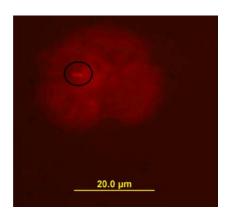
ECOHAB: Karenia

RECENT FINDINGS: Karenia Grazing on Picoplankton

Karenia brevis: The Mixotroph

Mixotrophy is the ability of an organism to assimilate organic carbon and inorganic carbon using a combination of heterotrophic and autotrophic modes of metabolism to acquire nutrients for growth. **Karenia brevis* is a mixotrophic dinoflagellate that is able to ingest picoplanktonic organisms as well as photosynthesize. Mixotrophy among dinoflagellates, especially harmful algal species, may have important ecological ramifications. The idea that many dinoflagellate species can switch from autotrophic to heterotrophic metabolism may be fundamental to understanding harmful algal bloom dynamics particularly in coastal systems.



Synechococcus cell that was ingested by a Karenia brevis cell during a laboratory grazing experiment

Grazing by *K. brevis* on picoplankton in the Gulf of Mexico may also shorten the trophic transfer of dissolved organic material through the <u>microbial loop</u> as dinoflagellates can be a direct link between <u>picoplankton</u> and <u>zooplankton</u>.

<u>Picoplanktonic organisms</u> are some of the most abundant organisms on earth and may provide the nutrients that provide the necessary nutrients to allow bloom initiation, permit intense and long lasting blooms to persist, as well as maintain 'seed' populations of *K. brevis* in offshore waters.

To date, efforts to quantify nitrogen (N) sources fueling *Karenia brevis* blooms in the Gulf of Mexico have not included phagotrophic grazing. Many dinoflagellates, including *K. brevis*, are known to be capable of grazing; however, grazing by *K. brevis* on co-occurring picoplankton in the Gulf of Mexico has not been examined. Initial laboratory and field studies have demonstrated that recent isolates and natural blooms of *K. brevis* can ingest *Synechococcus*.

In an effort to more accurately quantify the N budget for *Karenia brevis* including mixotrophic <u>grazing</u>, these questions are being examined:

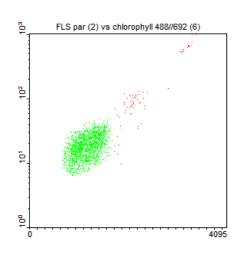
- 1: Are recent culture isolates of *K. brevis* able to ingest *Synechococcus*?
- 2: Are natural populations of *K. brevis* able to ingest *Synechococcus*?

3: Is there a difference in <u>grazing</u> rates by *K. brevis* growing under different light or nutrient conditions?

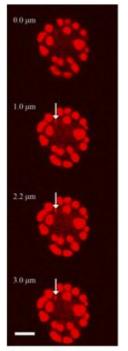
Quantifying Grazing Rates

Flow Cytometry:

Flow cytometry was originally used in the medical field and only recently has it been used for counting marine microorganisms. Researchers use flow cytometry to more efficiently count *Karenia brevis* and *Synechococcus* cells from the Gulf of Mexico and in laboratory cultures. It takes less than a minute to run one sample through the flow cytometer. Once the sample is run, an analysis is possible of the dot plot cytogram (shown here) by recognizing different cell populations, drawing a box, or 'gate', around each population, and then counting the dots (cells) within each gate.



A cytogram showing three different populations of autofluorescent autotrophs



Confocal Laser Scanning Microscopy:

Epifluorescence Microscopy (EM) is used to detect whether Synechococcus prey cells were ingested. Ingested prey cells would be seen as small orange-red cells within the cell wall of the much larger Karenia brevis cells. This, however, is inconclusive due to the fact that the EM results in a 2-dimensional image. Confocal laser scanning microscopy (CLSM) is used to verify that a prey cell was ingested by scanning a cell vertically, from top to bottom, in increments as small as 0.2 micrometers. After a complete scan, a 3-dimensional model can be created from the consecutive images, which can be used to verify that a prey cell was ingested or not.

A series of CLSM images showing a Synechococcus prey inclusion within a Karenia cell