Carbon-specific growth rates of the toxic dinoflagellate, *Karenia brevis*

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1. The Problem

Harmful algal blooms (HABs) are responsible for the loss of millions of dollars to the economy through their negative impacts on the fishing and tourism industries. The harmful effects come about because these algae produce potent neurotoxins that can be transferred through the food web, resulting in fish kills, closures of shellfish areas, marine mammal mortalities and respiratory irritation in humans. In the Gulf of Mexico, the organism most often responsible for HABs is called *Karenia brevis* (Fig. 1), a toxic dinoflagellate which blooms annually off the west coast of Florida and frequently off the coasts of Alabama, Louisiana, and Texas.

In order to understand how blooms of these algae form and decline in nature we need to understand how their growth rate varies in response to environmental factors like light and nutrients. This sounds simple, but in practice it is difficult to determine growth rates of one specific type of algae in a mixed community. Fortunately, *Karenia brevis* contains a unique pigment called **gyroxanthin-diester** (Fig. 2) that allows cells of this genus to be distinguished from others in the water.

Fig. 1 A scanning EM micrograph of *Karenia brevis* (K. Steidinger, FMRI)

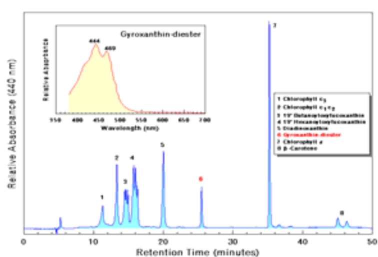


Fig. 2 HPLC chromatogram showing the *Karenia*-specific pigment, gyroxanthin-diester

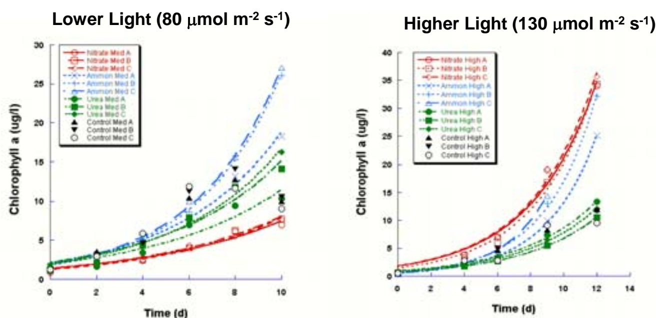
2. Objectives

Our EPA-STAR funded research examines the growth dynamics of Karenia brevis *in relation to prevailing light intensity, nutrient availability (different nitrogen forms) and growth rate.* Specifically, we are assessing the utility of a technique known as **photopigment radiolabeling**, which takes advantage of the presence of gyroxanthin-diester, in measuring the growth rates of *Karenia brevis* in natural samples. To see how well this technique works, we compared growth rates measured by the photopigment radiolabeling method to rates determined by standard methods in the lab (i.e. time-course measurements of cell numbers and chl *a* in batch cultures and to semi-continuous cultures with set dilution rates (= growth rates)).

3. Time-Course Batch Culture Experiments

Lower Light (80 μmol m^{-2} s^{-1})

Higher Light (130 μmol m^{-2} s^{-1})



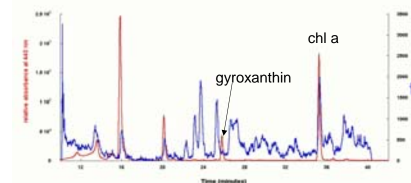
Nitrate $\mu = 0.18 \pm 0.01$ d^{-1}
Ammonium $\mu = 0.25 \pm 0.03$ d^{-1}
Urea $\mu = 0.19 \pm 0.02$ d^{-1}

There was no significant difference ($p<0.01$) in growth rates between nutrient treatments

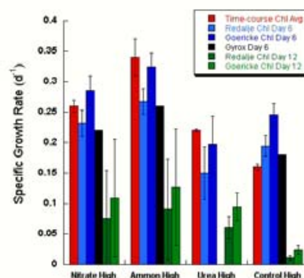
Nitrate $\mu = 0.26 \pm 0.01$ d^{-1}
Ammonium $\mu = 0.34 \pm 0.03$ d^{-1}
Urea $\mu = 0.22 \pm 0.002$ d^{-1}

There was no significant difference ($p<0.01$) between nutrient treatments but growth rates were sig. higher at higher light and there was a significant nutrient x light interaction.

4. Photopigment Radiolabeling: Batch Cultures



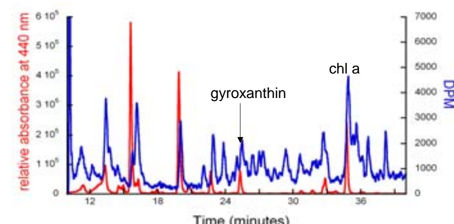
Chromatogram and radiogram for higher light treatment (nitrate grown cells). The radiolabeling peak associated with gyroxanthin (dpm) is small because pigment per cell is low and growth rates are relatively slow. Labeling was barely detectable and rates were difficult (but sometimes possible) to calculate. See results below.



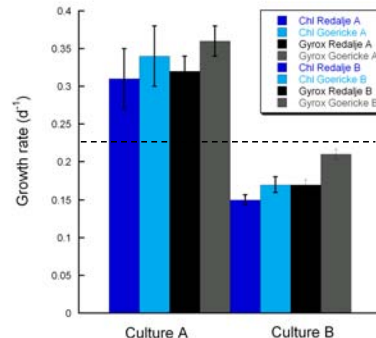
Comparison between radiolabeling and time-course growth rates (shown in panel 3)

There was no significant difference ($p<0.01$) between the time-course batch culture and "Goericke" radiolabeling estimates of growth rate when cells were in exponential growth (Day 6). This is good! Day 12 rates should not agree because cells were in stationary phase then.

5. Photopigment Radiolabeling: Semi-Continuous Cultures



Two semi-continuous cultures (with $\mu = 0.23$ d^{-1}; dashed line) were incubated with ^{14}C for 48h. We partly solved the "low detection" problem (see 4. above) by filtering more material. Gyroxanthin rates were detectable and agreed well with rates based on radiolabeling of chlorophyll *a*. Radiolabeling growth rates were different from the set dilution rate of 0.23 d^{-1}, however results were within the error bounds of the method and look promising. More semi-continuous cultures are currently underway.



6. Conclusion

The photopigment radiolabeling method may be used successfully to determine carbon-specific growth rates of *Karenia brevis* during monospecific blooms, however gyroxanthin-radiolabeling may be of limited use in mixed assemblages (when there is low *Karenia* biomass), or in early stages of bloom formation (when growth rates are slow) because low concentrations of gyroxanthin per cell and low turnover rates hinder detection.

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