

# Effects of Acidification on Coccolithophore and Diatom Cultures

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## Background

The largest sink for anthropogenic CO<sub>2</sub> emissions is the atmosphere; however, a substantial fraction has been absorbed into the global ocean. Once absorbed in seawater, CO<sub>2</sub> undergoes a series of chemical reactions that result in the release of H<sup>+</sup> ions, making the water more acidic. Lowering the pH of seawater may have important implications for the population health of marine organisms, as shifts in pH move certain species out of their optimal pH range. The excess H<sup>+</sup> ions consume carbonate ions in seawater to form bicarbonate, thus reducing the concentration of bio-available carbonate (Fig. 1). Reduction of carbonate ion may have negative impacts on marine calcifying organisms, which utilize carbonate to form calcium carbonate shells.

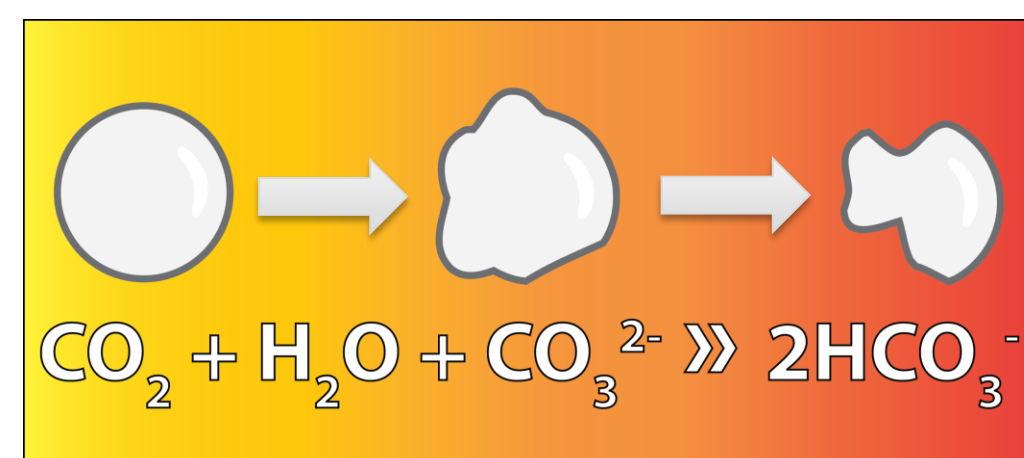


Figure 1. Carbonate consumption impedes calcification.

## Objectives

The purpose of this experiment is to investigate the effects of increased seawater acidity on calcareous and siliceous phytoplankton population growth and shell formation.

## Methods

1. A coccolithophore (*E. huxleyi*) and a diatom (*T. weissflogii*) were cultured in triplicate in seawater media at normal pH (initial pH 8.1) and in acidified media (initial pH 7.5).
2. Cultures were incubated for eight days under 24 hours light at 20°C. Cell counts were measured quasi daily throughout the incubation period.
3. Culture pH was measured at inoculation, and on the final day of sampling.
4. Grown cultures were preserved with formalin (borate-buffered for *E. huxleyi*, and unbuffered for *T. weissflogii*), collected on a 3 μm pore-size filter, air dried, and visualized on a bench-top electron microscope to assess the integrity of mineral tests.

## Results

### Growth Rates (Figure 2)

- Coccolithophores had reduced growth rates under acidic conditions  $0.49 \pm 0.07 \text{ day}^{-1}$  (control) vs.  $0.37 \pm 0.07 \text{ day}^{-1}$  (acid)
- Diatom growth rates were similar in control and acidic conditions
- $0.81 \pm 0.01 \text{ day}^{-1}$  (control) vs.  $0.79 \pm 0.01 \text{ day}^{-1}$  (acid)

### pH Changes (Figure 3)

- pH decreased by **0.39** in the control coccolithophore cultures and increased by **1.06** in the acidic cultures.
- pH increased in both the control and acidic diatom culture by **0.81** vs. **1.61**, respectively.

### Structure of mineral tests (Figure 4)

- Electron microscope pictures reveal that coccolithophore tests underwent apparent dissolution in the acidified medium, while diatom frustules were not visibly affected

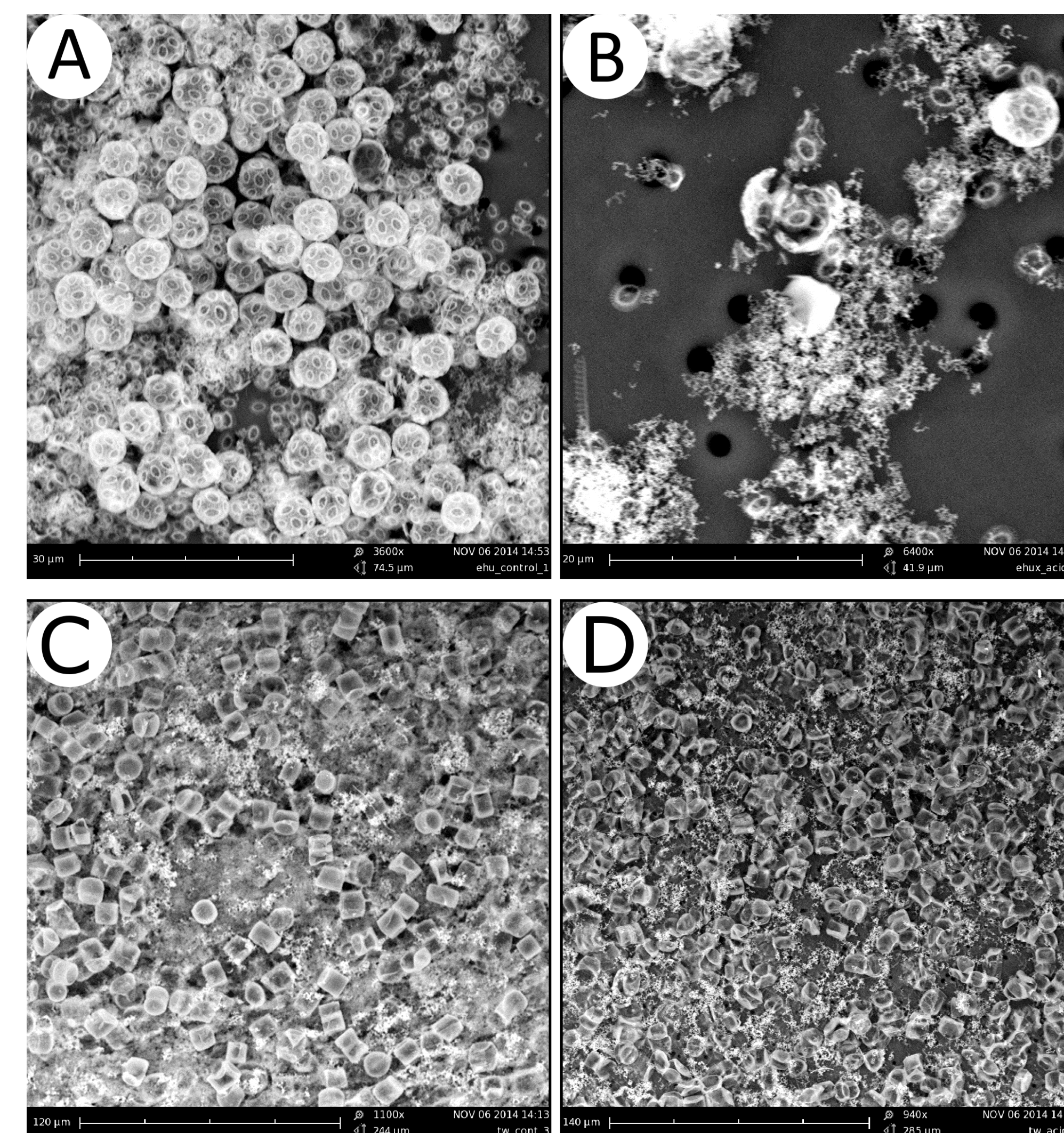


Figure 4. A) Electron microscope picture of *E. huxleyi* tests in control and B) acidified media. C) Picture of *T. weissflogii* frustules in control vs. D) acidified media.

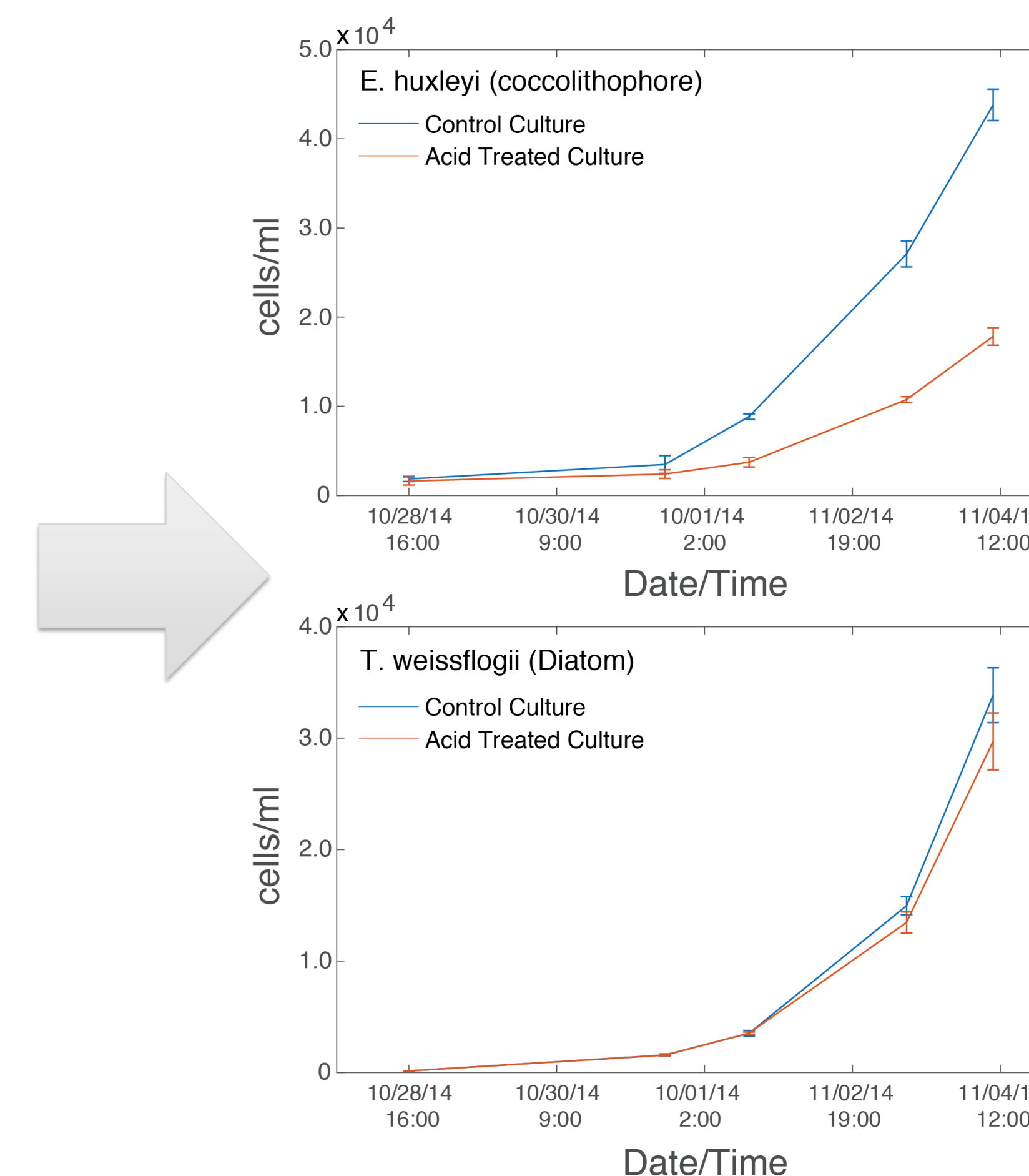


Figure 2. *E. huxleyi* (top) and *T. weissflogii* (bottom) cell numbers vs. time in control (blue) vs. acidified (orange) culture media. Triplicate averages ± standard deviation.

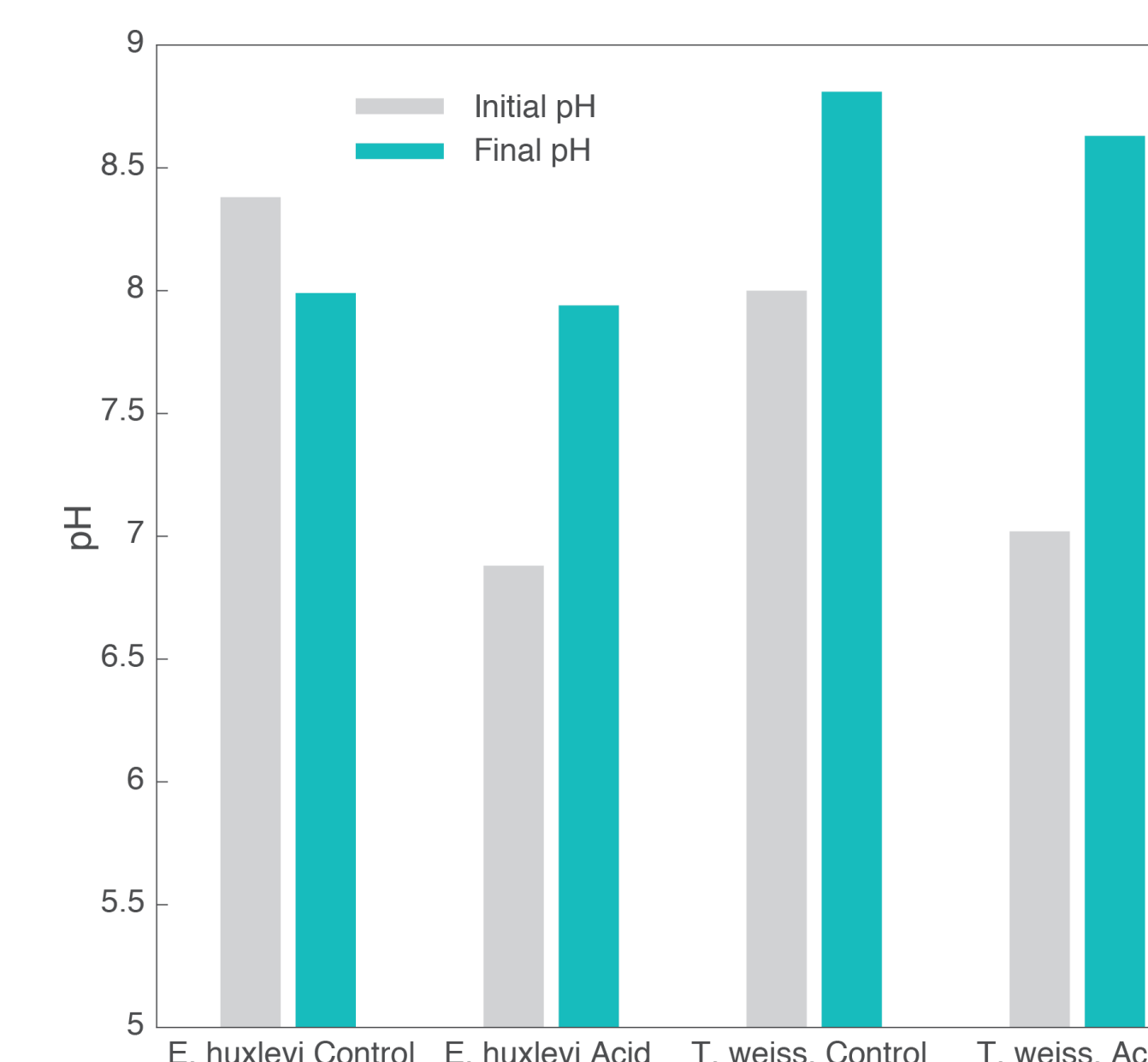


Figure 3. Initial (gray bars) and final media pH (green bars) for experimental cultures (average of triplicate culture). Note: A one unit change in pH equals a ten-fold change in H<sup>+</sup> concentration.

## Conclusions

- Coccolithophore growth rates were lower in the acidic culture than control culture. We speculate that under low pH conditions, coccolithophores must allocate more energy to shell formation - given higher propensity for dissolution - and/or to cellular regulation, thus lowering growth rates. Diatom growth rates were not affected by lower pH conditions, suggesting *T. weissflogii* is tolerant to a pH range of 7.5-8.2. Importantly, siliceous tests do not dissolve at reduced pH.
- The pH reduction in the control coccolithophore cultures is likely the result of shell formation, countering pH increases from CO<sub>2</sub> and nitrate consumption. The control culture formed healthy shells (Figure 3A), thus reducing [CO<sub>3</sub><sup>2-</sup>] and pH. In the acidified culture, shells appear to have undergone dissolution (Figure 3B), which has the opposite effect on [CO<sub>3</sub><sup>2-</sup>] and pH. Thus, pH increased due to photosynthesis and nitrate consumption.
- In the diatom cultures, pH changes are likely dominated by photosynthesis and nitrate consumption, as photosynthesis removes CO<sub>2</sub> from the water, which in turn reduces H<sup>+</sup> concentration, increasing pH. Nitrate consumption increases alkalinity (buffering), thus increasing pH.

## Broader Implications

- Average ocean pH has already declined from 8.2 to 8.1 since pre-industrial times, corresponding to a 30% increase in H<sup>+</sup> ions in ocean surface waters.
- Current IPCC (Intergovernmental Panel on Climate Change) projections predict further decreases of 0.2 - 0.5 by 2090 (Figure 5.). Our results suggest that calcifying organisms like *E. huxleyi* will be negatively impacted by an acidifying ocean; however, further research is necessary to determine species pH tolerance and adaptability in natural systems.

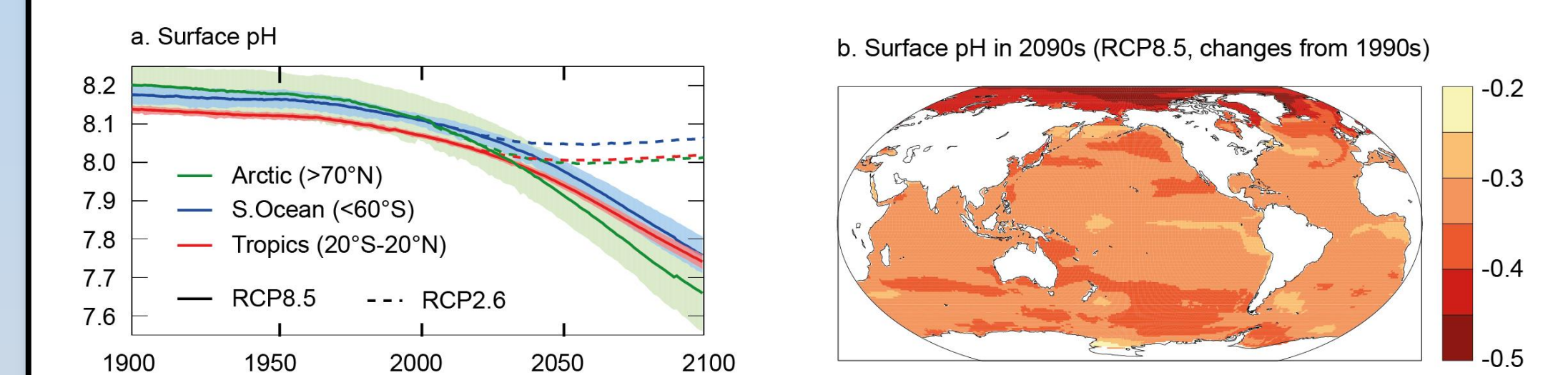


Figure 5. IPCC predictions for surface pH change by 2090. Cited from IPCC, 2013, Chapter 6.

## References

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## Further information

More information on ocean acidification and its effects on marine organisms can be found at the following websites.  
<http://www.pmel.noaa.gov/co2/story/What+is+Ocean+Acidification%3Fhttp://ocean.si.edu/ocean-acidification>