# Parasitic *Amoebophrya* Interactions with *Karenia brevis*

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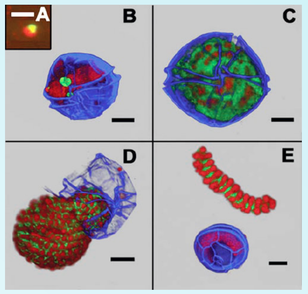
## Introduction

- *Karenia brevis* is a species of bloom-forming phytoplankton in the Gulf of Mexico that produce a neurotoxin called brevetoxin. The toxin build up in shellfish and other filter feeders. Its effects spread outward to marine and terrestrial vertebrates and can even aggravate respiratory problems in humans.
- *Amoebophrya* is a widespread genus of parasitic dinoflagellates that are well known to infect HAB species.




Life Cycle of *Amoebophrya*: Green shows probe ALV01 marker by FISH, red shows dyed DNA, blue marks theca of host cell (*A. minutum*).
A: Zoospore stage
B: Early infection
C: Trophont stage
D: Emergence
E: Vermiform stage
Scale bars: 3 µm in A, 10 µm in B through E

(Chambouvet et al, 2008)

- The purpose of this study was to determine if *Amoebophrya* can infect *K. brevis* or inhibit its growth in culture. Two strains of *Amoebophrya*, from hosts *Scrippsiella trochoidea* (IFREMER) and *Alexandrium sp*. (Woods Hole, containing unknown dinoflagellate contaminant), were tested with *K. brevis* cultured from Manasota Key.
- *S. trochoidea* is an armored, non-toxic host and *Alexandrium sp*. is an armored, toxic host. These were used because both can be infected by *Amoebophrya* despite differences in protection.

## Methods

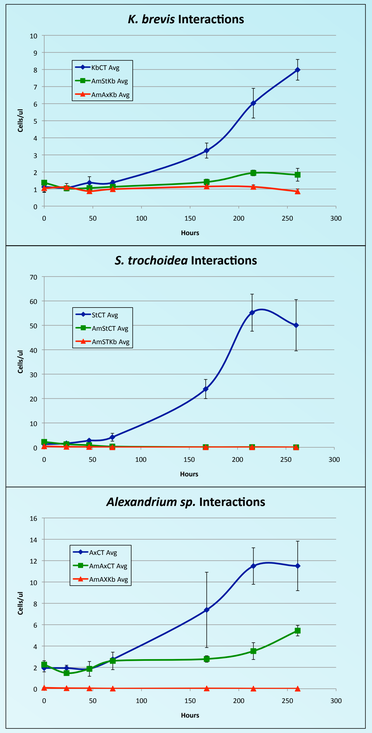
- All studies run in triplicate in tissue culture flasks at 18.0°C. Total volume of culture at Time 0: 30 ml. L1 media (MML recipe) used for nutrient base.
-Cultures monitored with fluorescent microscopy and regular cell counts: fixed with Utermohl's Iodine and counted on a Nageotte hemocytometer or wellplate depending on density.

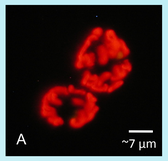
- Sonication in Challenge Study 1.3 occurred at 21% amplitude for 45 seconds with a Sonics VibraCell
  - Lysate was added to culture through a 0.45 um syringe filter.
  - Pre-sonication density~1.98 cells/ul; Post-sonication density~ 0.26 cells/ul

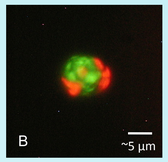
## Challenge Study 1

- Challenge Study 1 was the main experiment testing *K. brevis* against both *S. trochoidea* and *Alexandrium* strains of *Amoebophrya*. Culture flasks set up as follows with associated abbreviations:

Kb CT: *K. brevis* control
Ax CT: *Alexandrium sp* control
St CT: *S. trochoidea* control
AmexAx CT: *Amoebophrya* infected Ax control
AmexSt CT: *Amoebophrya* infected St control
AmexAx Kb: AmexAx with *K. brevis* (both Kb and AX counts respectively)
AmexSt Kb: AmexSt with *K. brevis* (both Kb and ST counts respectively)



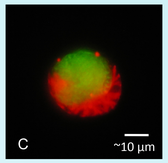




Fig. 1: Average growth of each triplicate category: *Amoebophrya* suppressed growth in Ax, St and Kb. In AmexAx, *K. brevis* appears to also have decreased the growth of *Alexandrium*. For all fluorescent microscopy pictures: red shows chloroplasts, green shows Amoebophrya trophont. Pic A: *K. brevis*; Pic B: infected *S. trochoidea*; Pic C: Infected *Alexandrium*

### Discussion

- Toxins released by lysed *Alexandrium* or a dinoflagellate contaminant could have decreased *K. brevis* growth. However since *K. brevis* growth was affected in both AmexSt and AmexAx, it is possible the decrease is due to *Amoebophrya*.
- Some *K. brevis* cells in AmexSt and AmexAx observed to be misshapen and, under epifluorescence, contain pale green dots not observed in the control Kb cultures.

## Challenge Study 1.2

- To determine why the growth of *K. brevis* had been restrained in Challenge Study 1, *K. brevis* in culture with uninfected *Alexandrium* was observed. Any growth different than the control would be from *Alexandrium* or the small dinoflagellate contaminant present in Ax CT cultures.

Kb CT: *K. brevis* control and AxKb: *Alexandrium sp.* with *K. brevis*

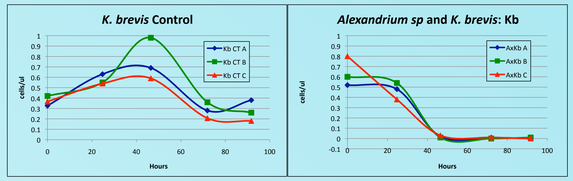



Fig. 2: *K. brevis* growth in culture with *Alexandrium* decreased compared to control growth.

- Unfortunately, starting Ax densities were significantly higher than Challenge Study 1 and *Alexandrium* overwhelmed *K. brevis*. There is a possibility that the *K. brevis* decreased due to resource competition.

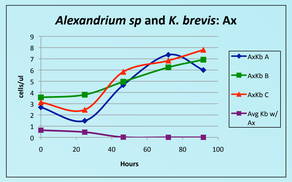



Fig. 3: Comparing *Alexandrium* growth with average *K. brevis* growth in test cultures.

## Challenge Study 1.3

- In case *K. brevis* was affected by toxins released from *Alexandrium* cells lysed by *Amoebophrya*, Ax culture was lysed using a sonicator, filtered, and then added to Kb culture.

Kb CT: *K. brevis* control and AxL Kb: *Alexandrium* lysate with *K. brevis*

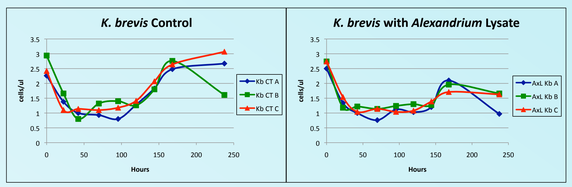



Fig. 4: *Alexandrium* lysate slowed *K. brevis* growth compared to control.

- The large initial drop in all cultures is most likely due to a temperature change from culture storage (24°C) to testing incubation (18°C).
- Kb avg growth rate: 0.0096±0.0059 cells/µm/hour
- AxLKb avg growth rate: 0.0020±0.0027 cells/µm/hour

## Conclusion

- Growth of *K. brevis* in culture was negatively affected by *Amoebophrya*. In both AmexSt and AmexAx, the growth rate of *K. brevis* was inhibited compared to the control.

| Average Growth Rates (cells*ul⁻*hr⁻) | | |
|---|---|---|
| Kb CT | AmexAx Kb | AmexSt Kb |
| 0.036±0.001 | 0.002±0.001 | 0.006±0.001 |
| Ax CT | AmexAx CT | AmexAX Kb |
| 0.073±0.015 | 0.006±0.010 | 0.0001±0.0002 |
| St CT | AmexSt CT | AmexST Kb |
| 0.353±0.043 | -0.041±0.012 | -0.009±0.002 |

Fig. 5: Control growth rates higher than counterpart with *Amoebophrya*, *K. brevis* also decreased host growth.

- This decrease in growth could be due to a compound produced by *Amoebophrya,* actual infection or other stressors.
- Observations under fluorescent microscopy suggest the possible presence of *Amoebophrya* infection in *K. brevis.*
- Resource competition or the dinoflagellate contaminant also had an effect on the growth of *K. brevis.*
-It is also possible that toxins from lysed *Alexandrium* can negatively affect *K. brevis* growth.

## Future Research

- Use CARD-FISH to determine if infection occurred
-Filter *Amoebophrya* from host then add to *K. brevis* culture
- Test *K. brevis* against additional strains of *Amoebophrya.*
- Test for *Amoebophrya* infection in various phytoplankton from the Gulf of Mexico, including *K. brevis.*
- Determine if *Amoebophrya* can survive at normal Mote Research Laboratory culturing temperature of 24°C.

## Acknowledgements

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