment was complete (so as not to disturb the water column).

Analysis—Chl *a* samples were filtered onto glass-fiber filters (GF/F; Whatman), extracted in 90% acetone, and analyzed on a Turner 10-AU fluorometer. For experiment 1, Chl *a* samples were collected hourly for the first 24 h but were lost during sampling and storage after hour 6. The Cyclops was calibrated to the collected Chl *a* (experiments 1 and 2), and raw fluorescence was converted to Chl *a*. For experiments 1 and 2, we report the fluorescence data converted to Chl *a* since this is the most complete record of biomass from the experiments and because the fluorometer provided fluorescence readings between ports, removing the error of "disappearing" Chl *a* (cells between sampling ports; Cullen and Horrigan 1981; Doblin et al. 2006). The Cyclops was not used for experiments 3 and 4, and Chl *a* is reported (Fig. 2).

Nitrate and P samples were collected from the Chl *a* filtrate, frozen at −20°C for 48 h, and then analyzed using a Lachat autoanalyzer. Total particulate N samples were filtered onto combusted (450°C for 5 h) GF/F filters and frozen at −20°C until analyzed using a ThermoFinnigan Delta Plus XP isotope ratio mass spectrometer for particulate nitrogen and isotopes (Dugdale and Wilkerson 1986). Total particulate Fe samples were filtered onto 0.4 μm polycarbonate (Poretics) 45 mm acid-cleaned filters and frozen at −20°C until analysis for leachable particulate Fe (bioavailable) and Fe isotopes by a Thermo Element XR Magnetic Sector inductively coupled plasma mass spectrometry (ICP-MS; Berger et al. 2008). Dissolved Fe samples were collected by acid-cleaned syringe, stored in acid-clean high-density polyethylene bottles, acidified to pH 2, and refrigerated at 4°C until analysis by ICP-MS (Biller and Bruland 2012).

Statistical analyses—Differences between initial and final values were calculated as a one-sample, one-tailed *t*-test. Integrated nutrient drawdown was analyzed using regression in SigmaStat version 6.0. Significance levels for statistical tests were set at $p = 0.05$.

Results

Vertical migration—Between 62 and 213 μg L^{-1} Chl *a* was initially added to the surface (Table 1) as either an *A. sanguinea* culture (experiments 1–3) or a *P. micans*–dominated natural bloom from Monterey Bay. Experiment 1 (surf. + N + P) and experiment 2 (surf. − N − P) had 250 mL of culture added, experiment 3 (bott. − N − P) had 250 mL of concentrated culture added, and experiment 4 (nat.) had 500 mL of natural water added. The first 24 h of Chl *a* for experiments 1 and 2 is presented in Fig. 3 and experiments 3 and 4 in Fig. 4. In all cases, cells began migration immediately, reaching the bottom port (port 1; 0.18 m above the bottom) between 3 and 4 h, with the bulk of the Chl *a*

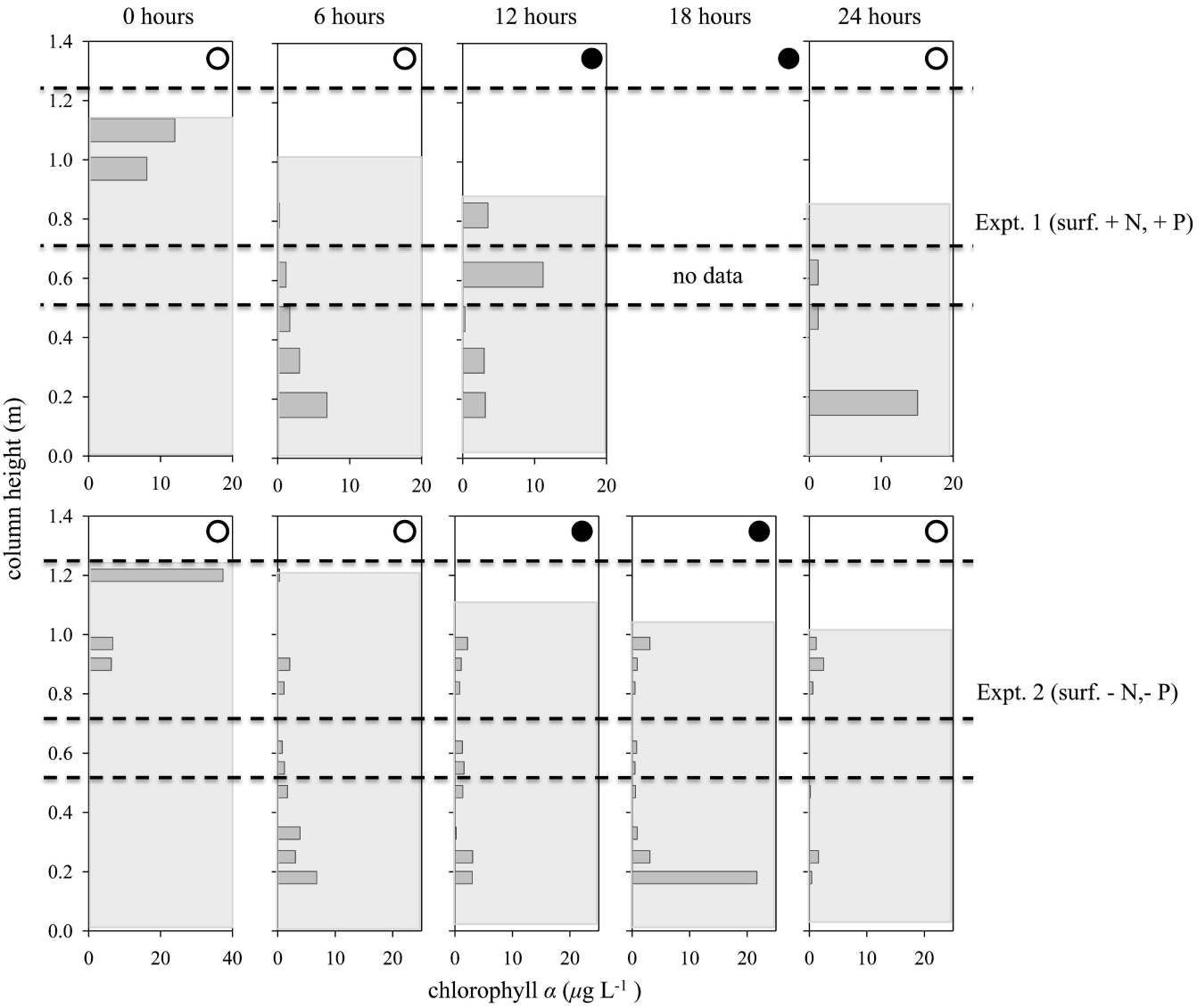

Fig. 3. Distribution of phytoplankton biomass for experiment 1 (surf. + N + P) and experiment 2 (surf. − N − P) for the first 24 h. Biomass is Chl *a* (μg L^{-1}) determined from the fluorescence proxy measured by the Cyclops-7 fluorometer. Experiment 2 measurements are in higher resolution than the other experiments. The shaded background indicates the water level within the column. Note the differences in biomass axes. The different salinity layers are indicated with dotted lines. Light and dark times are indicated with the presence of an open circle (light) or a closed circle (dark).

measured at port 1 for experiments with the *A. sanguinea* culture and split between port 1 and the surface for the *P. micans* natural bloom for the remainder of the experiment (Fig. 2). The pattern of Chl *a* was different for each experiment, but bulk migration resulted in elevated biomass at the surface 8–16 h after the experiment began. With the return migration, there was typically a remainder of Chl *a* present at the bottom of the column and directly above the nutricline at the light : dark interface (near port 4; 0.62 m above the bottom of the column). There were some nonmotile phytoplankton present in the *P. micans* experiment.

Nitrogen—The initial and experiment end sample values for nitrate drawdown from the surface and ports 1 and 2 (isotopically enriched water), total integrated nitrate from the column, total particulate surface N μg L^{-1}, ^{15}N % atom enrichment, and particulate surface μg N normalized to μg Chl *a* are provided in Table 2. Drawdown of nitrate for experiment 1 (surf. + N + P), experiment 2 (surf. − N − P), and experiment 4 (nat.) was accelerated for hours 12–72, with the greatest depletion recorded within the N and Fe isotope enriched bottom water at port 2 (0.33 m from the bottom; Fig. 5). Trapezoidal integration of the total column nitrate for experiment 1 (surf. + N + P) provided estimates of 30.0 mmol m^{-2}, 20.8 mmol m^{-2} for experiment 2 (surf. − N − P), and 51 mmol m^{-2} for experiment 4 (nat.). Drawdown of nitrate for experiment 3 (bott. − N − P) was minimal (1.5 mmol m^{-2}), as expected, since there was no additional nitrate added to the bottom. All of the integrated nitrate drawdown was statistically

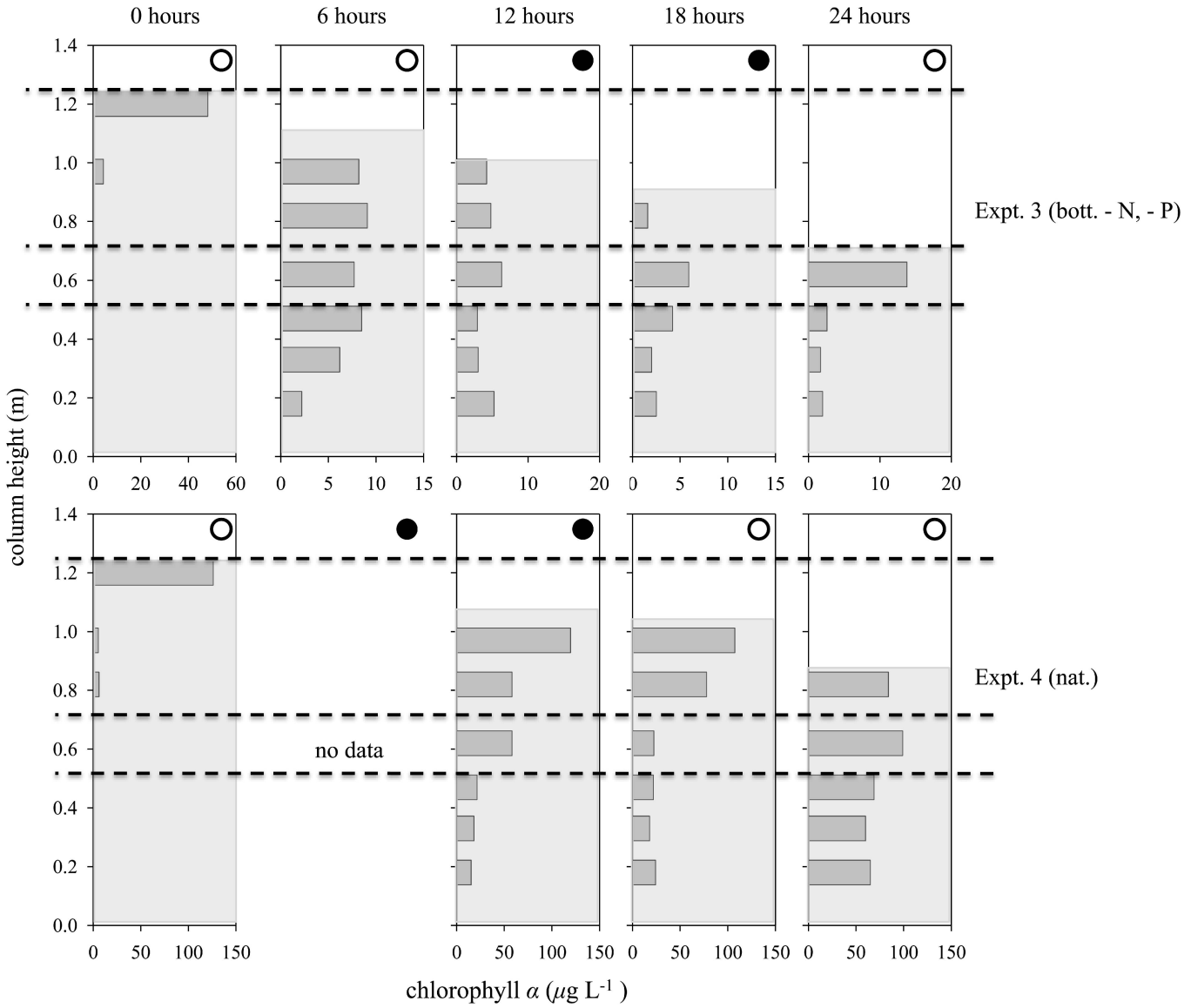

Fig. 4. Distribution of phytoplankton biomass for experiment 3 (bott. − N − P) and experiment 4 (nat.). Biomass is Chl *a* (μg L^{-1}). The shaded background indicates the water level within the column. Note the differences in biomass axes. The different salinity layers are indicated with dotted lines. Light and dark times are indicated with the presence of an open circle (light) or a closed circle (dark).

significant based on linear regression determination, and the significant data are presented in Table 4.

Total particulate N from the surface for all experiments increased with time, though there was a wide range of initial and end values (Table 2). Samples taken from the bottom ports (either port 1 or port 2) were always enriched, and we suspect that a mixture of senescent, dead, or detrital particles from the initial inoculation, along with viable migrating cells, were sampled. The greatest increase in particulate surface N was seen in experiment 4 (nat.), corresponding to $>$ 50 mmol m^{-2} of nitrate drawdown from the column. The surface particulate μg-atom N L^{-1} normalized to μg Chl *a* increased with time for all experiments, though there was variation in the enrichment across experiments (Table 2). All end values were statistically significantly enriched compared with the combined average initial values (Table 4).

There was measurable ^{15}N enrichment of the surface samples over time for experiments 1–2 and 4, excluding experiment 3, where no ^{15}N was added to the column (Tables 1 and 2; Fig. 6). Experiments 2 and 4 had a 3-fold increase in atom % enrichment, and the increase in experiment 4 was statistically significant compared to the initial starting % atom enrichment (Table 4). Experiment 1 (surf. − N − P) was also statistically significant, with a 2-fold increase compared to the initial value (Tables 2 and 4). Samples taken from the bottom ports (either port 1 or port 2; Fig. 1) were enriched from T_0 and do not correspond with the vertical migration of cells.

Iron—The initial and experiment end sample values for total particulate surface Fe ng L^{-1}, ^{57}Fe % atom enrichment, and particulate surface ng Fe normalized to μg Chl *a* are given in Table 3. Only experiments 1 (surf. + N

Table 2. Comparison of N at T_0 and the experiment end (T_{end}) for each of the four experiments. Nitrate samples are given for the two bottom ports and the surface. Replicate samples with standard deviation are given in parentheses where appropriate.

	Experiment 1 (surf. + N + P)		Experiment 2 (surf. − N − P)		Experiment 3 (bott. − N − P)		Experiment 4 (nat.)	
Parameters	T_0	T_{end}†	T_0	T_{end}‡	T_0	T_{end}§	T_0	T_{end}†
NO_3 (μmol L^{-1})								
P1‖	23.8	20.4 (0.2)	26.9 (0.2)	17.1 (0.1)	1.0	1.3	88.1 (0.3)	23.4
P2¶	21.9	11.4	24.7 (0.1)	2.5 (0.0)	1.6	1.1	86.0	2.3
Surface	58.0 (0.1)	45.8 (0.0)	3.4 (0.1)	1.9 (0.1)	1.5	1.7	6.8	0.0
Total NO_3 integrated (mmol m^{-2})	47.0	26.2*	12.3	5.7*	2.9	1.4*	58.1	7.6*
Total surface particulate N μg L^{-1}	3.7	5.3*	2.3	4.7*	0.8	19.2*	6.2	74.0*
Total surface particulate μg N normalized to μg Chl *a*	0.2	9.8*	1.2	73.1*	0.3	12.5*	0.25	0.73*
^{15}N % atom enrichment	0.36	0.51* (0.01)	0.36 (0.00)	1.538 (0.38)	0.38 (0.00)	0.38	0.37 (0.00)	1.03*

* $p<0.05$.
† 72 h.
‡ 120 h.
§ 24 h.
‖ 0.18 m from the bottom within the isotope-enriched water.
¶ 0.33 m from the bottom within the isotope-enriched water.

+ P) and 2 (surf. − N − P) had dissolved Fe samples collected. The concentration of dissolved Fe for experiment 1 (surf. + N + P) was initially 7.8 nmol L^{-1} while experiment 2 (surf. − N − P) was 6.9 nmol L^{-1}. Subsequent sampling for dissolved Fe (at the 24 and 72 h time points) were contaminated, likely during the filtering process, as 20–50 times more Fe was measured compared to the initial samples (Table 1). For all experiments, particulate surface Fe ng L^{-1} increased with time. Experiment 4 (nat.) had an initial concentration of 75.1 ng L^{-1} Fe compared to initial concentrations of 5.1–11.5 ng L^{-1} Fe for the other three experiments, but the total increase was similar. The surface particulate ng Fe normalized to μg Chl *a* increased with time for experiments 1–3 in varying amounts. Again, the initial and end values were different for all experiments, with a 7-fold-less increase for experiment 2 (surf. − N − P) compared to experiment 1 (surf. + N + P) and experiment 3 (bott. − N − P). The final value for experiment 4 (nat.) was not elevated compared to the initial concentration, though the greatest increase was seen at 18 h with 2.52 ng Fe (μg Chl *a*)$^{-1}$ (Table 3). Statistical data are presented in Table 4.

Since the amount of Fe to Chl *a* can be variable with the amount of Chl *a* present in the cell, of more importance is the ratio of ^{57}Fe to ^{56}Fe. There was measurable ^{57}Fe enrichment for all of the experiments. Experiment 1 (surf. − N − P) had the least amount of ^{57}Fe % atom enrichment from the initial sample to the end (0.05% increase), but the highest recorded enrichment was 3.89% at hour 18. The discrepancy in the enrichment is likely related to the migration of the cells, where the greatest portion of Chl *a* was seen at the bottom or above the nutricline after 12 h (rather than the surface). The change in enrichment for experiment 1 (surf. + N + P) was nearly significant, and the change in enrichment for experiments 2–4 were statistically significant (Table 4), where experiment 4 (nat.) had the greatest % atom enrichment at 4.05% compared to the initial value of 2.21% (Tables 3 and 4; Fig. 7).

Phosphorus and silicic acid—Experiment 1 (surf. + N + P) was replete for P throughout the column (2.8 μmol L^{-1} at the bottom of the column and an increasing gradient to 4.3 μmol L^{-1} at the surface). The integrated drawdown was 0.6 mmol L^{-1} over 72 h. Experiment 4 (nat.) was also replete for P and had 0.6 μmol L^{-1} of phosphorus at the surface and 0.2 μmol L^{-1} at the bottom and middle of the column, and integrated drawdown was 0.3 mmol L^{-1} over 72 h. Only experiment 4 (nat.) was replete for silicic acid and had 6.5 μmol L^{-1} at the surface initially. The integrated drawdown was 1.1 mmol L^{-1} over 72 h, attributable to the nondinoflagellate cells in the water. Experiment 1–3 were not grown in media with silicic acid, and experiments 2 and 3 did not have additional P so as to simulate bloom conditions where all macro- (and micro-) nutrients would be depleted.

Discussion

Many dinoflagellates vertically migrate to assimilate nitrate from depth, and our results are consistent with

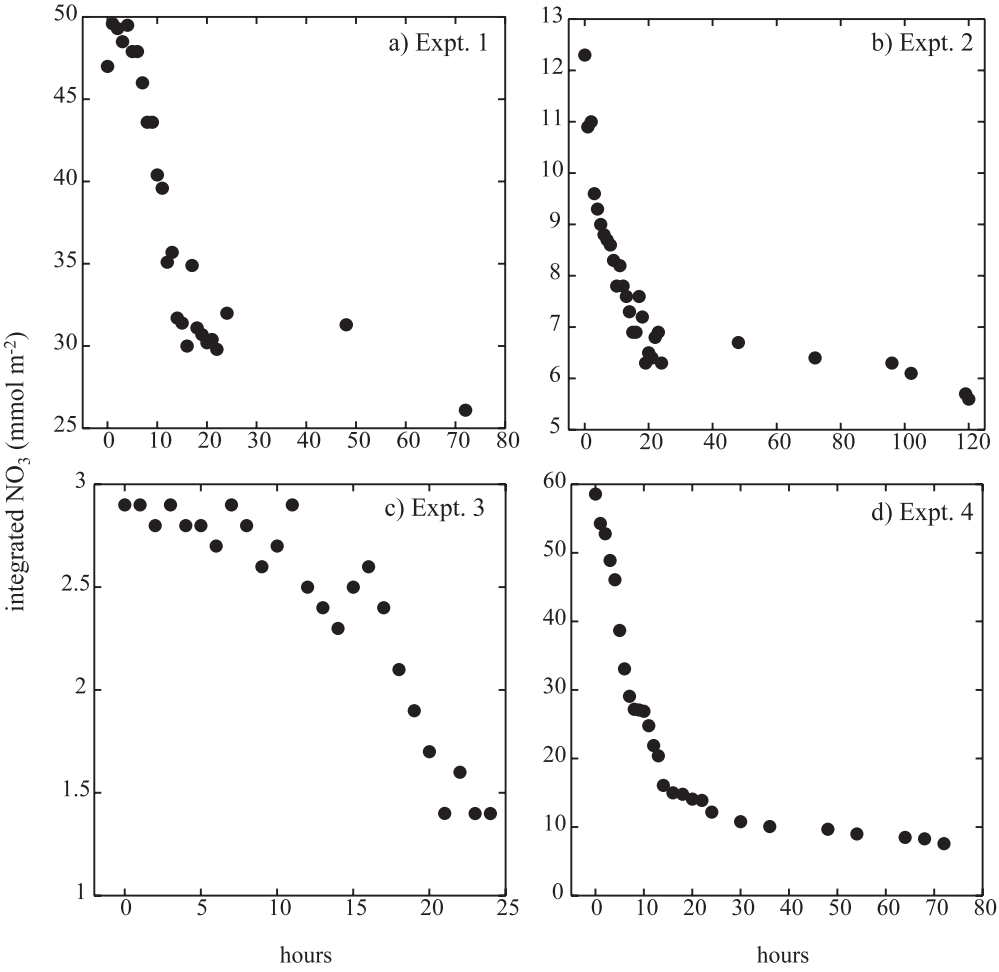

Fig. 5. Integrated NO_3 (mmol m^{-2}) drawdown determined by trapezoidal integration in the column for (a) experiment 1 (surf. + N + P), (b) experiment 2 (surf. − N − P), (c) experiment 3 (bott. − N − P), and (d) experiment 4 (nat.).

previous reports for *A. sanguinea* (Cullen and Horrigan 1981; Kamykowski 1981) and *P. micans* (Kamikowski 1981). Here, we document the assimilation of N and Fe during vertical migration by two dinoflagellate species. Previous N assimilation experiments have been supported by in situ observations (Cullen and Horrigan 1981; MacIntyre et al. 1997; Smayda 2010), and we assume that experiment 2 (surf. − N − P) and experiment 3 (bott. − N − P), laboratory-based Fe assimilation experiments during vertical migration, are similarly representative of dinoflagellates in the environment. All four experiments had appropriate temperature and salinity gradients for what would be expected of natural waters of Monterey Bay given that the column is only 1.2 m in height, while the nutricline in Monterey Bay is typically 15–20 m below the surface (Johnson et al. 1999; Bruland et al. 2001; Ryan et al. 2010). The atom % enrichment of ^{57}Fe and ^{15}N at the surface and the drawdown of nitrate at depth presents convincing evidence that *A. sanguinea* and *P. micans* are assimilating Fe and nitrate before returning to the surface. These results therefore suggest that *A. sanguinea* and *P. micans* under nitrate- and Fe-deficient surface conditions are capable of sustaining growth through vertical migration.

Vertical migration—A combination of factors can influence the start and completion of vertical migration while still allowing for the assimilation of nitrate from depth. These include changes in irradiance (Kamykowski 1981), anoxia (Whittington et al. 2000), density gradients (Kamykowski 1981), influence of other nonalgal particulates (Doblin et al. 2006), toxins (MacIntyre et al. 1997), and predator avoidance (Park et al. 2002). The mechanism is further complicated by internal factors such as circadian rhythm, growth stages, and internal orientation (e.g., phototaxis and geotaxis; Kamykowski et al. 1999). For these experiments, not all the cells were migrating to depth, especially experiment 1 (surf. + N + P) and experiment 4 (nat.), or were not migrating synchronously, indicating that under these conditions, vertical migration was not regulated solely by nitrate concentration. This has been reported with *A. sanguinea* (Cullen and Horrigan 1981), *P. micans* (Kamykowski 1981), and other dinoflagellates, such as *Lingulodinium polyedrum* (Heaney and Eppley 1981), *Heterocapsa niei* (Eppley et al. 1968; Kamykowski 1981), and *Alexandrium tamarense* (MacIntyre et al. 1997).

Both of the dinoflagellates in these experiments migrate in the field (Kamykowski 1981; Ryan et al. 2010; Du et al.

Table 3. Comparison of Fe at T_0 and the experiment end (T_{end}) for four experiments. Replicate samples with standard deviation are given in parentheses where appropriate.

Parameters	Experiment 1 (surf. + N + P)		Experiment 2 (surf. − N − P)		Experiment 3 (bott. − N − P)		Experiment 4 (nat.)	
	T_0	T_{end}†	T_0	T_{end}‡	T_0	T_{end}§	T_0	T_{end}†
Total surface particulate ng L^{-1} Fe	5.1	54.1	11.5	21.3*	5.3	22.1*	75.1	127.5*
Total surface particulate ng Fe normalized to μg Chl *a*	0.02	20.80*	0.06	3.53*	1.91	25.11*	0.70	0.63*
^{57}Fe % atom enrichment	2.20	2.25	2.33 (0.00)	3.23*	2.16 (0.04)	2.99*	2.21	4.05*

* $p<0.05$.
† 72 h.
‡ 120 h,
§ 24 h.

Table 4. Statistical *t*, *p*, and degrees of freedom (df) values for experiment 1 (surface nitrate and phosphorus replete), experiment 2 (surface nitrate and phosphorus deplete), experiment 3 (surface and bottom nitrate and phosphorus deplete), and experiment 4 (natural bloom). Total integrated nitrate statistics were computed with linear regression; all others were a one-sample, one-tailed *t*-test.

Experiment	Total NO_3 integrated (mmol $m^{-2)}$	Total surf part. μg L^{-1} N	Total surf part. μg N normalized to μg Chl *a*	^{15}N % atom enrichment	Total surface part. ng L^{-1} Fe	Total surf part. ng Fe normalized to μg Chl *a*	^{57}Fe % atom enrichment
1							
t-value	4.900	2.161	3.330	5.606	−0.288	14.375	1.842
df	26	6	4	6	4	4	4
p-value	<0.001	0.037	<0.0001	0.007	0.394	<0.0001	0.070
2							
t-value	5.083	13.795	2.896	1.415	−2.006	−2.077	6.476
df	29	8	8	8	9	9	9
p-value	<0.001	<0.0001	0.010	0.097	0.038	0.034	<0.0001
3							
t-value	4.919	6.176	4.375	nd*	−5.095	2.974	2.247
df	22	12	12	nd	11	11	11
p-value	<0.001	<0.0001	0.001	nd	0.002	0.006	0.002
4							
t-value	6.801	7.816	2.654	7.285	3.452	−5.499	5.665
df	23	10	6	10	8	6	8
p-value	<0.001	<0.0001	0.002	0.002	0.004	0.002	<0.0001

* nd, not determined.

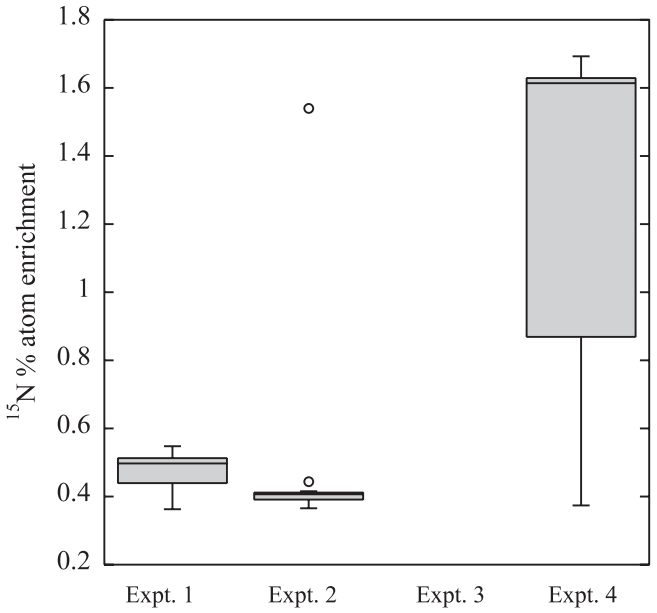

Fig. 6. Box-and-whiskers plot of ^{15}N % atom enrichment of particulate surface samples for experiment 1 (surf. + N + P), experiment 2 (surf. − N − P), and experiment 4 (nat.). Experiment 3 (bott. − N − P) did not include enriched nitrogen. Experiments 2 and 4 have a statistically significant enrichment compared to the initial enrichment (see Table 4). Whiskers indicate the minimum and the maximum, and outliers are plotted as singular points.

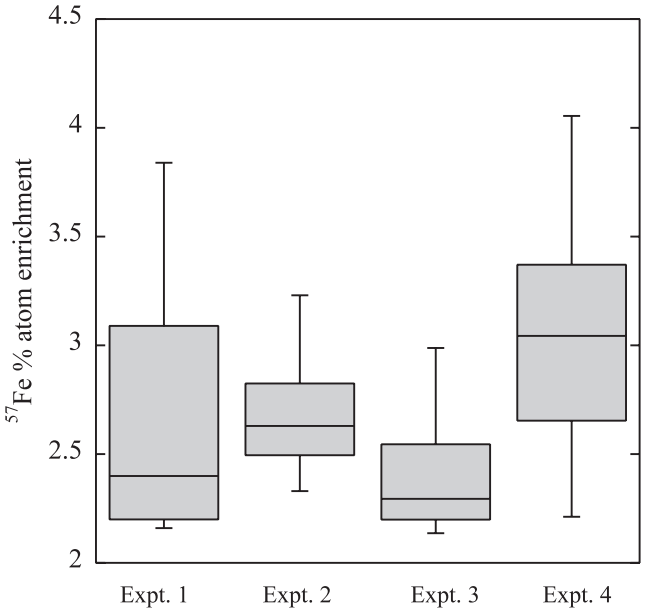

Fig. 7. Box-and-whiskers plot of ^{57}Fe % atom enrichment of particulate surface samples for experiment 1 (surf. + N + P), experiment 2 (surf. − N − P), experiment 3 (bott. − N − P), and experiment 4 (nat.). Experiments 2, 3, and 4 have a statistically significant enrichment compared to the initial enrichment (see Table 4). Whiskers indicate the minimum and the maximum, and outliers are plotted as singular points.

2011) and under various conditions in the lab (Cullen and Horrigan 1981; Kamykowski 1981; Kamykowski et al. 1992). These four experiments established that *A. sanguinea* in culture and field samples of *P. micans* migrated over a range of pycnocline, light, and nutrient conditions. Swimming speeds for *A. sanguinea* vary from 0.41 to 1.08 m h^{-1} (Kamykowski et al. 1992; Smayda 2010), with the descent faster than the ascent (Kamykowski et al. 1992) and with no explicit light : dark pattern to the migration (Cullen and Horrigan 1981). Swimming descent speeds documented here, at least 0.53 m h^{-1}, are consistent with this reported range. Only one experiment was completed with *P. micans*, but the descent rate of at least 0.4 m h^{-1} is similar to the reported rate of 0.47 m h^{-1} (Smayda 2010). The pattern of cell migration varied with the nutrient concentration in the column, with more active migration when the column was depleted of nitrate (experiments 2–4), consistent with prior studies (Doblin et al. 2006).

Strong vertical migration was observed in all experiments regardless of surface nitrate concentrations. For experiment 2 (surf. − N − P), cells (fluorescence) were not observed in abundance at the bottom port until after 72 h. This is likely due to a combination of a strong halocline (Olsson and Granéli 1991) and phosphorus stress, which could reduce the production of adenosine triphosphate and decrease the ability of the cells to migrate (Heaney and Eppley 1981). Experiment 3 (bott. − N − P) did not appear to be hindered by deplete P throughout the column, with the cells (fluorescence) reaching the bottom port by hour 4. *A. sanguinea* is often mixotrophic in the absence of available nutrients (Jeong et al. 2005), which could explain why the lack of P did not appear to retard migration. Even though the migration to the bottom port was delayed for experiment 2 (surf. − N − P), much of the fluorescence was in the nutricline, where drawdown of nitrate was also observed (Table 2). Once the halocline had relaxed, fluorescence increased at port 1. This flexible adaptation to changing conditions presumably allows *A. sanguinea* to thrive and enhance its growth potential in a temporally and spatially dynamic environment where light and nutrients are separated but accessible.

Nutrient acquisition at depth—Our results indicate that both nitrate and Fe were assimilated at depth with subsequent transfer to the surface as enriched particles (Tables 2, 3; Figs. 6, 7). It is well established that dinoflagellates migrate and incorporate inorganic nutrients at depth (Smayda 2010), and it has been speculated that along with nitrate and P, Fe is also acquired (Salonen and Rosenberg 2000; Naito et al. 2005). In situ experiments to determine macronutrient uptake during migration are difficult to execute (MacIntyre et al. 1997; Matsubara et al. 2007), and this difficulty increases for trace metals. Our results indicate that assimilation of nitrate and Fe occurred even when *A. sanguinea* was not strongly nutrient limited. This has been documented for nitrate with other dinoflagellates (Olsson and Granéli 1991; Doblin et al. 2006), but some reports suggest that nutrient limitation is required before vertical migration to depth is triggered (Paasche et al. 1984). We found that *A. sanguinea* migrated regardless of N deficiency, and while there was nitrate and Fe assimilation at depth for all experiments, enrich-

ment increased with the extreme nutrient stress seen in experiment 2 (surf. − N − P; Tables 2, 3; Figs. 6, 7). This is presumably due to the preexperiment nutrient starvation of the cells. The total particulate Fe samples from the bottom port exhibited enhanced atom % enrichment compared to the initial culture, though it was not possible to clearly identify this enrichment as intracellular since Fe may attach extracellularly (Hudson and Morel 1989). Loosely bound extracellular Fe would react with the DFB in the upper column, so surface particulate samples (after vertical migration) are presumably representative of intracellular enrichment (Hudson and Morel 1989). Sinking particles (i.e., senescent or dead cells) introduced with the addition of the culture at T_0 may also explain enhanced enrichment seen at the bottom port if these particles served to adsorb and concentrate the isotopes.

Evidence for vertical migration under Fe stress has been based primarily on conjecture and Fe requirements for phytoplankton, combined with their documented ability to incorporate N and P from depth (Salonen and Rosenberg 2000; Naito et al. 2005). Naito et al. (2005) determined growth yields with insoluble Fe ($FePO_4$ and FeS) for high-biomass (''red tide'') species (including five species belonging to Dinophyceae), demonstrating that the phytoplankton were able to utilize species of insoluble Fe that are associated with resuspended sediments or bottom water. This suggests that motile phytoplankton with access to bottom water may use insoluble Fe, supporting blooms when soluble Fe is insufficient to support the often very high surface biomass. Furthermore, *A. sanguinea* has a comparatively large cellular Fe requirement compared to neritic phytoplankton of similar size (Doucette and Harrison 1990), and if Fe is not available in the surface, it must be acquired from another source.

Our experimental enrichment rates were variable, based on a multitude of factors. Experiments 1 and 4 were significant for N enrichment, and experiments 2–4 were significant for Fe enrichment (Table 4). Experiments 1, 2, and 4 had a marginal relaxation of the pycnocline, between 48 and 102 h. For experiment 1 (surf. + N + P), the 48 and 72 h surface enrichments for both ^{15}N and ^{57}Fe are less than the 12–48 h time points. It is possible that the 48 and 72 h surface time points were dominated by cells that had not migrated, as most of the fluorescence was seen at the bottom ports, or cell division of *A. sanguinea* may have occurred, diluting the ^{15}N and ^{57}Fe signal. Cell counts were not completed during these experiments due to water volume constraints, as cell density became quickly diluted in the 24 liters of water in the column.

The lack of migration by some of the cells would be consistent with nitrate stress acting as a migratory trigger, as the column was not nitrate deficient and the cells were nitrate replete prior to the experiment. The presence of a strong halocline can trigger vertical migration, or, in experiments 2 and 3, where the columns were nitrate deficient, it may have slowed the rate of vertical migration, as cells will often form in tight bands above or below the halocline (Olsson and Granéli 1991; Hall and Pearl 2011), which could explain the slower rates of atom % enrichment for both nitrate and Fe for experiment 2 (surf. − N − P; Figs. 6, 7). It could also be a result of cell health, as the cells were P and Fe limited, which can slow both migratory rates (Heaney and Eppley 1981) and nitrate assimilation since Fe is needed for many of the nitrate and nitrite reductase assimilatory enzymes (Doucette and Harrison 1991). There was variation in the rate and amount of isotope uptake, which is expected based on the differences in experimental designs and the variable nutrient stress experienced during each experiment. For all experiments, total particulate surface N and the particulate N normalized to Chl *a* increased with time for the surface samples, though this measurement can be imprecise, as Chl *a* per cell content can change. For experiments 1–3, total particulate surface Fe and the particulate Fe normalized to Chl *a* had a subgroup of surface cells that did not appear to migrate because they included either nonmigrating *P. micans* or nonmotile phytoplankton, thus diluting the total particulate surface Fe : Chl *a* ratio. The increase in enriched particles detailed here is consistent with alleviation of N and Fe stress (Tables 2, 3, Figs. 6, 7). The clear drawdown of nitrate from depth for all experiments is in agreement with these observations, consistent with previous experiments that determined assimilation of intracellular nitrate during migration (Fig. 5; Doblin et al. 2006).

Ecological implications—Understanding dinoflagellate behavior and autecology is key to predicting dinoflagellate dominance and particularly important for those species considered HAB organisms. Our results advance our understanding of *A. sanguinea*, *P. micans*, and dinoflagellate adaptations leading to a competitive advantage during low-nutrient, stratified conditions when vertical migration provides a favorable strategy for acquisition of both N and Fe. HAB organisms from upwelling systems can often be prolific during periods of both low- and high-nitrate pulses (Kudela et al. 2010), making it difficult to determine the exact nutrient requirements that precede bloom events. Matsubara et al. (2007) reported optimal growth for *A. sanguinea* at a salinity of 20, under high light, and in temperatures of 25°C, producing a growth rate of 0.4 m d^{-1}. This indicates that the species does well in warm, stratified, lower-salinity water conditions. The strain MB1206 used in experiments 1–3 exhibits similar growth rates of 0.4 m d^{-1} when grown in a salinity of 35 at 25°C (Boyd et al. 2013). The growth rate for the *P. micans* used in experiment 4 is unknown, but *P. micans* can have a maximum growth rate of 0.99 d^{-1} in 18°C ± 10°C water (Costas 1990). In the California Current System, both dinoflagellates are associated with stratified, low-salinity, warm surface waters (Kudela et al. 2008; Ryan et al. 2010) and a relaxation of upwelling (Du et al. 2011) that typically occurs seasonally in the late summer and autumn. *A. sanguinea* often blooms immediately after diatom blooms (Kudela et al. 2008; Du et al. 2011), and Matsubara et al. (2007) described *A. sanguinea* as being suppressed when grown in cultures with *Chaetoceros* and *Skeletonema* spp.

Current modeling efforts include the average swimming patterns of dinoflagellates (Yamazaki et al. in press), but it is important to model the actual complex swimming

behaviors, which can be influenced by internal as well as external factors. Modeling behavior of dinoflagellates demonstrates that they will often undertake vertical migration when N stressed (Kamykowski and Yamazaki 1997; Ji and Franks 2007), but models including vertical migration for Fe requirements have not been tested, even though the importance of including Fe in modeling bulk primary productivity is well established. While most vertical migration nutrient experiments have focused on the assimilation of nitrate, studies have shown that phytoplankton are able to acquire P (Watanabe et al. 1988) and ammonium (Hall and Pearl 2011) under nutrient-limiting conditions. Therefore, it is not surprising that the dinoflagellates *A. sanguinea* and *P. micans* would actively migrate and assimilate Fe from depth under Fe-limiting conditions, providing an additional competitive advantage for these slow-growing dinoflagellates in seasonally high-nutrient low-Chl *a* waters.

Acknowledgments

We thank D. Biller and R. Franks for inductively coupled plasma mass spectrometry and D. Andreasen for isotope-ratio mass spectrometry assistance. We thank two anonymous reviewers for their valuable comments on the manuscript.

Funding was provided by the Earl and Ethel Meyers Foundation (MBP) and the National Oceanic and Atmospheric Administration Ecology and Oceanography of Harmful Algal Blooms (ECOHAB) program, award number NA11NOS4780030 (RMK).

This is a contribution to the Global Ecology and Oceanography of Harmful Algal Blooms Core Research Project on Harmful Algal Blooms in Upwelling Systems and is ECOHAB Publication #767.

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Associate editor: Heidi M. Sosick

Received: 03 April 2013
Accepted: 06 November 2013
Amended: 06 January 2014