THE EFFECTS OF OCEAN ACIDIFICATION ON GROWTH, PHOTOSYNTHESIS, AND DOMOIC ACID PRODUCTION BY THE TOXIGENIC DIATOM *PSEUDO-NITZSCHIA AUSTRALIS*

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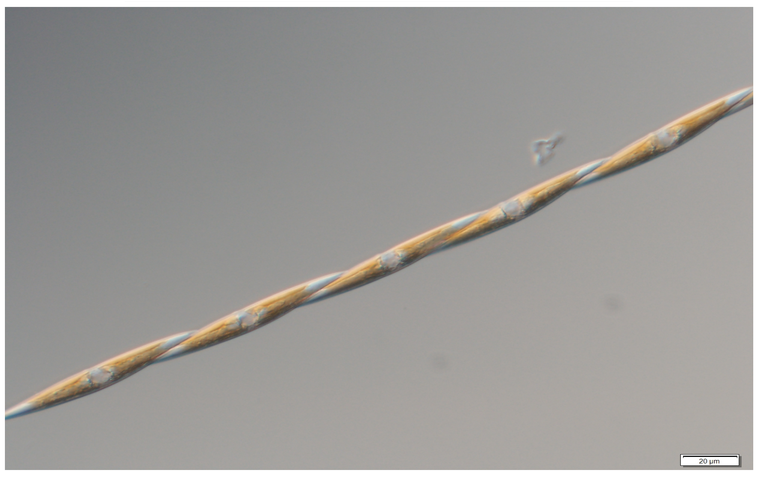
INTRODUCTION



Pseudo-nitzschia australis is one of the most problematic **toxigenic diatoms** on the west coast of North America (Trainer et al., 2012). It is capable of producing the potent neurotoxin **domoic acid (DA)**, responsible for amnesic shellfish poisoning in humans and impacts marine mammals and birds, as well as commercial and recreational fisheries. Blooms of *P. australis* are common in the nutrient-replete coastal waters of eastern boundary upwelling systems, including those off California, where **increased partial pressure of CO_2 (pCO_2)** and **decreased seawater pH** are well known. This is the first study to investigate the potential impacts of ocean acidification on this diatom.

Our study addresses the following issue:
Does reduced seawater pH (due to increased pCO_2) affect rates of growth, photosynthesis, and DA production by *P. australis*?

METHODS

The strain of *P. australis* (HAB 200) used here was isolated in April 2015 by Dr. Holly Bowers (MBARI) from the Santa Cruz Wharf in Santa Cruz, California.

HAB 200 was grown in triplicate (n=3) 6-L glass boiler flasks at four pH levels (8.1, 8.0, 7.9, 7.8) using nutrient-replete batch cultures maintained in 4.0 L of enriched natural seawater medium (ESNW; modified from Harrison et al., 1980).

Light was provided on a 14h light : 10h dark cycle at a saturating irradiance of 240 µmol photons • m^{-2} • s^{-1} using banks of white fluorescent bulbs (Mitsubishi FL40SS-W/37) and temperature was maintained at 13°C within environmental test chambers (Sanyo MLR-352H).

The exponential growth rate of each culture was determined from daily measurements of *in vivo* florescence (Turner AU-10 fluorometer) and microscopic cell counts (Olympus IX-83) using a 1-mL gridded Sedgewick-Rafter counting chamber. Cells for microscopy were preserved with acidic Lugol's solution (2.5% v/v). A minimum of 1,000 cells were counted per replicate.

Photosynthesis vs. irradiance (P v E) experiments were conducted to measure ^{14}C fixation over a gradient of light intensities for 30 minutes, using a temperature-controlled photosynthetron. However, dissolved inorganic carbon (DIC) data are still forthcoming so photosynthetic measurements are not presented here.

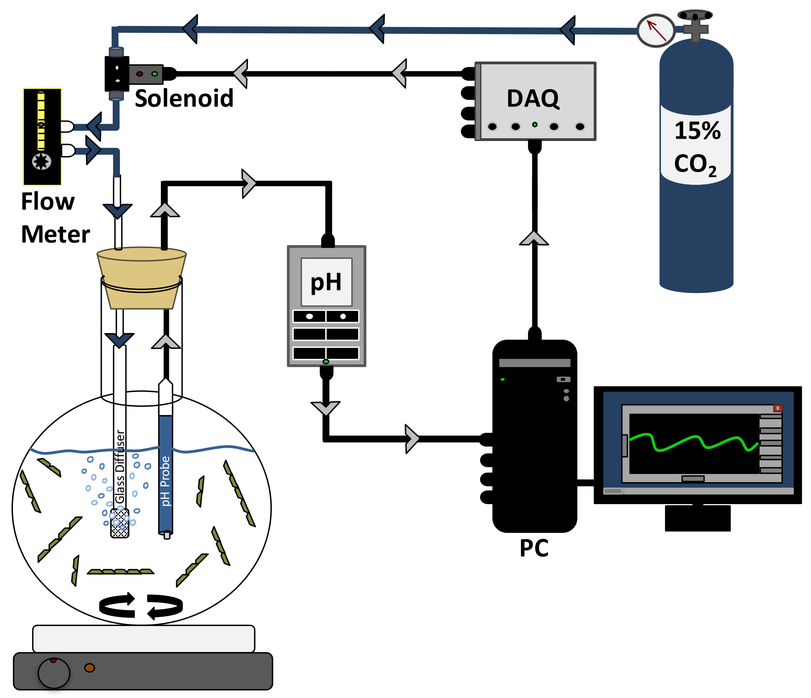


Figure 1. A schematic illustrating how pH of each culture is independently monitored and controlled throughout the experiment. Four (4) cultures are simultaneously regulated by a single computer and DAQ (data acquisition) interface.

Samples for particulate and dissolved domoic acid were collected during both nutrient-replete exponential growth and nutrient-deplete stationary growth phases. DA concentrations were determined using a competitive enzyme-linked immunosorbent assay (cELISA). Triplicate samples were filtered from each biological triplicate in every pH treatment.

RESULTS

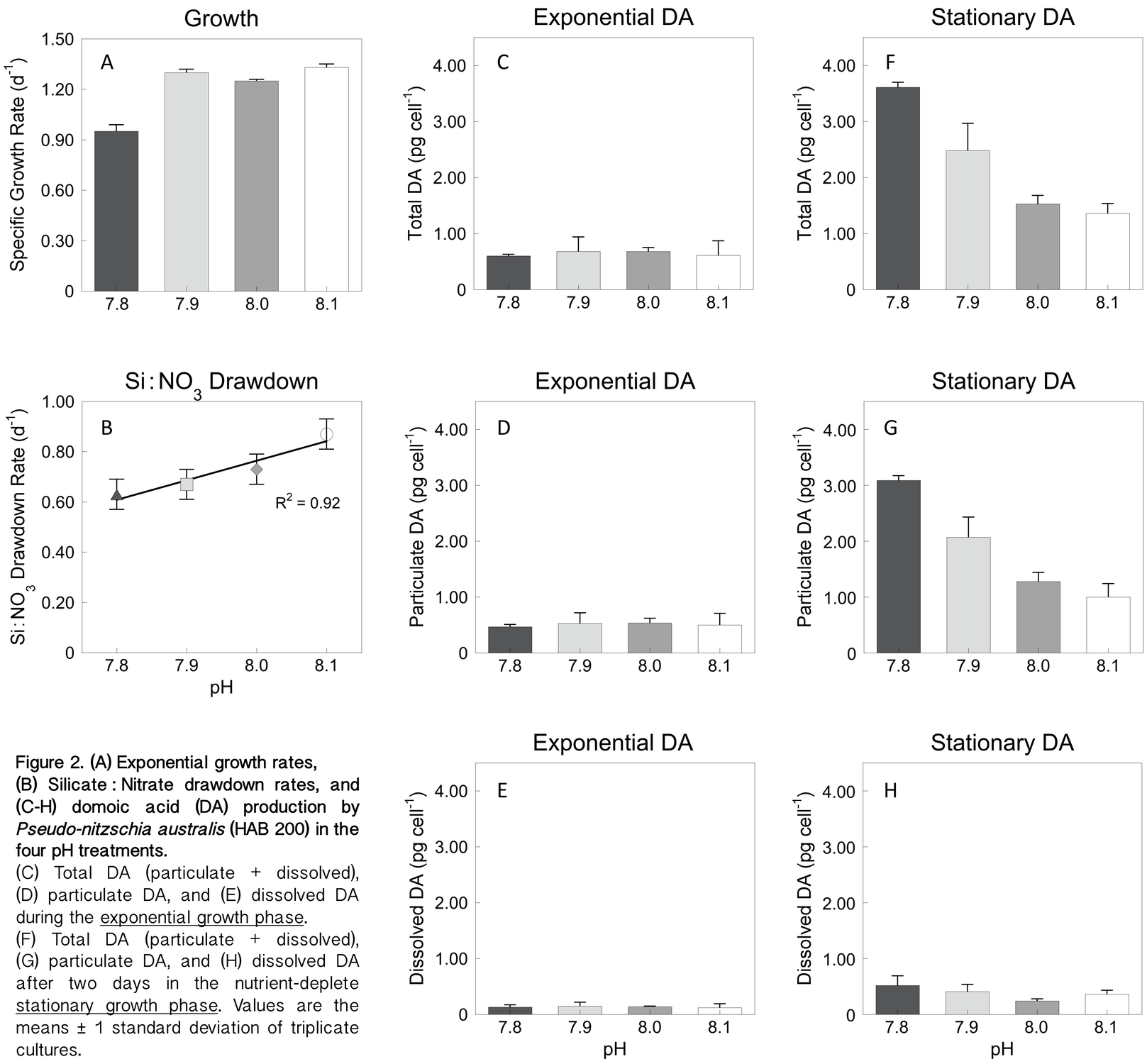


Figure 2. (A) Exponential growth rates, (B) Silicate : Nitrate drawdown rates, and (C-H) domoic acid (DA) production by *Pseudo-nitzschia australis* (HAB 200) in the four pH treatments.
(C) Total DA (particulate + dissolved), (D) particulate DA, and (E) dissolved DA during the exponential growth phase.
(F) Total DA (particulate + dissolved), (G) particulate DA, and (H) dissolved DA after two days in the nutrient-deplete stationary growth phase. Values are the means ± 1 standard deviation of triplicate cultures.

CONCLUSIONS

pH/pCO_2 affects the exponential growth rate of *P. australis* at a specific level:
- Growth rates of cultures maintained at pH 8.0 (1.25 ± 0.01 d^{-1}) and pH 7.9 (1.30 ± 0.02 d^{-1}) grew at the same rate as the control pH of 8.1 (1.33 ± 0.04 d^{-1}).
- However, at pH 7.8 (0.94 ± 0.02 d^{-1}) growth rates declined by 30% compared to all other pH treatments.

pH/pCO_2 affects the total DA production by *P. australis* during the stationary growth phase but not during the exponential growth phase:
- During stationary growth, total cellular DA production increased progressively from pH 8.1 to pH 7.8.
- Total DA was 2.7x greater in cultures at pH 7.8 (3.61 ± 0.09 pg $cell^{-1}$) compared to cultures at pH 8.1 (1.36 ± 0.18 pg $cell^{-1}$).
- During exponential growth, no differences in total DA production were observed as a function of pH.

pH/pCO_2 affects the Si:NO_3 nutrient drawdown rate:
- Cultures grown at reduced pH utilized nitrate faster than silicate, compared to cultures grown at higher pH, so potential production of DA may have been limited by nitrate availability.

REFERENCES

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