



Effects of nitrogen source, concentration, and irradiance on growth rates of two diatoms endemic to northern San Francisco Bay

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ABSTRACT: Impacts on growth rates from exposure to ammonium (NH_4^+) and nitrate (NO_3^-), at non-limiting concentrations, in combination with irradiances varying from 25 to 600 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ were investigated in the pelagic diatom *Thalassiosira weissflogii* and the benthic diatom *Entomoneis paludosa* recently isolated from Suisun Bay in northern San Francisco Bay. Growth rates were higher in *T. weissflogii* ($0.76 \pm 0.3 \text{ d}^{-1}$) compared with *E. paludosa* ($0.58 \pm 0.2 \text{ d}^{-1}$) across all irradiances and nitrogen (N) treatments. Differences in growth rates with N source were regulated by irradiance in both diatoms and were greatest at the intermediate irradiance due to saturation of rates at 85 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ when growing on NH_4^+ and at 200 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ when growing on NO_3^- . Notable physiological differences between these 2 diatoms included a larger range in the quantum yield of photosystem II (F_v/F_m) and in chlorophyll *a* per cell as a function of irradiance in *T. weissflogii* compared with *E. paludosa*. In addition, a negative interaction of high NH_4^+ concentration (1000 $\mu\text{mol l}^{-1}$) and irradiance ($\geq 200 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) was observed on growth rates in *E. paludosa* that was not evident in *T. weissflogii*. Differences in physiological parameters of these diatoms are discussed in relation to their distributions and frequency of occurrence in Suisun Bay.

KEY WORDS: Ammonium · Irradiance · Diatoms · *Entomoneis* · *Thalassiosira* · Suisun Bay · San Francisco Bay

1. INTRODUCTION

Suisun Bay, situated in the northern part of San Francisco Bay, USA, has experienced numerous environmental changes in the last 2 to 3 decades, some of which have contributed to a general decline in phytoplankton biomass (Alpine & Cloern 1992, Lehman 1996, 2000, Jassby 2008). These changes include increased grazing pressure following an invasion by the Asian clam *Potamocorbula amurensis* (Carlton et al. 1990, Alpine & Cloern 1992), increases in nitrogen (N) inputs from wastewater discharge (Jassby 2008), exposure to a broad suite of herbicides (Orlando et

al. 2014), changes in water residence times, and increases in water temperature (Jassby 2008), to mention a few. These changes were added on top of a system that already experienced acute light limitation (Cole & Cloern 1984, 1987, Jassby et al. 2002) resulting from relatively high levels of sediments carried with the Sacramento River and localized wind-wave sediment resuspension (Goodwin & Denton 1991, Ruhl & Schoellhamer 2004, Moskalski & Torres 2012, Schoellhamer et al. 2012). In turn, the decrease in phytoplankton biomass has been hypothesized to be partially responsible for decreases in biomass of higher trophic levels, including zooplankton and

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pelagic fish (Kimmerer 2002, Müller-Solger et al. 2002, Sommer et al. 2007, Kimmerer et al. 2017).

Of the bottom-up factors that could negatively impact phytoplankton growth, the two that have received the most attention are excessive ammonium (NH_4^+) build-up from wastewater effluent (Dugdale et al. 2007, Parker et al. 2012, Esparza et al. 2014, Kraus et al. 2017) and light limitation due to high turbidity (Cole & Cloern 1984, 1987, Alpine & Cloern 1988, Jassby et al. 2002). These factors are linked in that light limitation, resulting in low levels of primary productivity and biomass, allows dissolved nutrients to accumulate in the water column. As a result, the median chlorophyll *a* concentration in Suisun Bay ($2.0 \mu\text{g l}^{-1}$) is ~12-fold lower than what could be expected if the ambient nitrogen available in the water column was converted into phytoplankton biomass (Cloern & Jassby 2012).

While the impacts of elevated NH_4^+ concentrations and low light have been studied separately in Suisun Bay, they have not been studied simultaneously under controlled conditions. The interaction between light and nutrients is particularly pertinent to Suisun Bay as it is a mosaic of different light–nutrient environments varying from deep, dark channels to shallow, well-lit banks (Alpine & Cloern 1988) superimposed by steep concentration gradients in inorganic nutrients (Dugdale et al. 2007, Jassby 2008). To investigate the impact of changes in light and nutrients simultaneously on phytoplankton growth in Suisun Bay, several species of phytoplankton were isolated into pure cultures (Berg et al. 2017). By using recent isolates from upper San Francisco Bay, we avoided issues related to extrapolation of results using strains from other geographic regions to our locale as well as issues related to genetic adaptations resulting from growth at unnaturally high N (in most cases, nitrate only) concentrations for many decades (Lakeman et al. 2009). In addition, by using pure cultures rather than field populations, we were able to ascribe physiology associated with growth on different N sources to a particular phytoplankton taxon.

Two of the isolated species, *Thalassiosira weissflogii* and *Entomoneis paludosa*, were endemic to Suisun Bay and belong to genera that dominate phytoplankton community composition depending on season and location (Lehman 1996, Cloern & Dufford 2005, Glibert et al. 2014). These 2 species are ecologically distinct in that *T. weissflogii* is a centric diatom residing in the pelagic zone, whereas *E. paludosa* is a pennate diatom comprising part of the microphytobenthos that typically resides on the sediment surface (Ribeiro et al. 2003, Forster & Martin-Jézéquel

2005). Both pelagic and microphytobenthic communities may be exposed to gradients in irradiance and nutrients, but these gradients may differ in steepness. For example, gradients in irradiance may be less steep at the sediment surface compared with what is experienced by a phytoplanktoner that is mixed from the bottom to the surface of the water column. Because of lower mean irradiances, benthic productivity may be less than pelagic water column productivity (Kromkamp et al. 1995, Cullen & MacIntyre 1998, Underwood & Kromkamp 1999). In contrast, gradients in nutrients may be steeper in the sediments where organic matter deposition and nutrient remineralization are more concentrated compared with the water column (Admiraal 1977b, Krom 1991). In a previous report, both *T. weissflogii* and *E. paludosa* isolated from Suisun Bay were found to tolerate ammonium concentrations in excess of $1000 \mu\text{mol l}^{-1}$ (Berg et al. 2017). Environmental parameters such as salinity and temperature are also important with respect to the distributions of both pelagic and benthic phytoplankton (Admiraal 1977a, MacIntyre et al. 1996, Underwood & Provot 2000, Boyd et al. 2013).

In addition to gradients at the sediment–water interface, microphytobenthic communities are frequently exposed to wind-induced waves and tides that scour the surface of the sediments and suspend them into the water column (de Jonge & van Beusekom 1995). In San Francisco Bay, currents, tides, river flow, and wind combine to provide a high level of turbulence and water column churn (Cloern 1991, 1996, Moskalski & Torres 2012). Benthic diatoms in this system become suspended together with sediments into the water column and increasingly dominate pelagic phytoplankton community composition (Glibert et al. 2014, Kraus et al. 2017). Given recent increases in contribution of benthic diatoms to pelagic phytoplankton community composition in Suisun Bay, high tolerance of benthic diatoms to ammonium (NH_4^+), and elevated concentrations of NH_4^+ in this system, we wanted to investigate whether tolerance to varying combinations of light and nutrients could help characterize the ecological niches of *T. weissflogii* and *E. paludosa*.

To characterize the extent to which growth rates of these diatoms would be modulated by exposure to varying NH_4^+ concentrations in combination with varying irradiances, the impacts to growth rates of 3 different nitrogen (N) concentrations (under non-limiting conditions), using nitrate (NO_3^-) or NH_4^+ as the sole source of N for growth, under 4 different irradiances, were investigated in *T. weissflogii* and *E. paludosa*. When *T. weissflogii* and *E. paludosa* were

isolated from Suisun Bay for the present study, both were key members of the pelagic phytoplankton community. We also wanted to investigate whether any of the 3 factors tested (N source, N concentration, or irradiance) influenced growth rates to the extent that the factor could affect the outcome of competition between these 2 species.

2. MATERIALS AND METHODS

2.1. Sampling

Samples of phytoplankton were surveyed and collected along a cruise track that traversed Suisun Bay and its principal freshwater source, the Sacramento River, on 28 to 29 January 2014. During the survey, 33 discrete stations were sampled from the R/V 'Questuary' from the northernmost Stn 32 at the I-80 bridge in the Sacramento River to the southernmost Stn 1 in Suisun Bay. Samples for enumeration of phytoplankton abundance and isolation of phytoplankton into pure cultures were collected at 1 m depth from a smaller subset of stations (12 stations) along this survey track using a rosette sampler. Temperature and salinity data were obtained from a CTD attached to the rosette sampler. In addition, data on turbidity at each station was obtained using a YSI 6600v2 sonde.

2.2. Experimental conditions

Whole water was brought back to the laboratory where clonal, non-axenic cultures of the estuarine diatoms *Thalassiosira weissflogii* and *Entomoneis paludosa* were established by micropipette isolations of single cells from Suisun Bay according to Berg et al. (2017). Sterile techniques were used during all aspects of culturing. Stock cultures were maintained at 85 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ at 15.5°C and a salinity of 10 using NO_3^- as the N source. Before the start of an experiment, cultures were spun down, rinsed with N-free medium, and re-suspended in triplicate into dilute batch cultures containing 200 ml of medium with f/2 nutrient + silica solution lacking N (Goldman & McCarthy 1978). The nutrient solution was modified by the addition of N to give final concentrations of 20, 100, and 1000 $\mu\text{mol l}^{-1}$, either in the form of NH_4^+ or NO_3^- . In the case of the cultures with NH_4^+ added, the fraction of total ammonia ($\text{NH}_4^+ + \text{NH}_3$) that was composed of unionized NH_3 at a salinity of 10, temperature of 15.5°C, and pH of 8.3 (i.e. 3–6 %;

Khoo et al. 1977) was calculated to range from 0.6 to 1.2 $\mu\text{mol l}^{-1}$ in the 20 $\mu\text{mol NH}_4^+ \text{l}^{-1}$ cultures, 3 to 6 $\mu\text{mol l}^{-1}$ in the 100 $\mu\text{mol NH}_4^+ \text{l}^{-1}$ cultures, and 30 to 60 $\mu\text{mol l}^{-1}$ in the 1000 $\mu\text{mol NH}_4^+ \text{l}^{-1}$ cultures. Nitrogen concentrations were checked periodically throughout the growth phase of the cultures. Strains were cultured at 4 incident irradiances representing low (25 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$), intermediate (85 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$), high (200 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$), and inhibiting (600 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) light. The cultures were maintained on a 12 h light:12 h dark (L:D) cycle under cool-white fluorescent lights at a temperature of $15.5 \pm 1^\circ\text{C}$. Each culture was sampled daily, at noon, for physiological evaluations as described below.

2.3. Sample analyses

The physiology of each species was evaluated through daily measurements of the quantum yield of photosystem II (PSII), cell abundance, and chlorophyll *a* (chl *a*) concentration. Nitrogen concentrations (NH_4^+ and NO_3^-) were assayed periodically. The quantum yield of PSII (F_v/F_m) was measured by pulse-amplitude-modulated (PAM) fluorometry (Heinz-Walz WATER-PAM). The PAM was blanked with 0.2 μm filtered culture media. For assessment with PAM, aliquots were removed from the primary culture and dark-adapted for at least 10 min. After dark adaptation, background chl *a* fluorescence (F_0) and maximal chl *a* fluorescence following a saturating pulse (F_m) were measured to derive the variable (F_v) over maximum chl *a* fluorescence according to:

$$\frac{F_v}{F_m} = (F_m - F_0) / F_m \quad (1)$$

Samples for cell enumeration were preserved with acid Lugol's solution (20 μl Lugol's per ml culture volume) and stored cool (4°C) in the dark until enumeration with a Zeiss Axiovert 200 inverted microscope using a Parsons counting chamber. Geometric shapes of the cells were based on measurements of 10 organisms per taxon. Cell volumes were estimated by applying the geometric shapes that most closely matched the cell shape (Hillebrand et al. 1999). Abundances were estimated by random field counts totaling 400 unicells. Cell-specific growth rates (d^{-1}) were computed by fitting the exponential function:

$$C = C_0 e^{\mu t} \quad (2)$$

to the data from the exponential growth phase, where C is the cell abundance, C_0 is the starting cell abun-

dance, μ is the growth constant (d^{-1}), and t is time (d). All calculations and statistical tests were carried out using R software (R Core Team 2016).

Samples for chl *a* determination were collected onto glass-fiber filters (Whatman GF/F) and processed immediately using the non-acidification method (Welschmeyer 1994). Samples for nutrient (NO_3^- and NH_4^+) analysis were filtered (Whatman GF/F) and stored frozen until processing. Ammonium was analyzed using the orthophthaldialdehyde (OPA) method, and relative fluorescence units were obtained via fluorometry (TD-700, Turner Designs) according to Holmes et al. (1999). Nitrate was analyzed using a Lachat QuikChem 8500 Flow Injection Analyst System and Omnion 3.0 software (Lachat Instruments, Hach).

3. RESULTS

At the time of sampling, phytoplankton belonging to the *Thalassiosira* genus dominated phytoplankton community biomass throughout Suisun Bay, whereas *Entomoneis paludosa* dominated phytoplankton community biomass at stations in the X2 region where the salinity changes from freshwater to brackish water (Fig. 1). For the current study, *T. weissflogii* was isolated from Stn 6, while *E. paludosa* was isolated from Stn 19. Salinities were 11.5 and 1.4, and turbidities were 5 and 15 NTU, at Stns 6 and 19, respectively (Fig. 1A,B). The temperature was 11°C at both stations. These newly isolated diatom species were relatively large (Fig. 2). The dimensions for *E. paludosa* were approximately 50 μm length by 20 μm width,

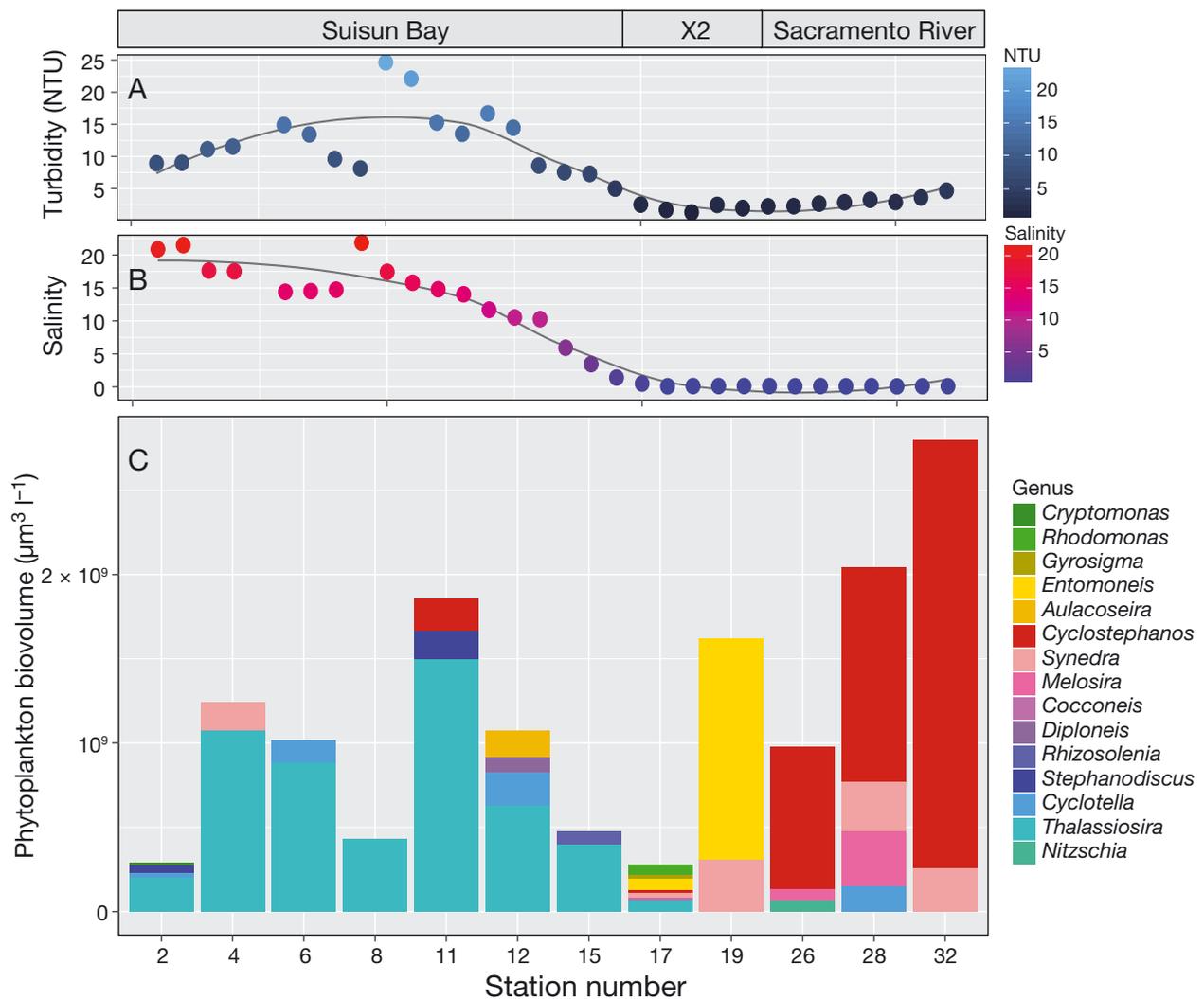


Fig. 1. (A) Turbidity, (B) salinity, and (C) phytoplankton community composition based on biovolume along the salinity gradient from the Sacramento River to Suisun Bay. *Thalassiosira* genus represented in teal, and *Entomoneis* genus represented in yellow. Grey line represents loess smoothing

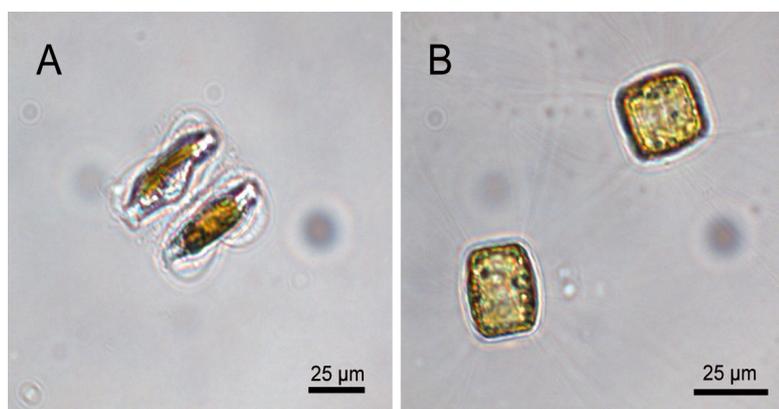


Fig. 2. Light microscopy image of a culture of (A) *E. paludosa* and (B) *T. weissflogii*. Scale bar = 25 µm

yielding a mean cell volume of $15\,580 \pm 2\,500 \mu\text{m}^3$. The mean diameter of *T. weissflogii* was 32 µm, yielding a mean cell volume of $6\,447 \pm 804 \mu\text{m}^3$ (Fig. 2).

F_v/F_m was slightly greater in *E. paludosa* (ranging from 0.49 to 0.76) than *T. weissflogii* (ranging from 0.39 to 0.74) particularly at the intermediate irradiance (Fig. 3A–D). However the difference in F_v/F_m as a function of species was not statistically significant ($F_{1,142} = 3.4$, $p = 0.07$). Nor was the difference in F_v/F_m as a function of N source statistically different in either *E. paludosa* ($F_{1,70} = 0.02$, $p = 0.89$) or *T. weissflogii* ($F_{1,70} = 1.2$, $p = 0.27$). Similarly, there was no significant effect of N concentration on F_v/F_m in either *E. paludosa* ($r^2 = 2.11 \times 10^{-5}$, $F_{1,70} = 0.0015$, $p = 0.97$) or *T. weissflogii* ($r^2 = 0.025$, $F_{1,70} = 1.8$, $p = 0.18$). In contrast to the aforementioned factors, F_v/F_m decreased significantly with increasing irradiance in both *E. paludosa* ($r^2 = 0.75$, slope = -3.20×10^{-4} , $F_{1,70} = 209$, $p < 2 \times 10^{-16}$) and *T. weissflogii* ($r^2 = 0.76$, slope = -3.43×10^{-4} , $F_{1,70} = 222$, $p < 2 \times 10^{-16}$). In *E. paludosa*, the decrease in F_v/F_m was greatest between 85 and 200 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (Fig. 3A,B), whereas in *T. weissflogii*, it decreased with each step increase in irradiance (Fig. 3C,D).

Chl *a* quota (chl *a* cell⁻¹) was significantly less for *E. paludosa*, ranging from 32 to 91 pg cell⁻¹, compared with *T. weissflogii* ($F_{1,142} = 179$, $p < 2 \times 10^{-16}$), where it ranged from 61 to 283 pg cell⁻¹ (Fig. 3E–H). There was no significant effect of N source on cell quota in either species (*E. paludosa* $F_{1,70} = 0.8$, $p = 0.38$; *T. weissflogii* $F_{1,70} = 0.5$, $p = 0.48$). There was a slight effect of N concentration on cell quota in *E. paludosa* ($r^2 = 0.06$, slope = 0.01, $F_{1,70} = 4.8$, $p = 0.03$) but not in *T. weissflogii* ($F_{1,70} = 0.5$, $p = 0.48$). The effect of irradiance also differed with respect to the chl *a* quota in the 2 diatoms. There was no significant effect of irradiance on the chl *a* quota in *E. paludosa* ($F_{1,70} = 0.08$,

$p = 0.78$), which decreased between the low and intermediate irradiance followed by a slight increase above the intermediate irradiance (Fig. 3E,F). In contrast with *E. paludosa*, there was a significant negative effect of irradiance on the chl *a* quota in *T. weissflogii* ($r^2 = 0.45$, slope = -0.21 , $F_{1,70} = 57$, $p = 1.2 \times 10^{-10}$). In *T. weissflogii*, the chl *a* quota decreased with each step increase in irradiance between low and high irradiance when grown on NH_4^+ (Fig. 3G) and between intermediate and high irradiance when grown on NO_3^- (Fig. 3H).

Growth rates differed significantly between the 2 diatoms tested here ($F_{1,142} = 18$, $p = 3.7 \times 10^{-5}$), with rates being greater in *T. weissflogii* ($0.76 \pm 0.3 \text{ d}^{-1}$) compared with *E. paludosa* ($0.58 \pm 0.2 \text{ d}^{-1}$) across all treatments (Fig. 3I–L). Growth rates in both *E. paludosa* ($r^2 = 0.17$, slope = 0.0038, $F_{1,70} = 14$, $p = 0.00038$) and *T. weissflogii* ($r^2 = 0.41$, slope = 0.0008, $F_{1,70} = 48$, $p = 1.6 \times 10^{-9}$) were significantly impacted by irradiance. In both diatoms, the largest increase in growth rate occurred between the low and intermediate irradiances when grown on NH_4^+ (Fig. 3I,K). When grown on NO_3^- , there was an additional increase between the intermediate and high irradiance (Fig. 3J,L).

In contrast with irradiance, there was no significant impact on growth rate in *E. paludosa* ($F_{1,70} = 0.3$, $p = 0.6$) or *T. weissflogii* ($F_{1,70} = 3.5$, $p = 0.07$) by either N source or N concentration ($F_{1,70} = 4$, $p = 0.06$ and $F_{1,70} = 0.02$, $p = 0.88$, respectively) across all irradiances (Fig. 3I–L). However, there was a negative interaction between growth on the highest NH_4^+ concentration and the 2 highest irradiances in *E. paludosa* (Fig. 3I). As a result, growth rates in *E. paludosa* decreased 40% from the intermediate to high irradiance when grown at 1000 $\mu\text{mol NH}_4^+ \text{ l}^{-1}$ (Fig. 3I). No such decrease was observed when grown on NO_3^- (Fig. 3J).

There was an effect of N source on growth rate at the intermediate irradiance in *T. weissflogii* (Fig. 3K–L, $F_{1,16} = 187$, $p = 3.05 \times 10^{-10}$). At this irradiance, growth on NH_4^+ was 69% faster than growth on NO_3^- (Fig. 4). Although not statistically significant, growth rates were also faster (28%) on NH_4^+ than on NO_3^- in *E. paludosa* at the intermediate irradiance (Fig. 4). This difference in growth rates with N source at the intermediate irradiance can be attributed to the difference in irradiance at which maximum growth rates were reached when growing on NH_4^+ vs NO_3^- . In both species, near-maximum growth rates were reached at the intermediate irradiance when grow-

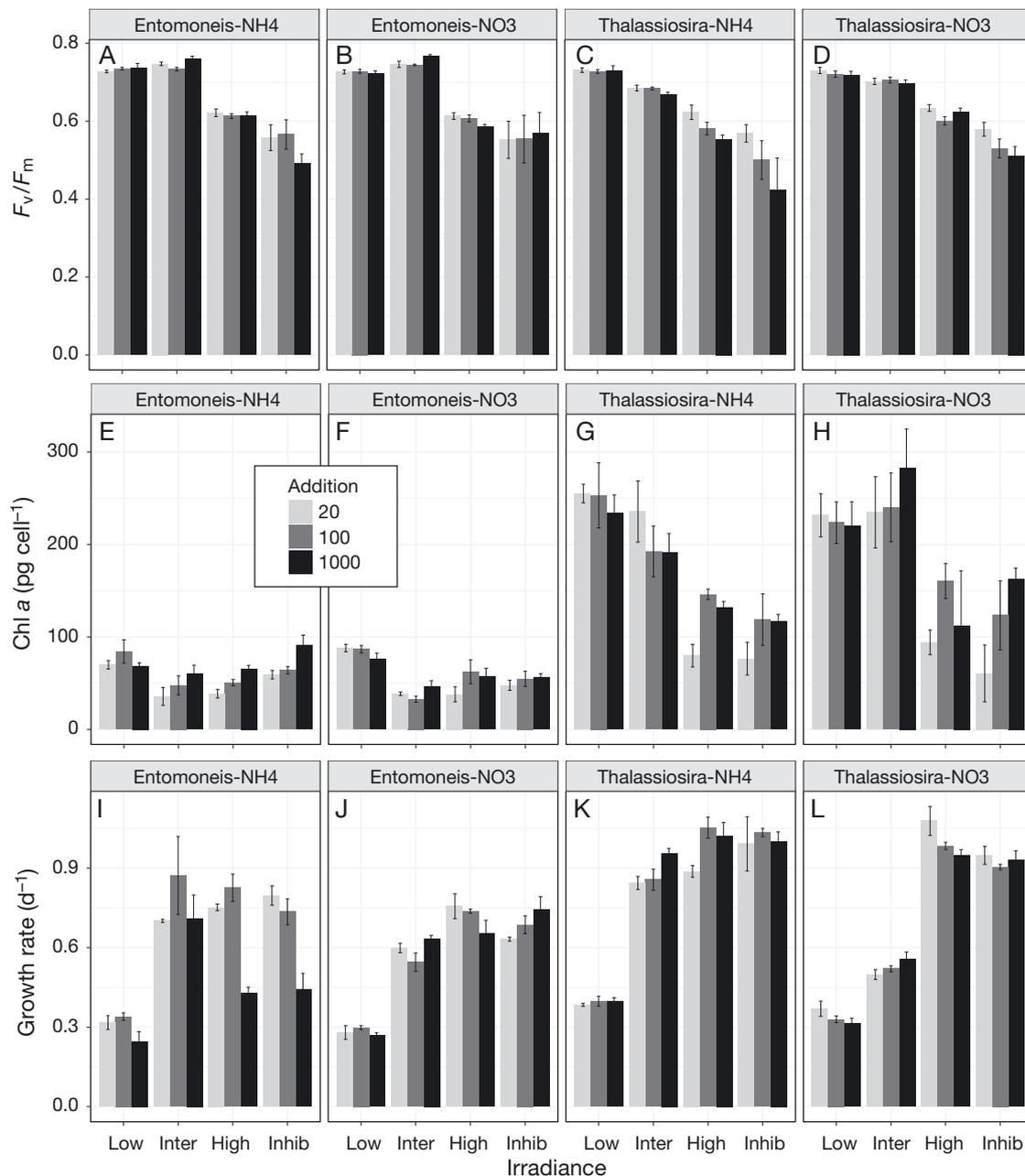


Fig. 3. Species-specific differences with N source and N addition in mid-exponential growth phase of (A–D) F_v/F_m , (E–H) chl *a* (pg cell⁻¹), and (I–L) growth rates (d⁻¹) as a function of irradiance for *E. paludosa* and *T. weissflogii*. Mean of triplicate cultures \pm standard error of the mean. Inter: intermediate; Inhib: inhibitory

ing on NH₄⁺, while the maximum was not reached until the high irradiance when growing on NO₃⁻ (Fig. 3J,L). As a result, the difference in growth rates between the 2 N sources was greatest at the intermediate irradiance. At the highest irradiances, growth rates on NO₃⁻ had caught up to rates on NH₄⁺, and there was no substantial difference. At the lowest irradiance, growth rates were similarly low on both N sources in both species (Fig. 4).

4. DISCUSSION

In recent years, blooms of benthic diatoms have become more common in Suisun Bay in the northern portion of San Francisco Bay. In particular, occasional blooms of the benthic chain-forming diatom *Entomoneis paludosa* have been observed throughout Suisun Bay (e.g. Glibert et al. 2014). Because *E. paludosa* is a benthic species and therefore acclimated to

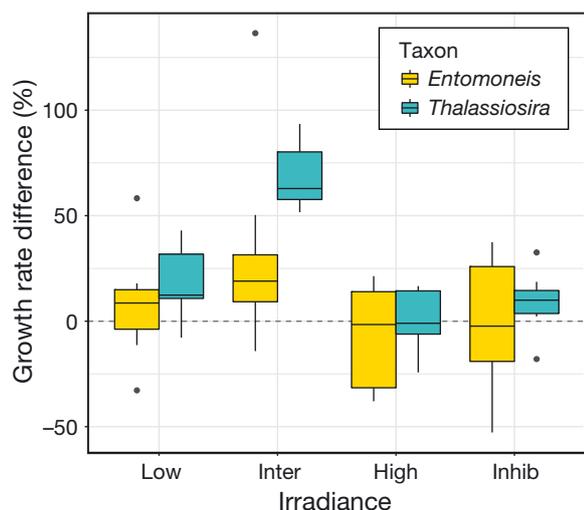


Fig. 4. Box plots of growth-rate differences with respect to N source as a percentage ($[\mu_{\text{NH}_4}/\mu_{\text{NO}_3} - 1] \times 100$) as a function of irradiance in *E. paludosa* and *T. weissflogii*. Dotted line represents no growth rate difference with N source. Line within box: median; box: interquartile range (IQR); whiskers: max./min. values $\leq 1.5 \times \text{IQR}$ above/below box; circles: outliers

high NH_4^+ concentrations fluxing out of the sediments (i.e. Admiraal 1977b, Jauffrais et al. 2016), and water column NH_4^+ concentrations in Suisun Bay are relatively high (Jassby 2008), it is possible that pelagic blooms of this species are related to shifts in N concentration and species. It has been hypothesized that growth of pelagic diatoms is inhibited by NH_4^+ at concentrations above $4 \mu\text{mol l}^{-1}$ (Dugdale et al. 2007) although this has not been demonstrated on a physiological level (Collos & Harrison 2014, Berg et al. 2017). We wanted to investigate how growth rates of the pelagic diatom *Thalassiosira weissflogii*, potentially more sensitive to NH_4^+ and less sensitive to irradiance, and the benthic diatom *E. paludosa*, potentially less sensitive to NH_4^+ and more sensitive to irradiance, would compare under varying conditions of irradiance and N sources and concentration at non-limiting levels of nutrients. We specifically tested non-limiting levels as earlier analyses had hypothesized that changes in N speciation at non-limiting levels could affect phytoplankton growth rates and competitive interactions (Dugdale et al. 2007, Glibert et al. 2011). In contrast with earlier reports, no significant impact of N source on growth rates were demonstrated in the 2 diatom species tested here. Of the 3 environmental variables tested, irradiance had the largest and most significant influence on growth rates.

At the temperatures used in the current experiments (15.5°C), *T. weissflogii* outgrew *E. paludosa*

across all treatments with an average growth rate difference of 0.18 d^{-1} . This growth rate difference was greater at high light (0.3 d^{-1}) compared with low light (0.07 d^{-1}). We noted physiological differences between these 2 diatoms that could underpin the pattern of a larger growth rate difference at high light compared with low light. One was a smaller range in F_v/F_m in *E. paludosa* compared with *T. weissflogii*, 0.27 vs. 0.35, respectively. Another was less plasticity in chl *a* quota, varying 5-fold in *E. paludosa* and 11-fold in *T. weissflogii*.

Being able to vary photosynthetic efficiency (short-term acclimation response to excess irradiance) and chl *a* quota (longer-term acclimation response to excess irradiance) can enable a species to acclimate more readily in response to change and thereby avoid damage to the cell under high light. Effective dissipation of excess energy at the point where light energy is harvested, as heat or fluorescence, called non-photochemical quenching, can prevent the build-up electrons that combine with oxygen to form oxygen radicals that damage the D1 protein of PSII (Vass et al. 1992, Aro et al. 1993, Clarke et al. 1993, Wu et al. 2012). This damage results in inhibition of photosynthesis (photoinhibition) which may or may not be reversible depending on how efficient the cell's repair mechanisms are (Vass et al. 1992, Clarke et al. 1993, Nixon et al. 2010, Campbell & Tyystjärvi 2012, Wu et al. 2012). Temporary diversion of excess energy reduces the photosynthetic efficiency of the cell but prevents long-term damage and mortality of the cells. Cells that do not have the ability to dissipate excess energy do not experience short-term decreases in photosynthetic efficiency but may not be able to adapt and as a consequence may not survive (Cullen & MacIntyre 1998). It is possible that the ability of *T. weissflogii* to decrease its photosynthetic efficiency and its chl *a* quota to a greater degree than *E. paludosa* enables it to maintain a better level of photoprotection and to attain a higher growth rate at high irradiances.

In addition to having less plasticity in photophysiological parameters, there was a negative interaction of high irradiance and high NH_4^+ concentration in *E. paludosa*, resulting in a 40% decrease in the growth rate, which was not evident in *T. weissflogii*. Such an interaction has been observed previously for *E. paludosa* (Admiraal 1977b), and it can be explained physiologically by direct damage to PSII from competitive binding of NH_3 with the oxygen evolution complex (Kallqvist & Svenson 2003, Drath et al. 2008, Collos & Harrison 2014). In other words, addition of high concentrations of NH_4^+ , a fraction of which will be com-

posed of NH_3 depending on temperature and pH (e.g. Khoo et al. 1977), will allow NH_3 to diffuse freely into the cell, resulting in photodamage. The impact of this damage will depend on the photorepair mechanisms present in the cell (Drath et al. 2008). A negative impact on growth of high NH_4^+ at high irradiances suggests that *E. paludosa* has an inferior photodamage repair mechanism compared with *T. weissflogii*. At the lower irradiances (25 to 85 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$), growth in *E. paludosa* was not inhibited by the highest NH_4^+ concentration tested here.

The combination of the high NH_4^+ concentration with constant high irradiance used here would not be expected to occur in Suisun Bay where water column NH_4^+ concentrations typically range from 2 to 11 $\mu\text{mol l}^{-1}$, fluxes of NH_4^+ out of the sediments to the water column are below 100 $\mu\text{mol NH}_4^+ \text{m}^{-2} \text{h}^{-1}$ (Cornwell et al. 2014), and depth-averaged integrated irradiances are typically below 100 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (Table 1). The intermediate irradiance used in the current experiments would probably be more applicable to depth-averaged irradiance conditions encountered in Suisun Bay (Table 1). Interestingly, the difference in growth rates between NH_4^+ and NO_3^- was the greatest in both species at the intermediate irradiance.

A large growth rate difference between NH_4^+ and NO_3^- at intermediate irradiances, but not at low or high irradiances, has been observed in several other phytoplankton species (Paasche 1971, Thompson et

al. 1989; Wood & Flynn 1995, Tong et al. 2016). This difference can be explained by the maximum growth rate being reached at a lower irradiance when growing on NH_4^+ than when growing on NO_3^- (i.e. Wood & Flynn 1995), resulting from the requirement of additional energy (i.e. 25–40%) and enzymatic steps associated with the reduction of NO_3^- (Thompson et al. 1989, Turpin 1991, Raven et al. 1992, Levasseur et al. 1993, Herrero et al. 2001, Brown et al. 2009). The difference in growth rates with N source may not be easy to detect unless a range of irradiances are tested as the difference disappears at higher (and lower) irradiances. Many studies have demonstrated no difference in growth regardless of N source, and some have greater growth rates on NO_3^- compared with NH_4^+ (Thompson et al. 1989, Levasseur et al. 1993, Clark & Flynn 2000, Herndon & Cochlan 2007, Strom & Bright 2009, Thessen et al. 2009, Berg et al. 2017). Whether a lack of growth rate difference with N source is due to testing at irradiances that are too high, and/or because the extra energy cost to the cell when using NO_3^- is compensated for by changes in metabolic parameters, is not clear. Previous studies have demonstrated variations in chl *a* quota, N quota, cellular C:N ratio, and photosynthetic efficiency with growth on NO_3^- versus NH_4^+ (Levasseur et al. 1993, Wood & Flynn 1995, Flynn 2001).

While there was a difference in growth rates with N source at the intermediate irradiance in the current experiment, averaged across all the irradiances, this difference was no longer significant in either diatom. Because phytoplankton in Suisun Bay are mixed from the top to the bottom of the water column (i.e. mixed layer depth is the same as the total water column depth; Cloern 1991) and therefore experience a range of irradiances in each mixing cycle, N source effects should be averaged across a range of irradiances. Another important note with respect to the use of different N sources is that, as demonstrated here and in other studies (Paasche 1971, Thompson et al. 1989, Wood & Flynn 1995, Tong et al. 2016), irradiance is the principal driver. At any given irradiance, phytoplankton are likely to use the same N source or N mix. Consequently, all phytoplankton are more likely to use NH_4^+ under the low-intermediate irradiances and non-limiting NH_4^+ conditions that prevail in Suisun Bay, and competition among species will be driven by intrinsic growth rate differences rather than use of different N sources.

The reason that *E. paludosa* is able to bloom in parts of Suisun Bay, despite growing at a slower rate than species of the *Thalassiosira* genus, may not be linked to either irradiance or NH_4^+ concen-

Table 1. Typical water column depth, NH_4^+ concentrations, and irradiances (depth-averaged integrated irradiance) for select stations along the cruise track in Suisun Bay during summer and winter. Corresponding USGS long-term monitoring station designations in parentheses

Station	Depth (m)	NH_4^+ ($\mu\text{mol l}^{-1}$)	Irradiance ($\mu\text{mol photons m}^{-2} \text{s}^{-1}$)
Summer			
17 (USGS3)	11.4	2.4	20.7
12 (Grizzly Bay)	6.4	4.5	62.0
8 (Middle Suisun)	5.9	4.7	62.7
6 (USGS5)	11	3.8	46.1
4 (USGS6)	12.5	5.9	41.3
2 (USGS7)	18	10.9	31.4
Winter			
17 (USGS3)	11.4	13.2	30.4
12 (Grizzly Bay)	6.4	10.2	78.6
8 (Middle Suisun)	5.9	10.2	15.4
6 (USGS5)	11	10.4	17.7
4 (USGS6)	12.5	10.9	26.1
2 (USGS7)	18	10.9	61.5

trations. As illustrated in Fig. 1 the distribution of these 2 genera, when they co-occur, is at opposing ends of the salinity spectrum in Suisun Bay. Diatoms of the *Thalassiosira* genus tend to dominate at salinities >1.5, whereas *E. paludosa* dominates at salinities around 1.5 and less. A number of studies have indicated that salinity is an important factor with respect to distributions of phytoplankton and microphytobenthos and competitive interactions (McIntire 1978, Admiraal et al. 1984, Underwood et al. 1998, Underwood & Provot 2000). Temperature is another potentially important factor in regulating growth rates and competitive interactions (Eppley 1972, Admiraal 1977a, Anderson 2000, Butterwick et al. 2005, Boyd et al. 2013). Studies to date suggest that the temperature optimum for growth of the *Thalassiosira* genus, between 15 and 20°C (Boyd et al. 2013), is lower than the temperature optimum of *E. paludosa*, which is 25°C (Admiraal 1977a). Because water temperatures have been increasing by up to 0.21°C per year in upper Suisun Bay and in the Delta (Jassby 2008), it may be that the warming of the water has provided an opportunity for *E. paludosa* to out-grow pelagic diatom species such as *Thalassiosira* spp. within a specific salinity range.

In summary, typical NH_4^+ concentrations in Suisun Bay do not inhibit growth of either *T. weissflogii* or *E. paludosa*. At irradiances and mixing regimes characteristic of Suisun Bay, the pelagic diatom *T. weissflogii* would likely be at a competitive advantage given its higher growth rates and photophysiological adaptations suitable for exploiting variable irradiance. Our findings are consistent with global findings that pelagic diatoms are adapted for growth in low-light environments with a high degree of turbulence (Margalef 1978, Kudela 2010, Edwards et al. 2015, Richardson et al. 2016) and that pelagic diatoms typically have higher rates of primary productivity than benthic diatoms (Kromkamp et al. 1995, Underwood & Kromkamp 1999). Episodic blooms of pelagic versus microphytobenthic species in Suisun Bay could be controlled by variations in water column stability, temperature, and salinity. Increased residence time, and thereby increased stratification and temperatures, could accelerate growth of *E. paludosa* at the sediment surface, allowing it to build up a large seed community. With an episodic increase in turbulence, this community could become suspended into the water column and outcompete centric pelagic diatoms from the *Thalassiosira* genus, as observed during the spring bloom of *E. paludosa* in 2014 at the height of drought conditions (Glibert et al. 2014).

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