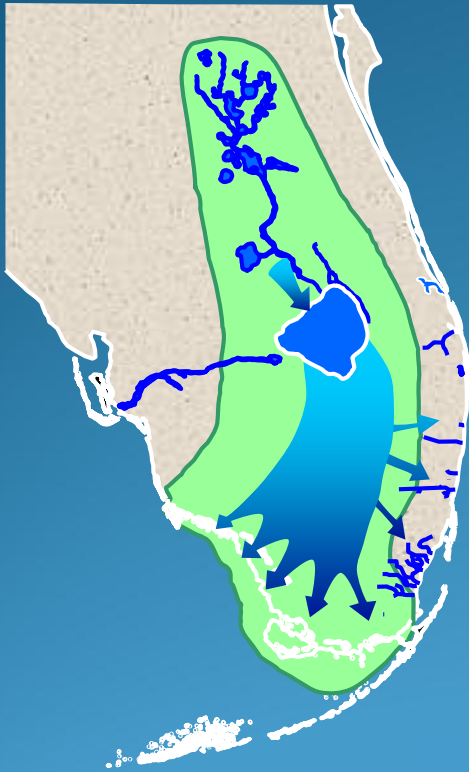


The Effects of Multiple Environmental Stressors on Hard Clam Survival and Physiology

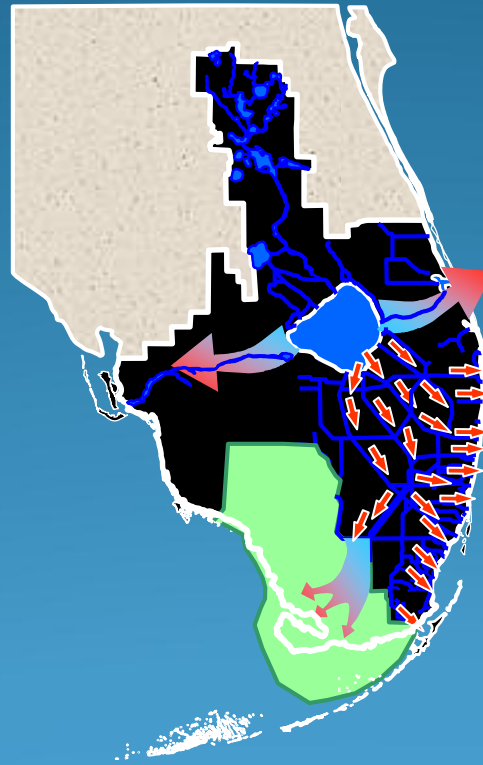
Encomio, Vincent¹; Goncalves, Madeleine²; Volety, K. Aswani²
Florida Oceanographic Society¹ , Florida Gulf Coast University²



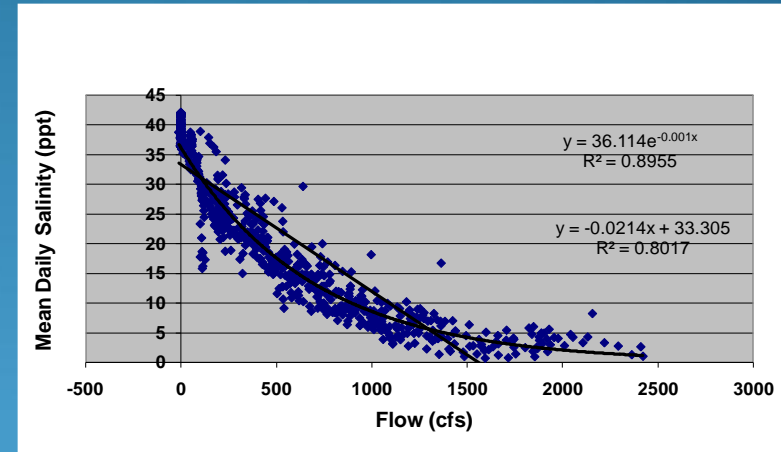
Freshwater releases



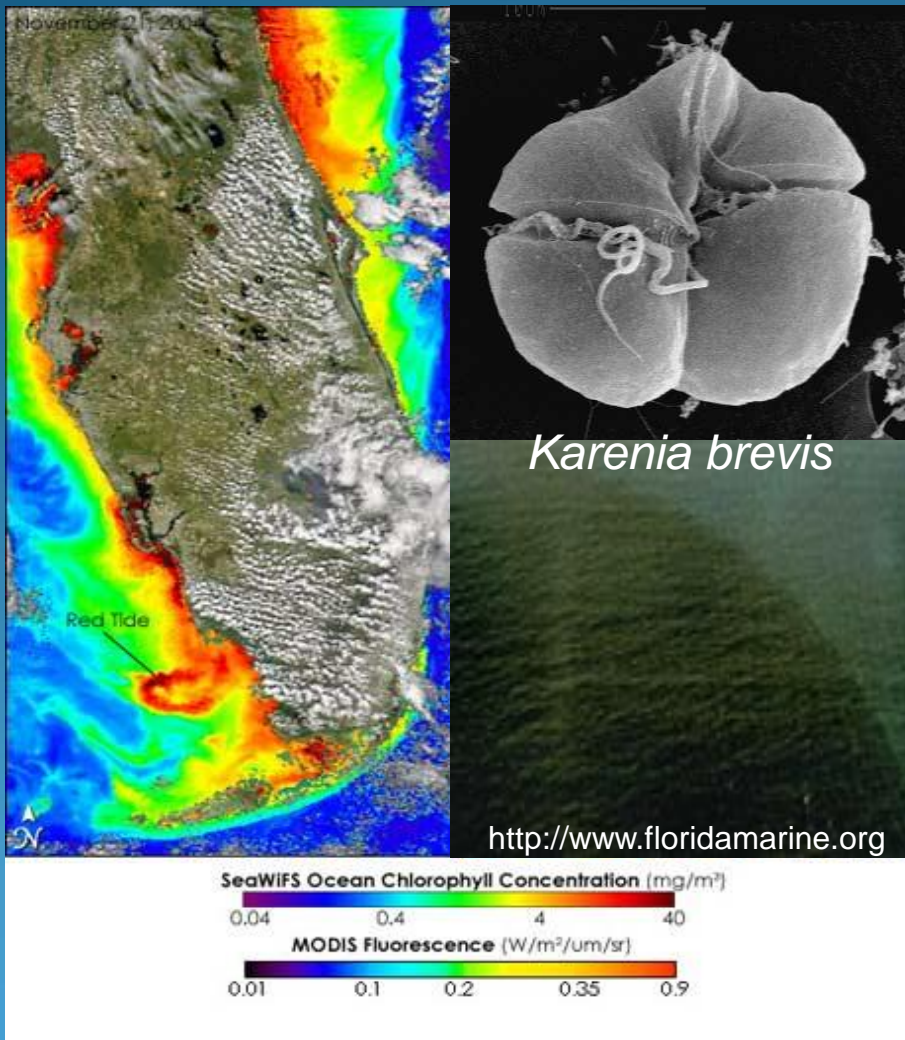
Historic Flow



Current Flow



Red Tide



Blooms can occur and persist year round (e.g. 2005) over wet and dry seasons

Also:

High temperature

Low dissolved oxygen

Red Tide choking life from gulf

A giant patch of the algal bloom is sucking oxygen from waters off Pinellas and Pasco counties, killing sea life and wreaking environmental harm.

By EMILY ANTHERS and CURTIS KRUEGER

Published August 17, 2005

St. Petersburg Times

Disastrous Red Tide Attacks West Coast

*Tampa Bay, Sarasota Bay and other stretches of peninsular west Florida experienced massive fish kills this summer during blooms of *Karenia brevis*, the planktonic critter that causes red tide.*

By Jeff Weakley,, Editor Florida Sportsman

Persistent Red Tide Takes Toll on Florida Sea Life and Tourism

by Abby Goodnough [New York Times](#) October 8, 2005
ANNA MARIA ISLAND, Fla.



Red Tide dampens Florida tourism.

Byline: Steve Huettel
St. Petersburg Times



Broad Questions

- Is there an effect of *K. brevis* on hard clams?
 - (Harvest-related problems regardless)
- Effects of multiple environmental stressors on hard clams?
- Particularly, their interactions (timing)

Specific Questions

I. Effect of *K. brevis* on tolerance to altered salinities?

- dry season to wet season

II. Effect of altered salinities on tolerance to *K. brevis*?

- wet season to dry season

Experiments

I. *Karenia brevis* → Δ Salinity

II. Δ Salinity → *Karenia brevis*

- Δ Salinity – 10, 20 and 30 ppt
- *Karenia brevis* – 500,00 cells L⁻¹ @ 30 ppt, 2x per week
- Clams heat shocked at 97 °F, 1 hour (sublethal treatment)
- Each experiment 3-4 weeks long

Factors: **Salinity, *K. brevis*, Heat shock, sampling time**



What is red tide?

In Florida, the algae that causes most red tides is *Karenia brevis*. This organism produces a toxin that can affect the central nervous system of fish. At high concentrations (called a bloom), the organisms may discolor the water.

Anatomy of a red tide cell

Chloroplast

Contains chlorophyll and where photosynthesis takes place.

Apical process/ carina

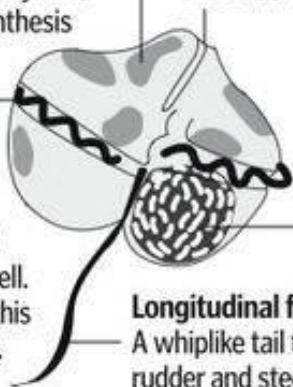
A hole at the top of the cell.

Nucleus

Contains chromosomes and where replication takes place.

Transverse flagellum

A ribbonlike tail encircling the cell. When beating, this propels the cell.



Longitudinal flagellum

A whiplike tail that acts like a rudder and steers the cell.

How toxins become airborne



1 Toxins are created by concentrated algal blooms in the Gulf.



2 The toxins cling to the air bubbles in the water as they rise.



3 The bubbles burst and eject toxic droplets into the air.

Possible effects

Cells per liter

- **Less than 1,000:** None
- **1,000 to 5,000:** Possible respiratory irritation
- **5,000 to 10,000:** Possible respiratory irritation and shellfish harvesting closures
- **10,000 to 50,000:** Respiratory irritation, but chlorophyll levels too low to be detected by satellites
- **50,000 to 100,000:** Respiratory irritation, maybe fish kills, and bloom chlorophyll probably detected by satellites
- **100,000 to 1,000,000:** Respiratory irritation and possible fish kills
- **More than 1,000,000:** All of the above plus discoloration

Cells per Liter

Experimental dosage
(500,000 per Liter)

Parameters

Physiological condition:

Condition Index

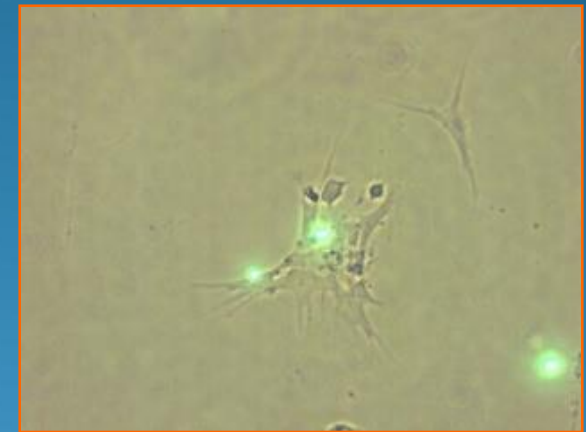
RNA:DNA

Physiological function:

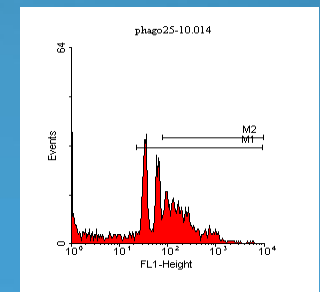
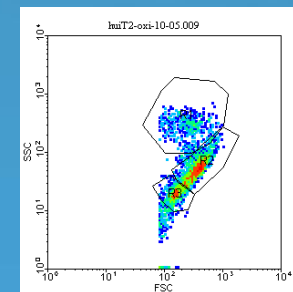
Hemocyte functions -
(**phagocytosis**, cell viability [% of dead cells], reactive oxygen species production)

Measured by flow cytometry

Heat shock response (Hsp70)
(indicator of thermal tolerance)



Hemocytes phagocytosing, or ingesting fluorescent beads



Flow cytometry measurements

Experiments

I. Karenia brevis → Δ Salinity

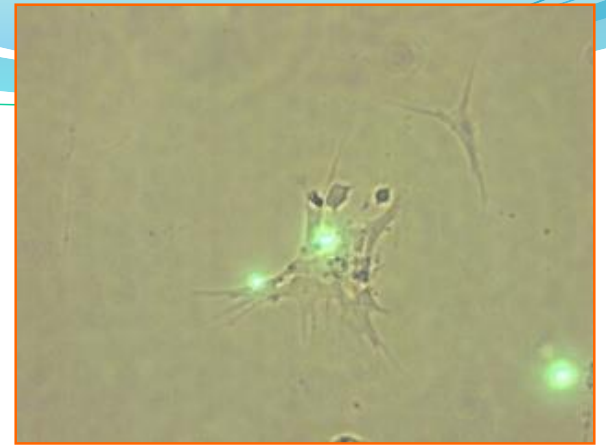
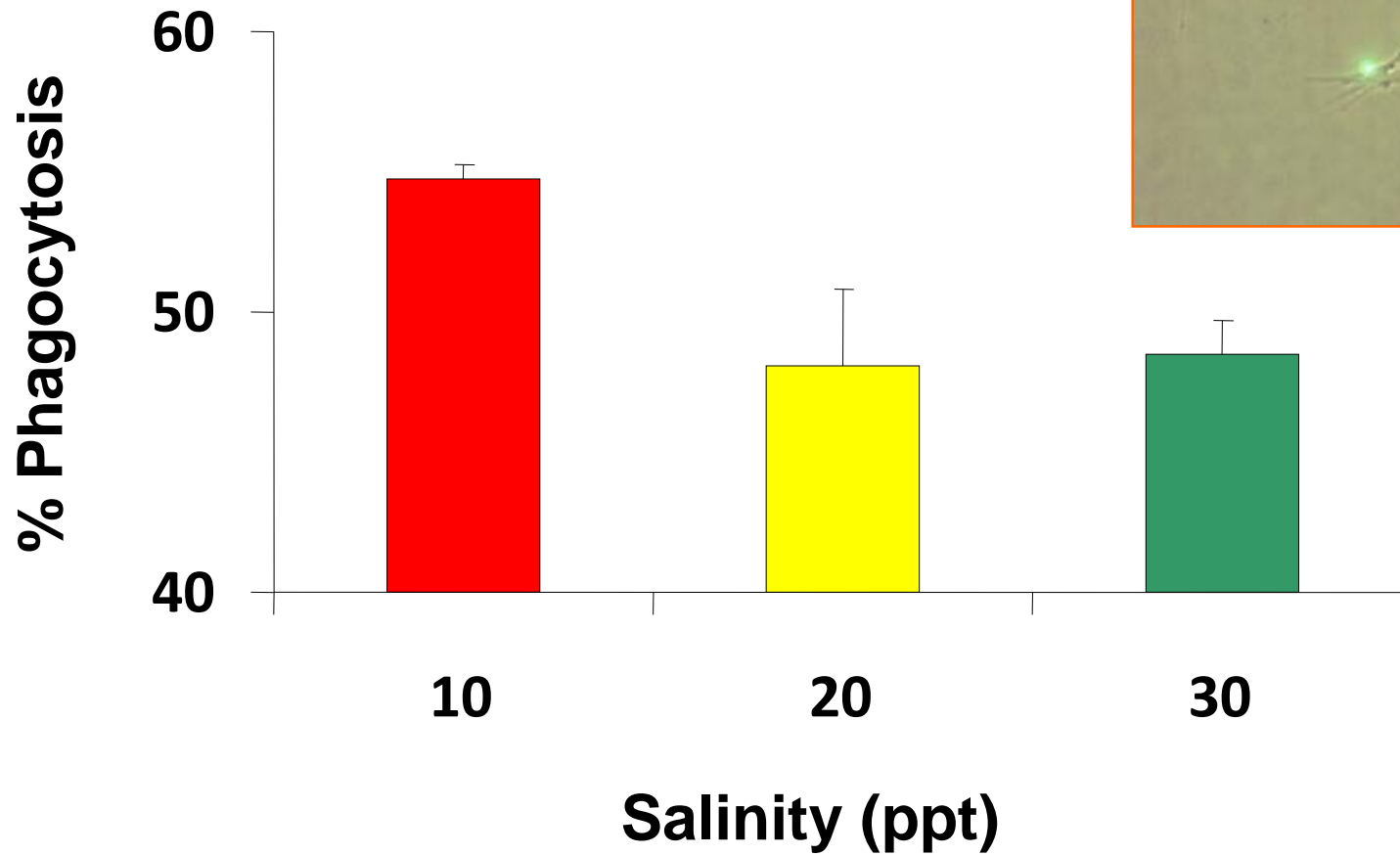
- A. Sublethal response
- B. Survival

II. Δ Salinity → *Karenia brevis*

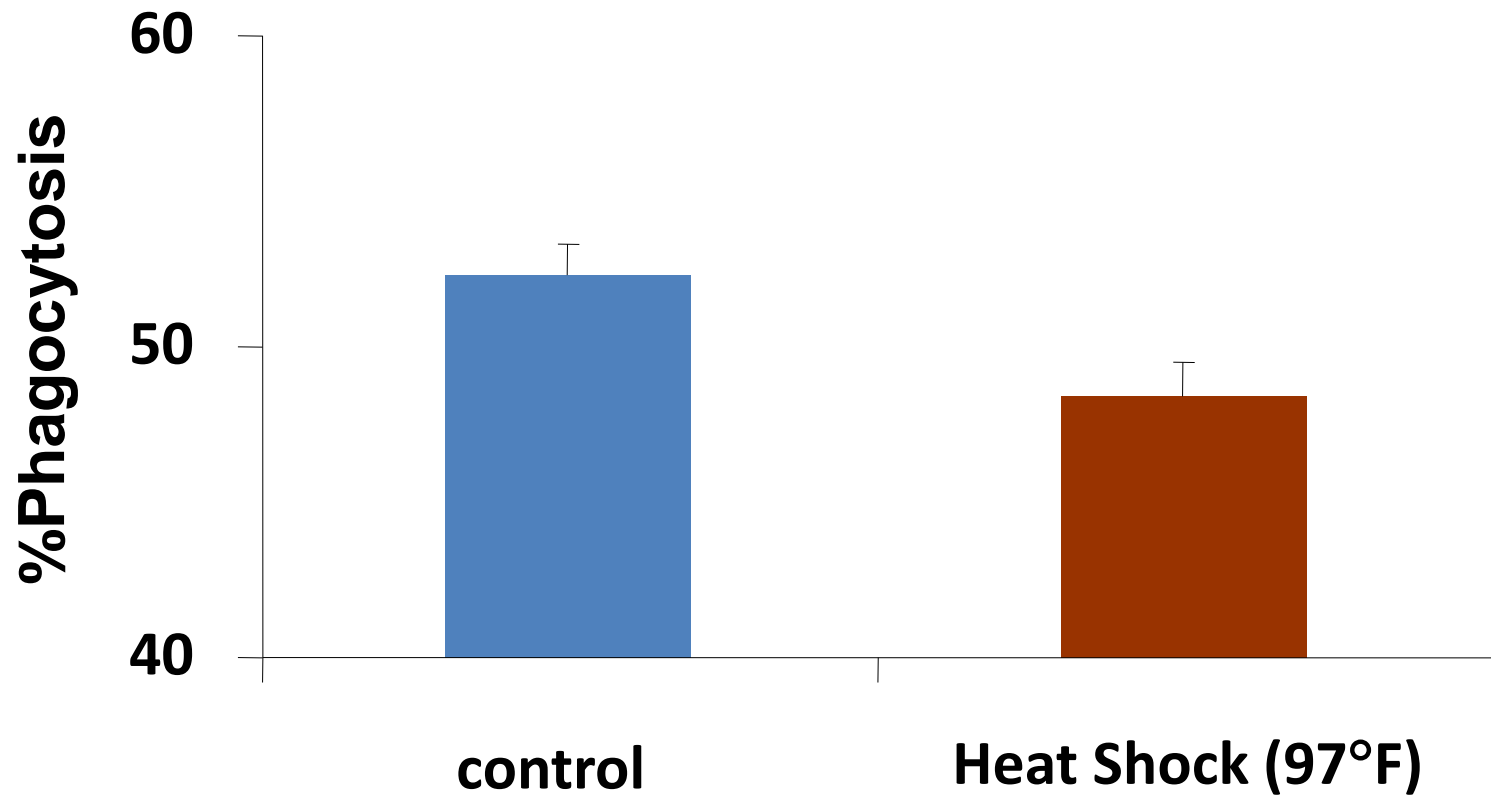
- A. Sublethal response

Experimental Design

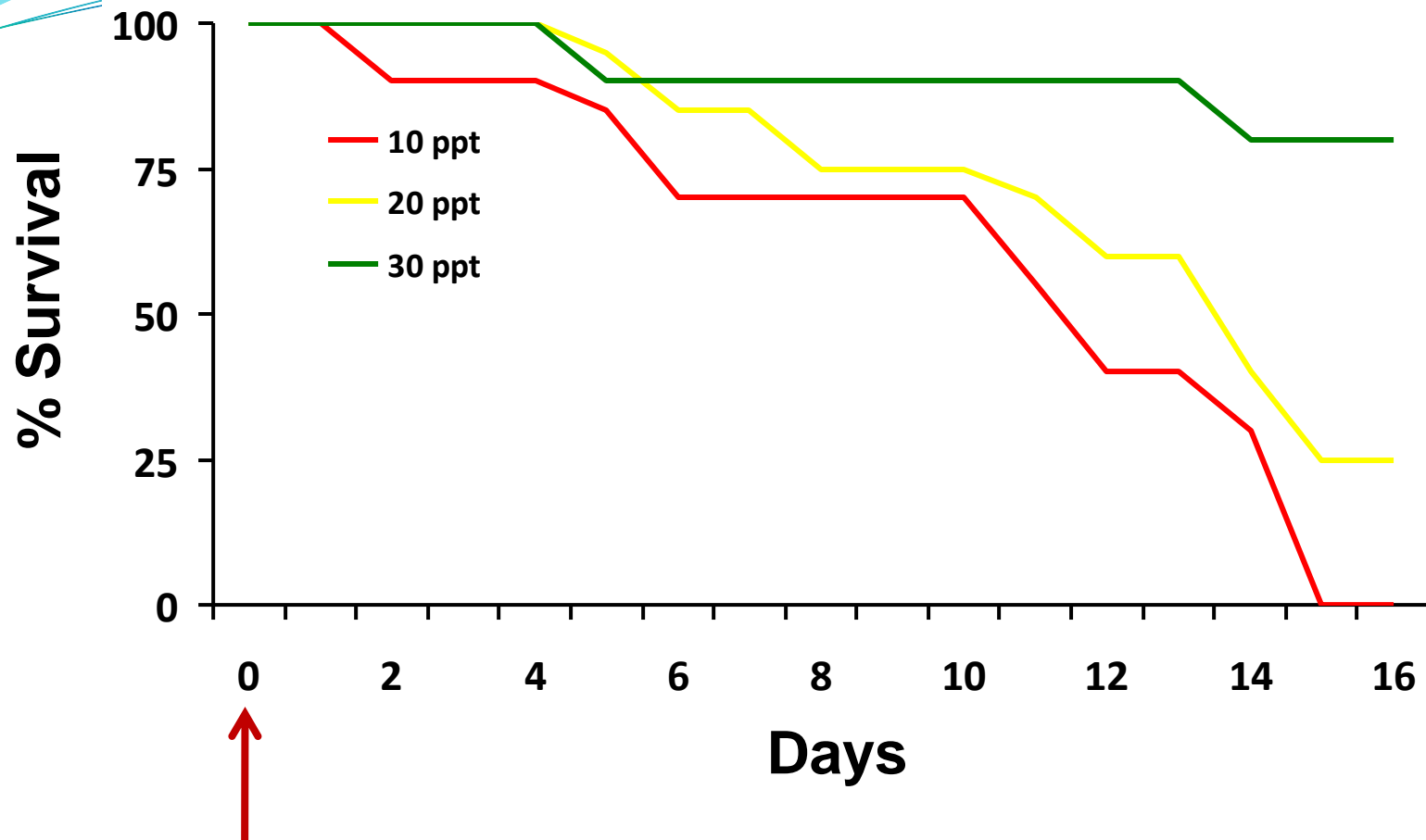
- *Karenia brevis* → Δ Salinity
- Clams exposed to red tide (500,000 cells per Liter, 30 ppt) for 2 weeks
- Clams then exposed to salinities of 10, 20, or 30 ppt for 2 weeks
- Heat shock response (non-lethal heat shock 97°F, 1 hr)
- Corresponding controls (no *K. brevis*, no salinity change, no heat shock)
- Examined both sub-lethal responses and survival



Cellular response elevated at low salinity



Heat shock (non-lethal) suppressed cellular response



Lethal heat exposure (100 °F, 1 hr)

Survival (post-lethal temperature) lowest at 10 ppt but, no additional effect due to *K. brevis*

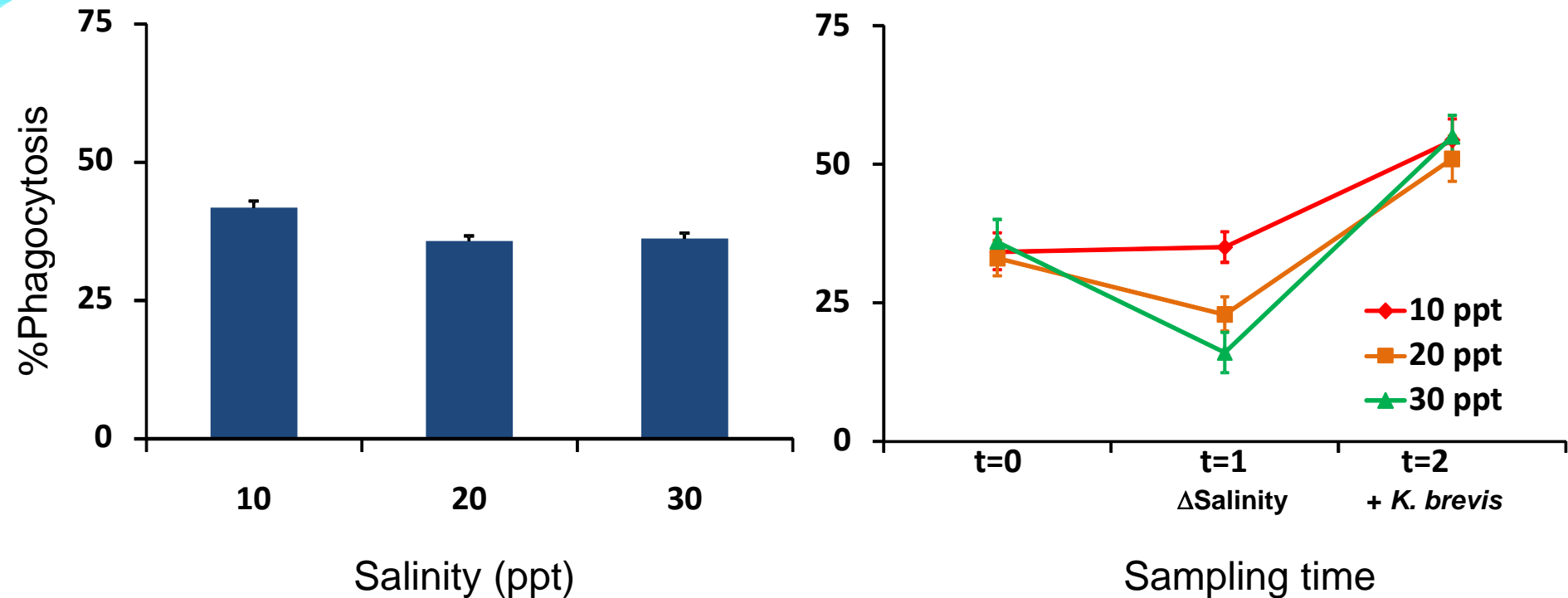
Experiments

I. *Karenia brevis* \rightarrow Δ Salinity

II. Δ Salinity \rightarrow *Karenia brevis*

Experimental Design

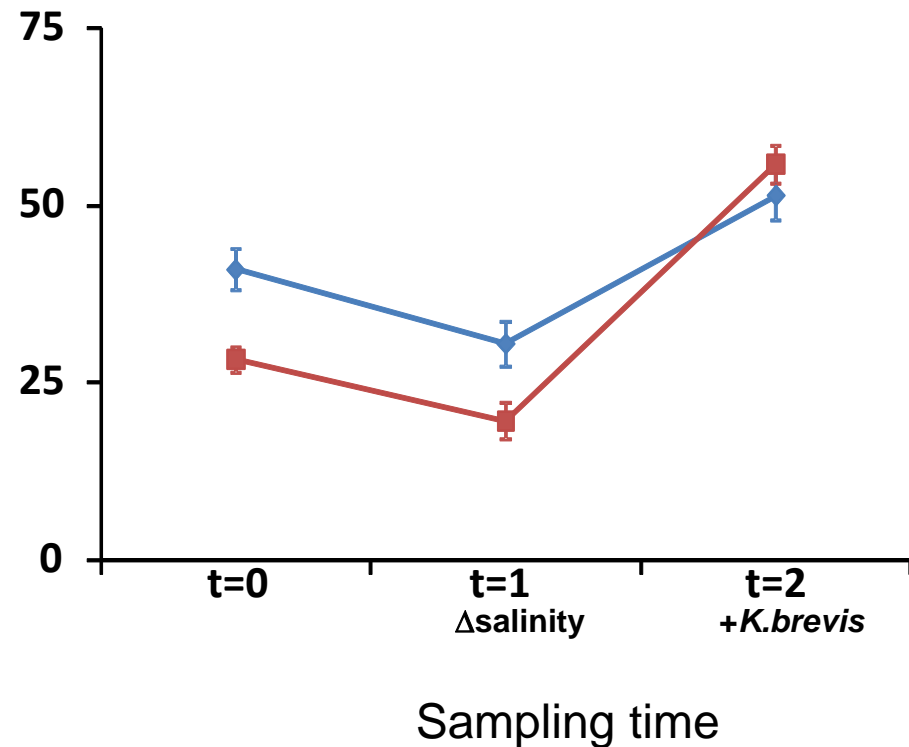
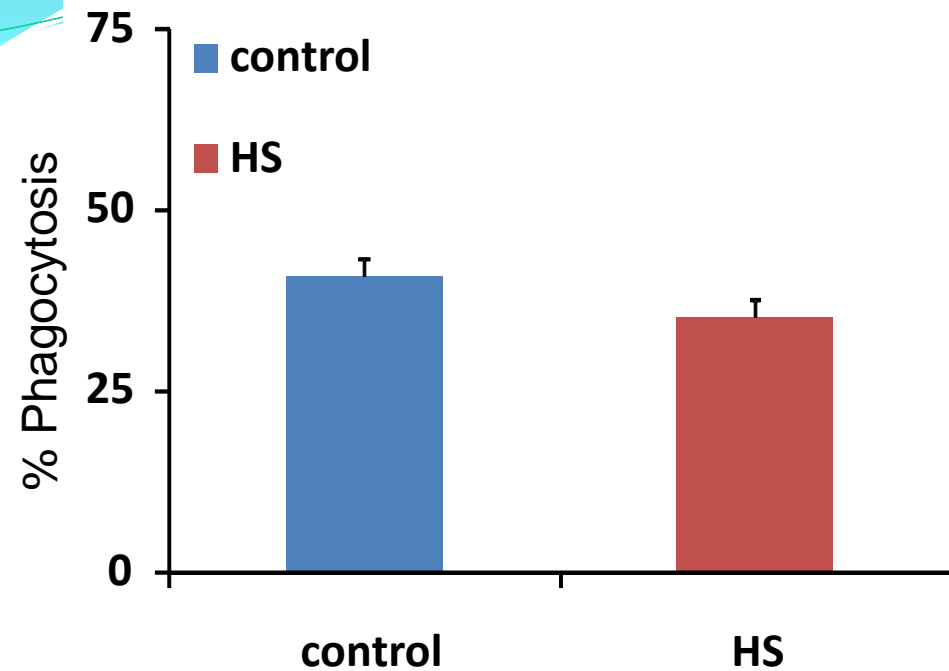
- Δ Salinity \rightarrow *Karenia brevis*
- Clams exposed to salinities of 10, 20, or 30 ppt for 2 weeks
- Clams exposed to red tide (500,000 cells per Liter, 30 ppt) for 2 weeks
- Heat shock response (non-lethal heat shock 97°F, 1 hr)
- Corresponding controls (no *K. brevis*, no salinity change, no heat shock)
- Examined *only* sub-lethal responses at this point



- *Phagocytosis higher at 10 ppt*

- *Overall increase after *K. brevis* exposure*

II. Δ Salinity \rightarrow *Karenia brevis*



- *Phagocytosis lower in heat shocked clams vs. controls*
- *Increase after K. brevis exposure*

Conclusions

Expt I & II:

Survival decreased at 10 ppt

Phagocytosis higher at 10 ppt

Phagocytosis lower after sub-lethal heat shock

Low salinity induced cellular stress response

Effects were chronic

Effects of *K. brevis* not obvious

What does this mean?

- Effects of low salinity may be long-lasting
- Recovery from low salinity events may be slow
- Harvest closures leave clams vulnerable to environmental stressors even if direct effects of red tide may not be apparent.

Future Work

Heat shock protein (measure of temperature response)

Simultaneous exposure to *K. brevis* and lower salinities

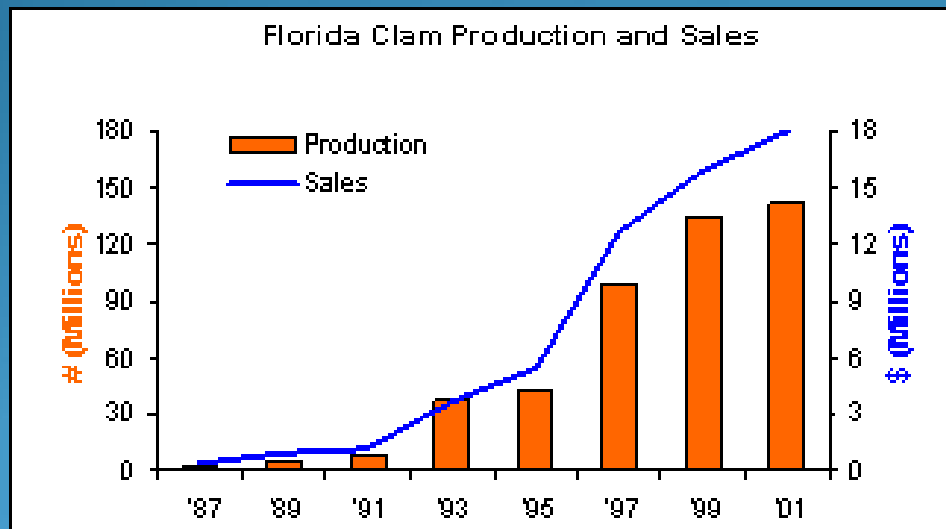
Chronic exposure to high temperatures

Interactive effect of hypoxia

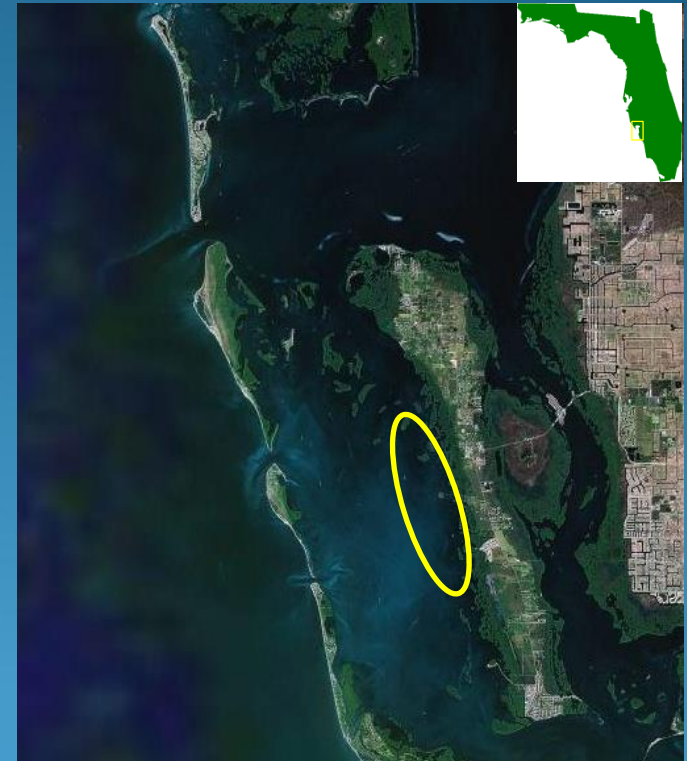
Acknowledgements

- **Environmental Protection Agency**
- **Tony Heeb at Cutthroat Clams**
- **Drs. Mike Parsons, Mike Savarese and Greg Tolley**
- **Patty Barreto**
- **Staff & students of FGCU's Coastal Watershed Institute**
 - **Holly Abeels, Andy Griffith, Lesli Haynes, Amanda Booth and Heather Benton**

Hard Clam Industry in SW Florida

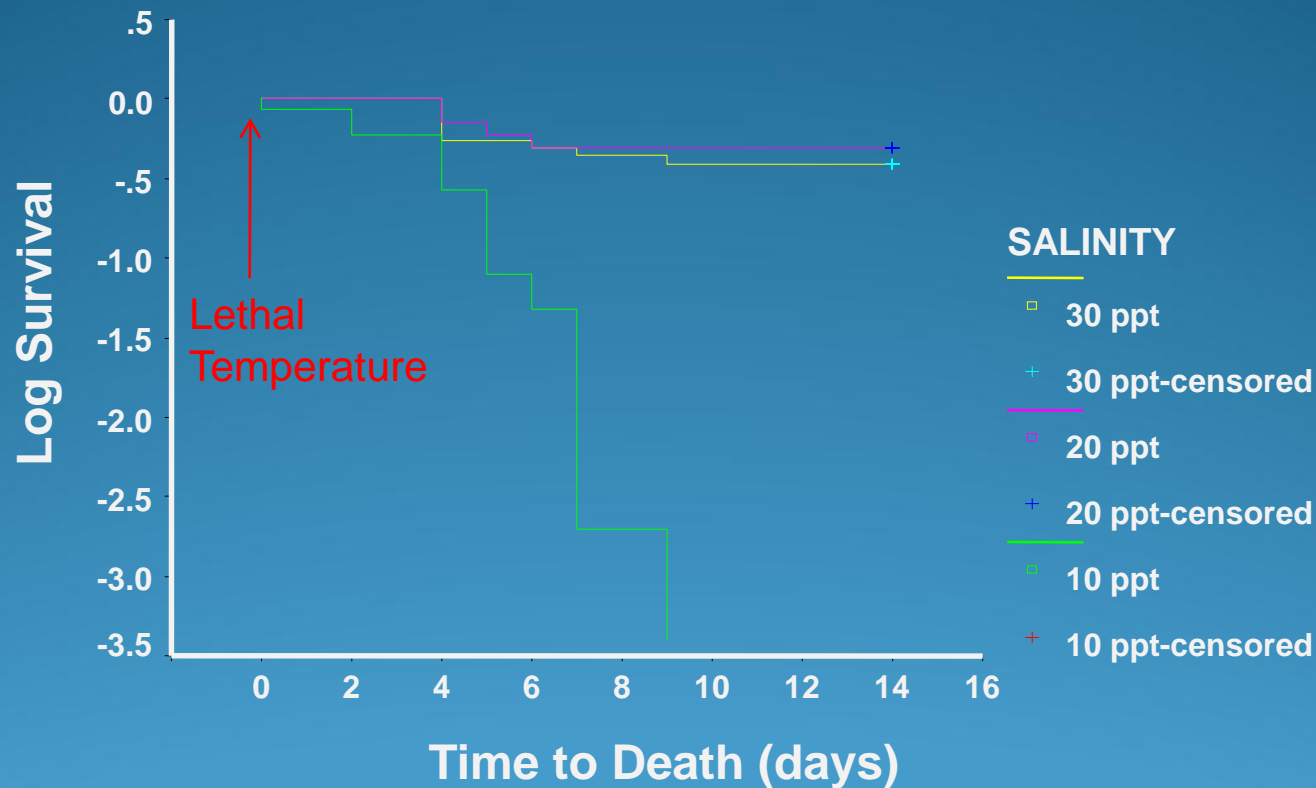


<http://shellfish.ifas.ufl.edu/industry.htm>

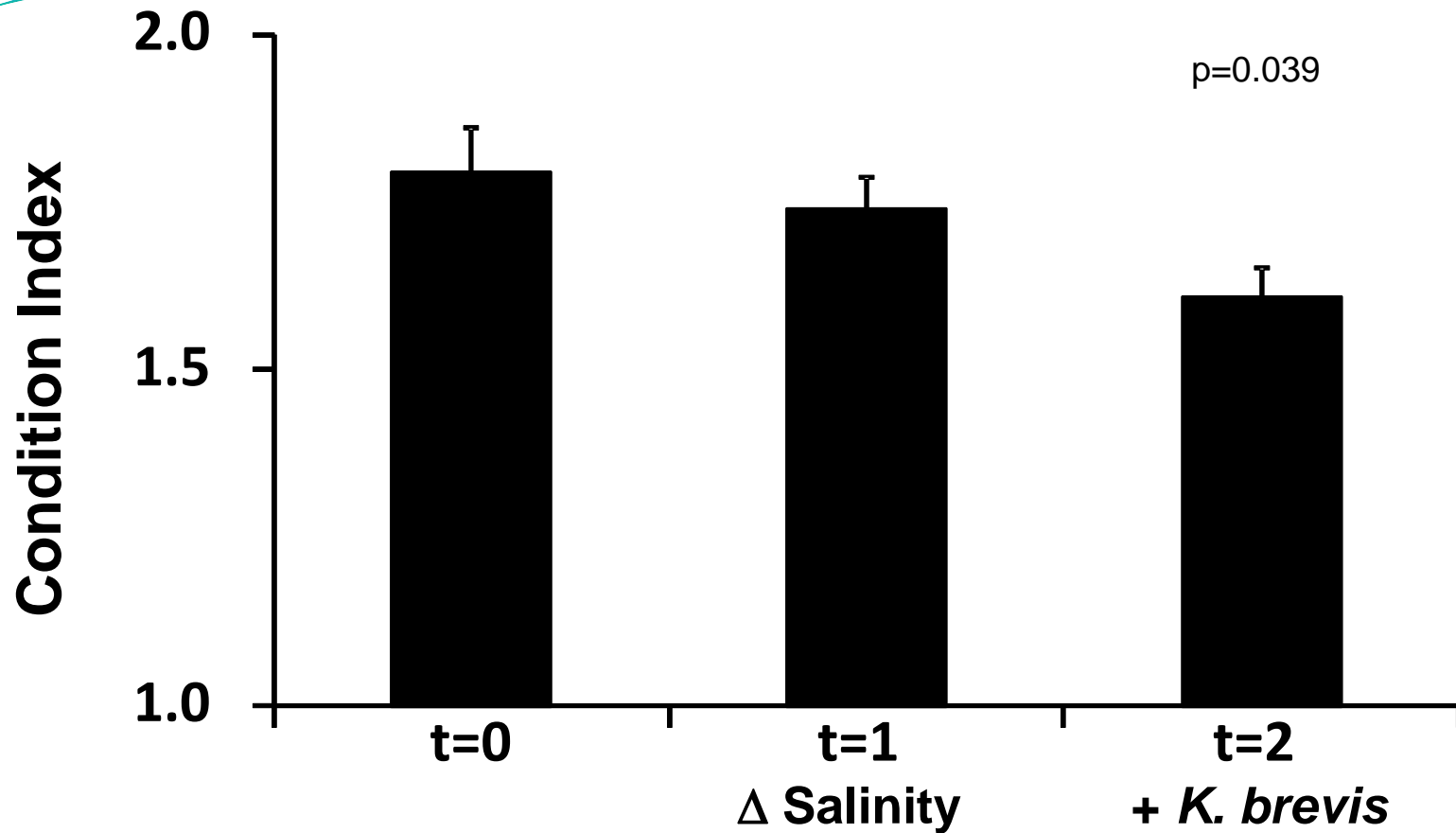


Pine Island Sound, SW Florida

Salinity effects on survival

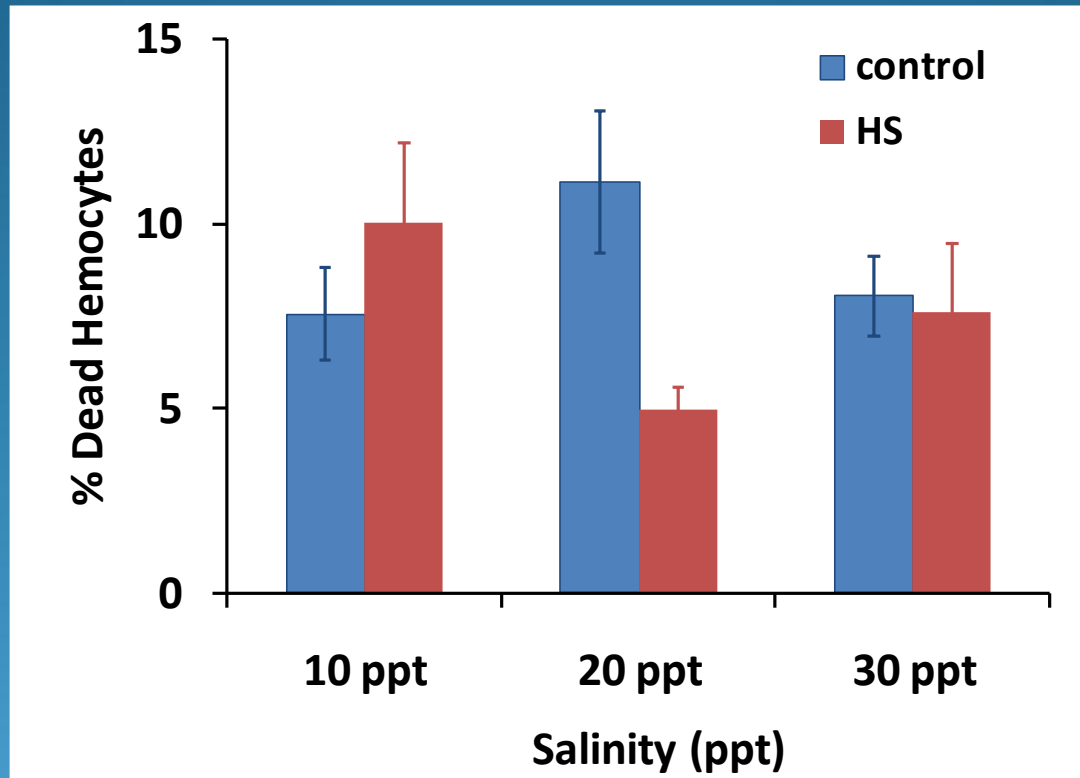


Clams exposed to 10, 20 & 30 ppt for 3 weeks, then heat shocked at 100 °F, 1 hour (lethal heat treatment) to induce mortalities

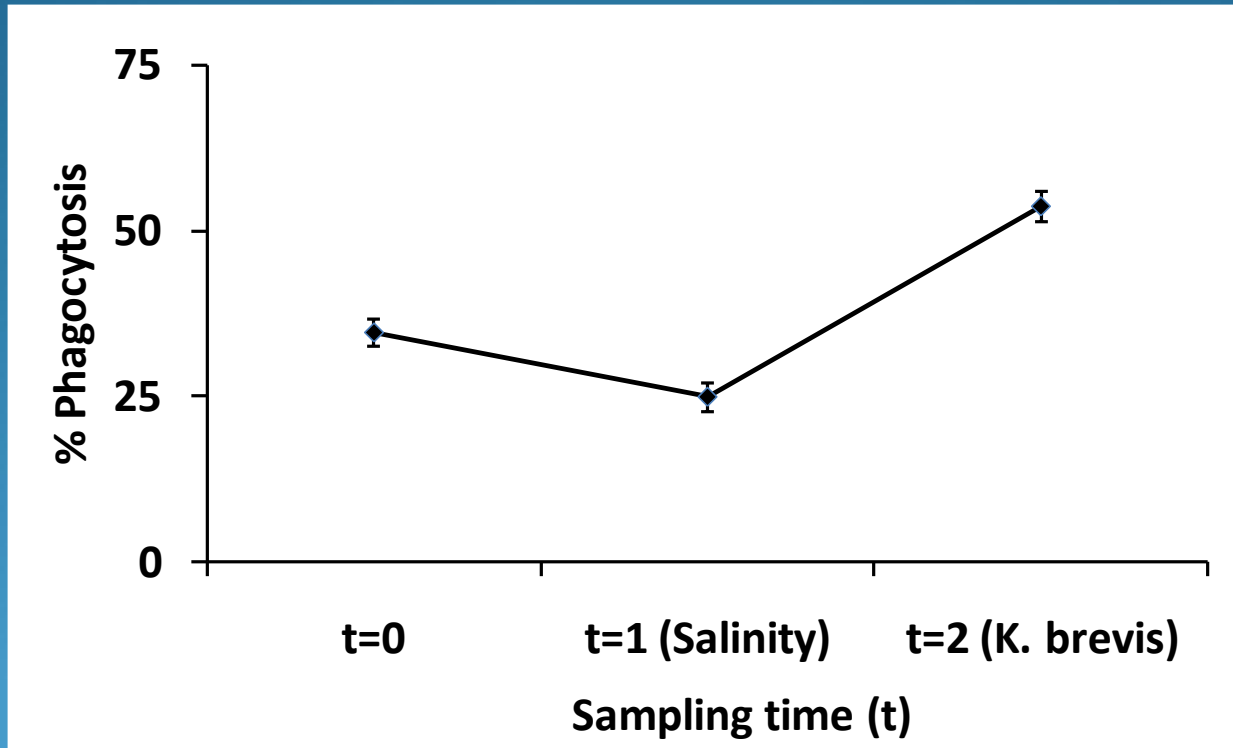


Decrease in condition index with time

II. Δ Salinity \rightarrow *Karenia brevis*



II. Δ Salinity \rightarrow *Karenia brevis*

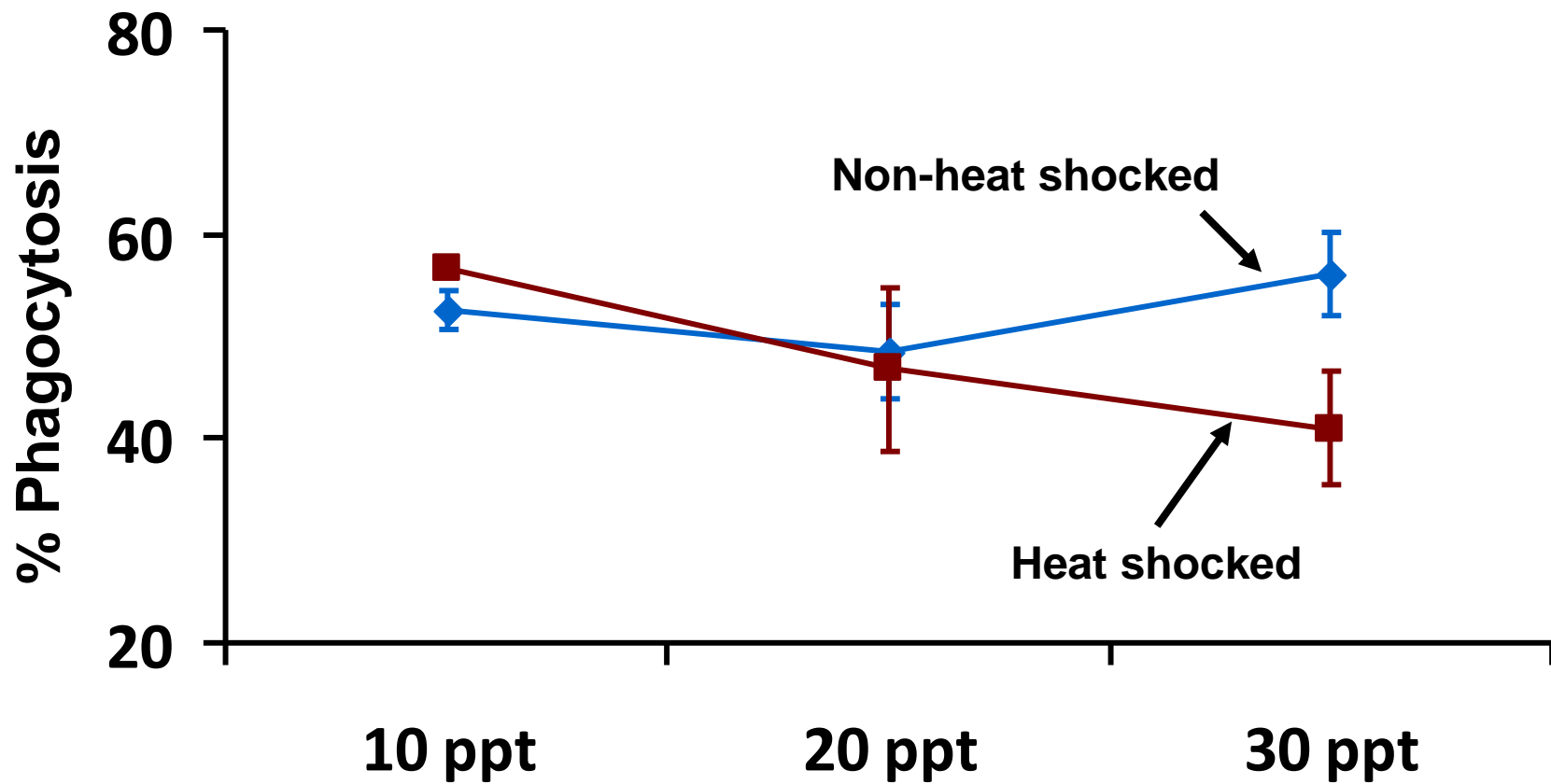


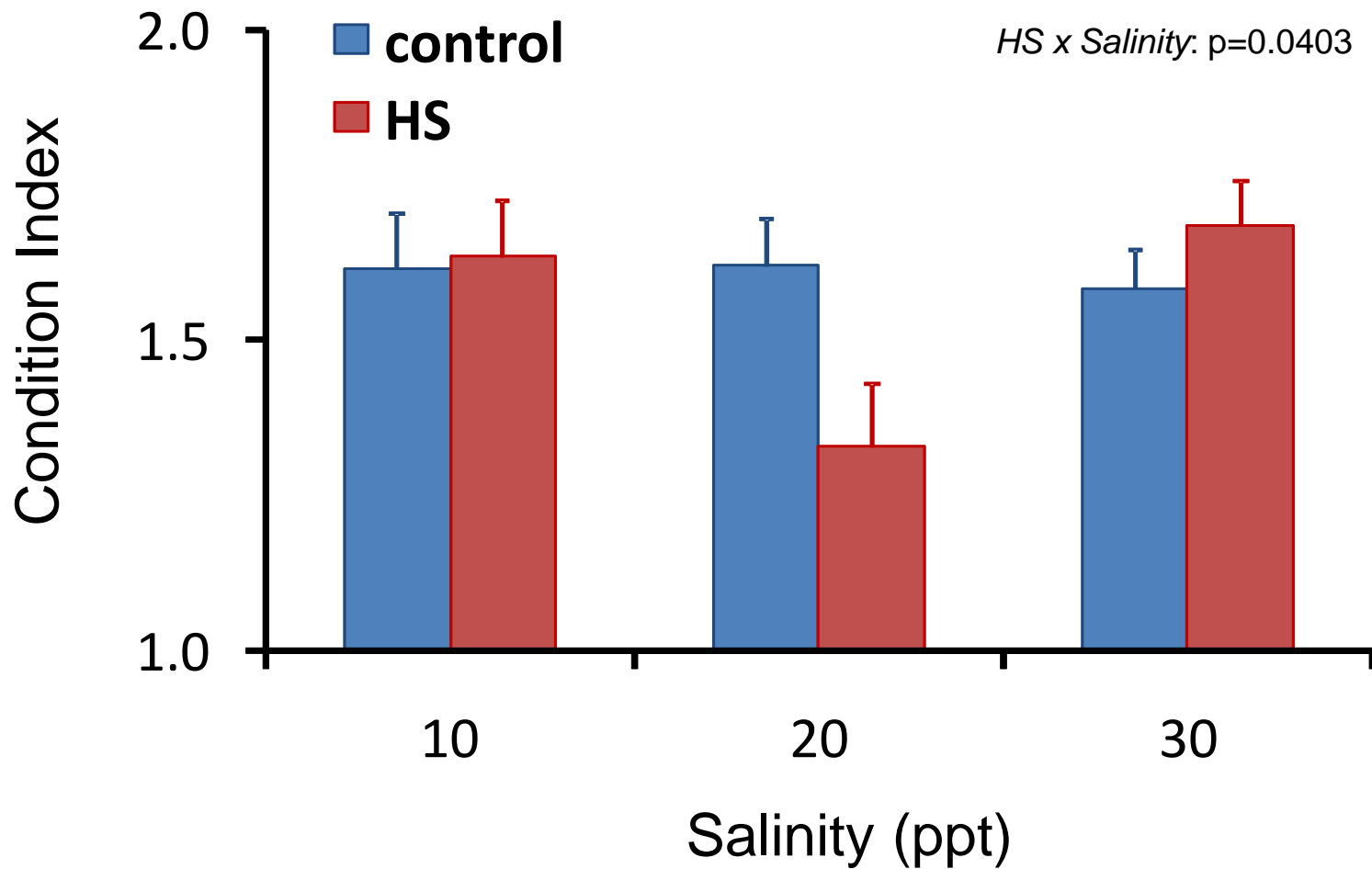
Experiments

I. *Karenia brevis* → Δ Salinity

II. Δ Salinity → *Karenia brevis*

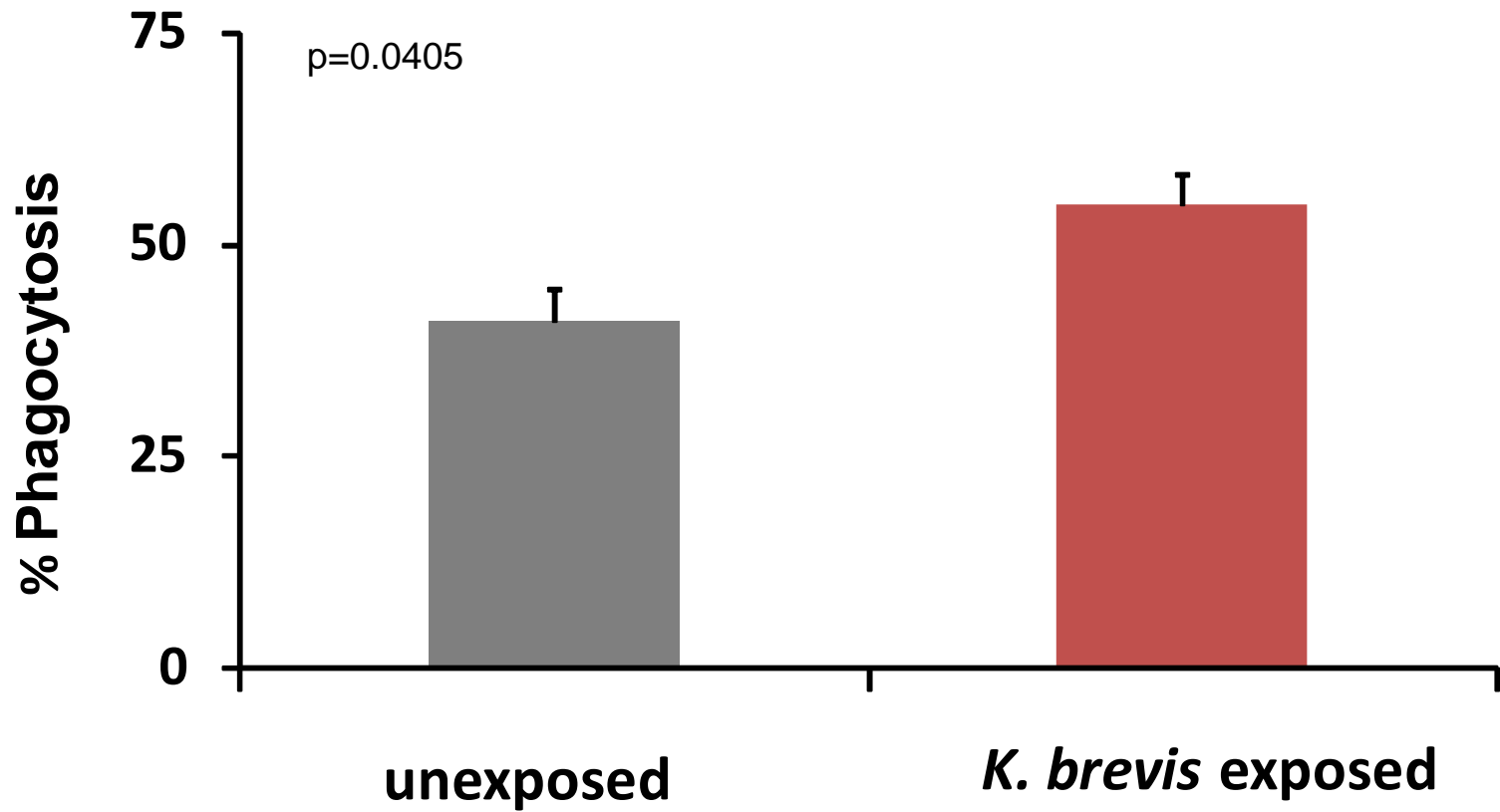
Also examined heat shock response as an indicator of thermal tolerance





Condition index affected by HS and salinity (20 ppt)

I. *Karenia brevis* → Δ Salinity



Phagocytosis increased in *K. brevis* exposed clams

I. *Karenia brevis* → Δ Salinity



FISH KILLS CAUSED BY RED TIDE

Oxygen-starved water cannot support most marine life off our coast. Here's a look at typical Red Tide vs. this year's outbreak:

A TYPICAL YEAR

■ The algae that cause Red Tide, which are attracted to sunlight, travel toward the surface of the gulf. Red Tide usually occurs in the fall when water temperatures are well mixed:



THIS YEAR

■ A thermocline, an area of great temperature change, prevents algae from swimming to the surface.

■ Algae stays beneath the thermocline, where it causes fish kills. Bacteria, which decompose the fish, use up oxygen in the water:

SURFACE LAYER

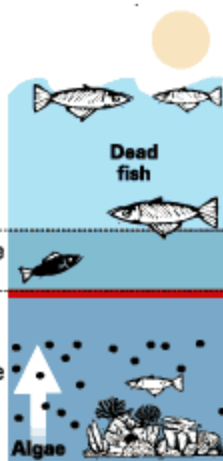
Surface layer where the temperature is warmer.

THERMOCLINE

The middle zone where water temperature changes abruptly.

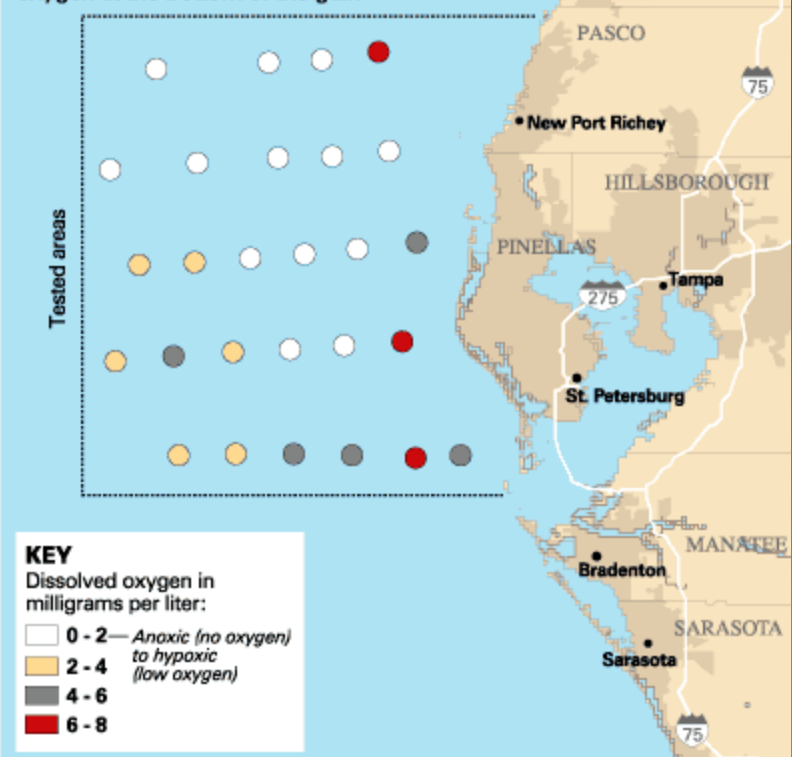
DEEP WATER

Bottom layer where the temperature is cooler.



OXYGEN LEVELS

Preliminary tests performed between Wednesday and Friday show areas of low oxygen at the bottom of the gulf:



Source: Fish and Wildlife Research Institute

Source: Florida Fish and Wildlife Conservation Commission

Times graphic