

# **Assessing Coral Health in the Florida Keys Using a Cellular Diagnostic System**

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**NOAA NOS**

**CCEHBR**

**What work have we been conducting?**

**Coral Disease and Health Consortium  
(CDHC)**

**Bioinformatics**

# *Ecosystem Health*

# *Current methods of Ecosystem Health Assessment*

- Document quality and quantity of contaminants
- Detect gross responses by organisms
  - Dead vs. Alive
  - Present vs. Absent
  - Rare vs. Common
- **CANNOT** directly link the contaminant to its effect on organism and ecosystem health

**Ecosystem**

**Community**

**Species**

**Population**

**Individual**

**Organ**

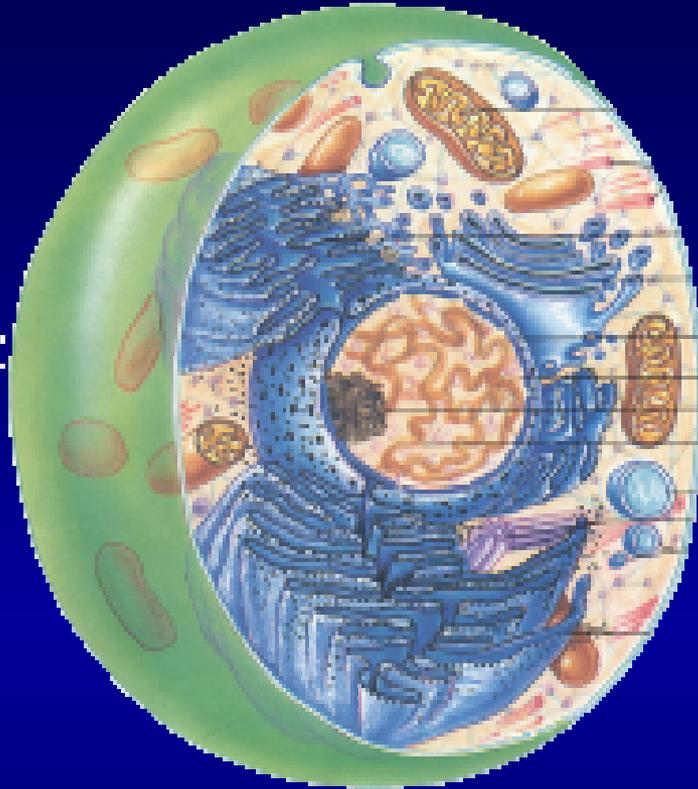
**Tissue**

**Cell**

**Major components of biological systems are not assayed**



# Molecular & Cellular Biomarkers



Cell

## • Genotoxic Stress

- DNA adducts
- micronuclei

Tissue

Individual

Species

Population

Community

Ecosystem

## • Cellular Integrity

- LPO
- Glutathione
- Ubiquitin
- Hsp 60 & 70

## • Toxic Stress

- P450s
- metallothioneins

## • Oxidative Stress

- Superoxide dismutases
- Hsp60, Hsp70, sHsps

# ***Goal:***

- **To determine health condition**
- **To determine susceptibility**
- **To identify stressor**
- **To predict outcomes (*prognosis*)**

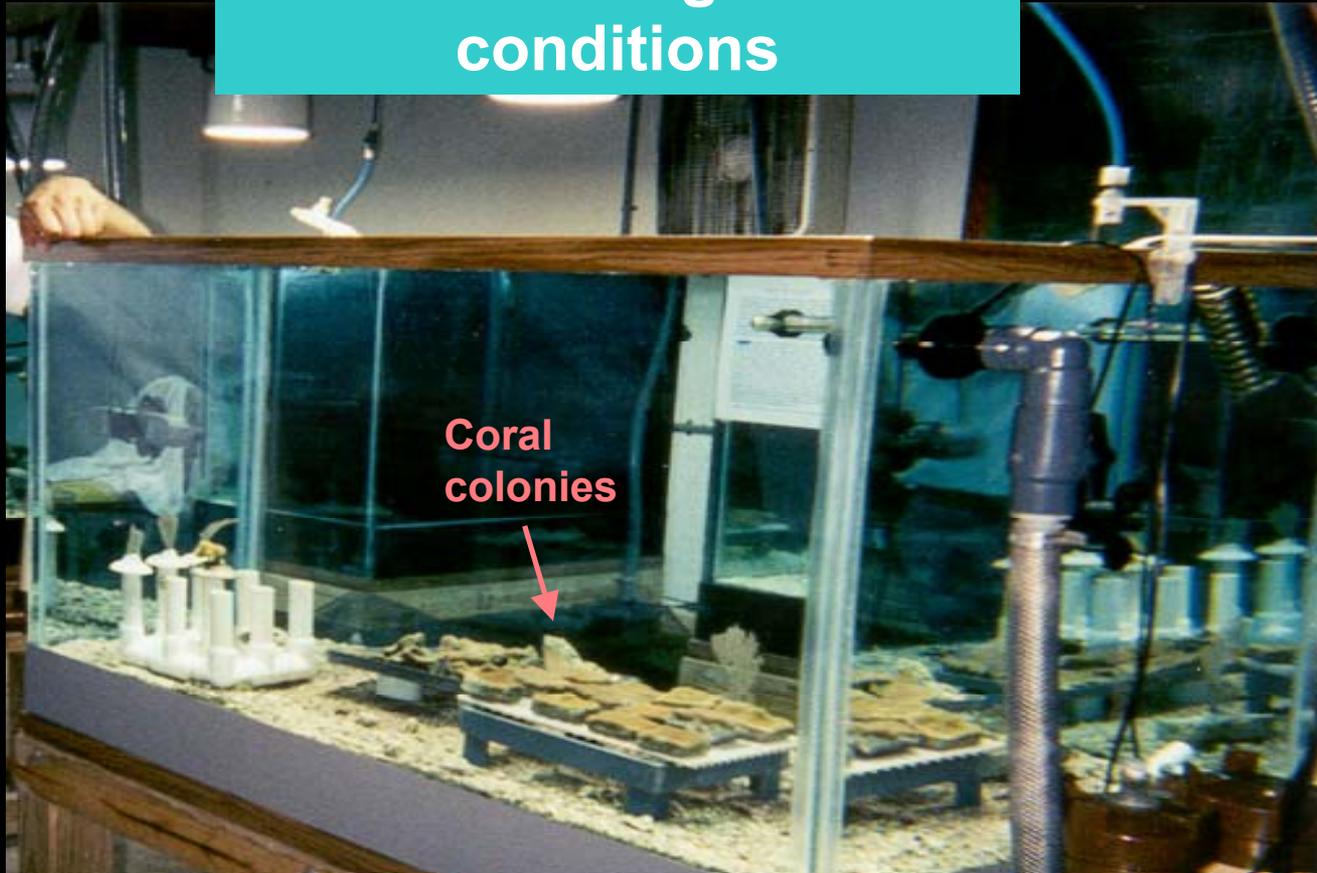
***... on the cellular level***

# *Coral Bleaching*



# Laboratory Experiments

Controlled growth conditions

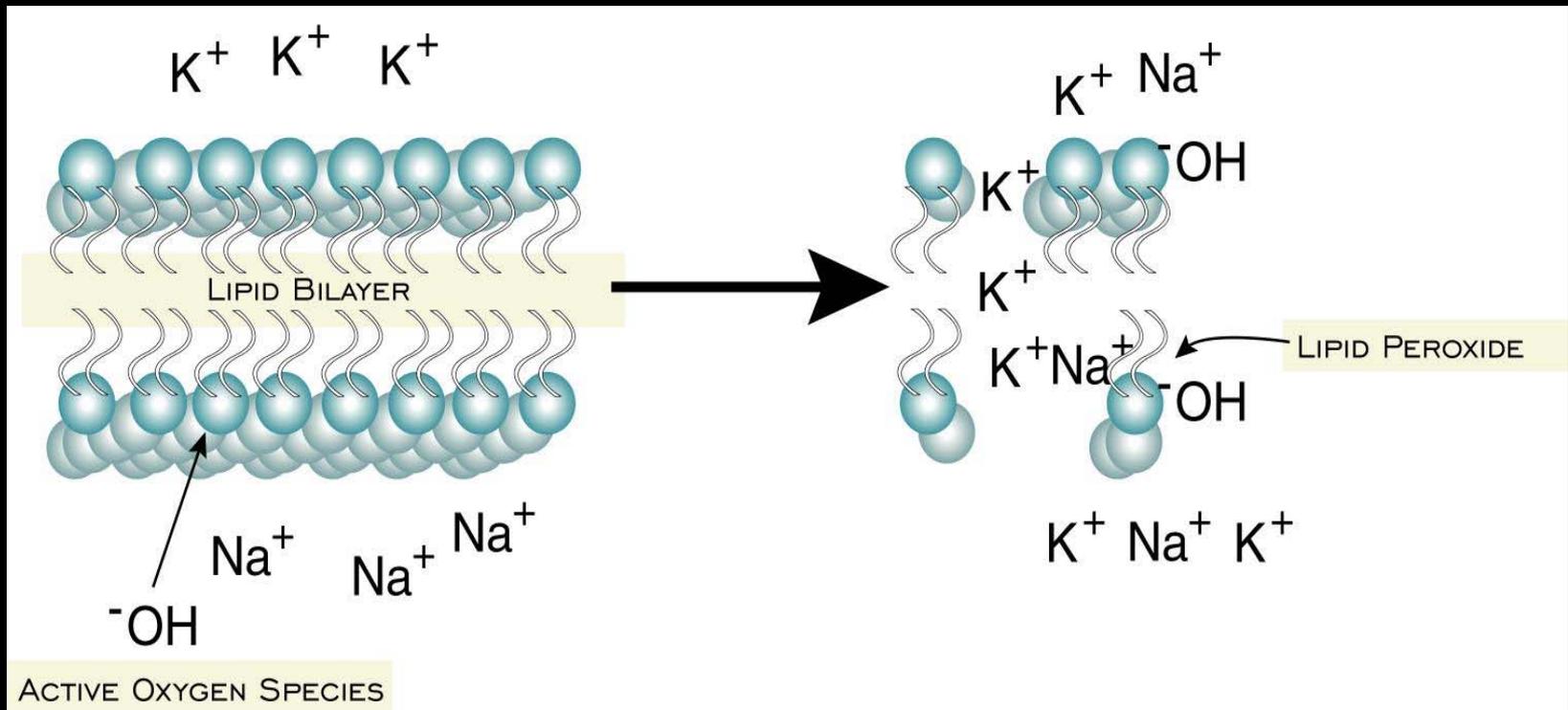


Coral colonies

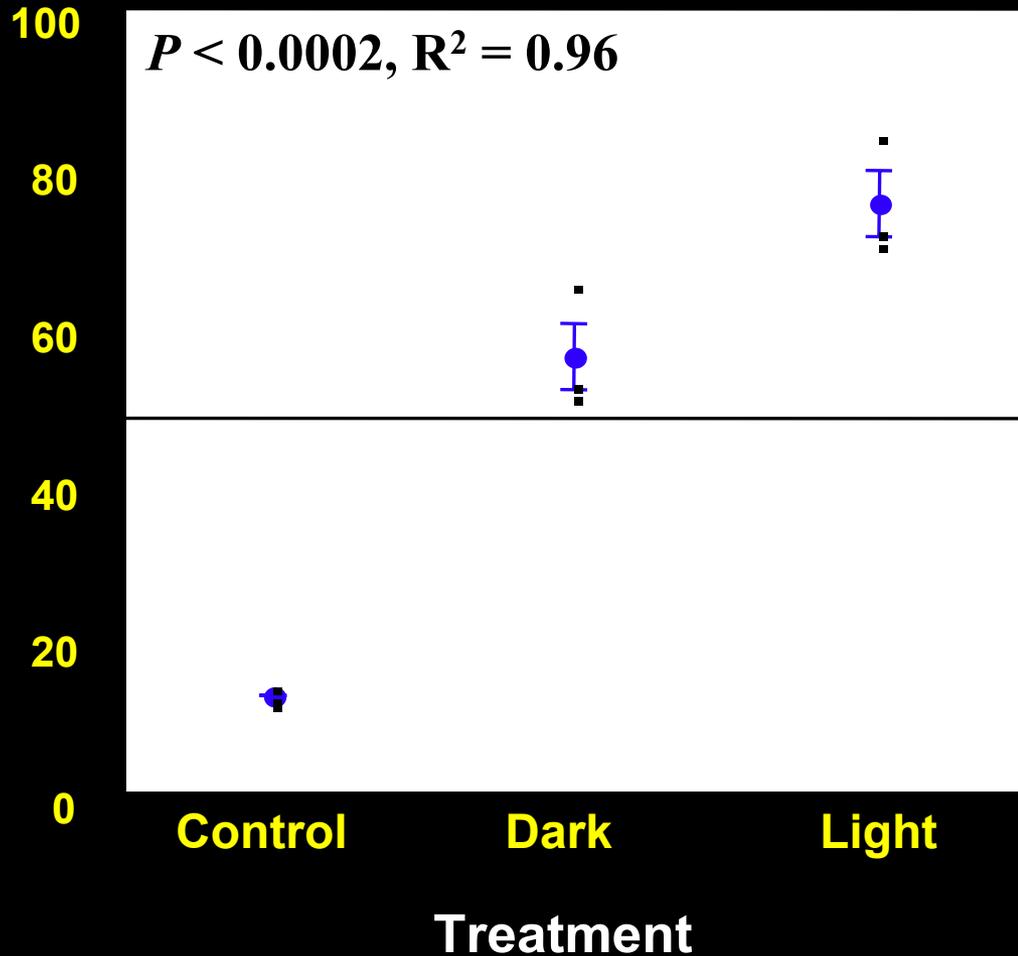
High Temperature Stress (33C) in the Dark

High Temperature Stress (33C) in the Light (PAR)

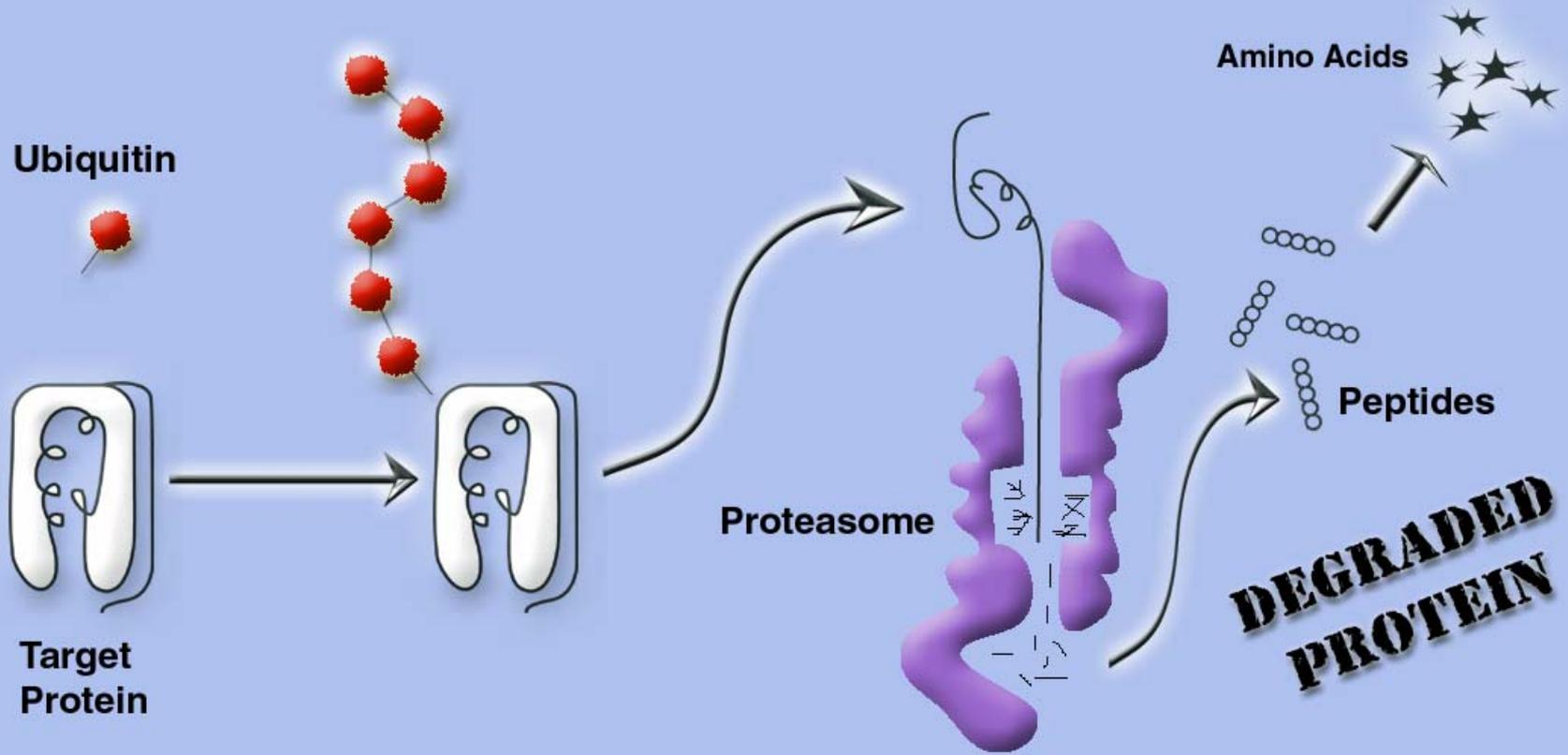
# Lipid Peroxidation



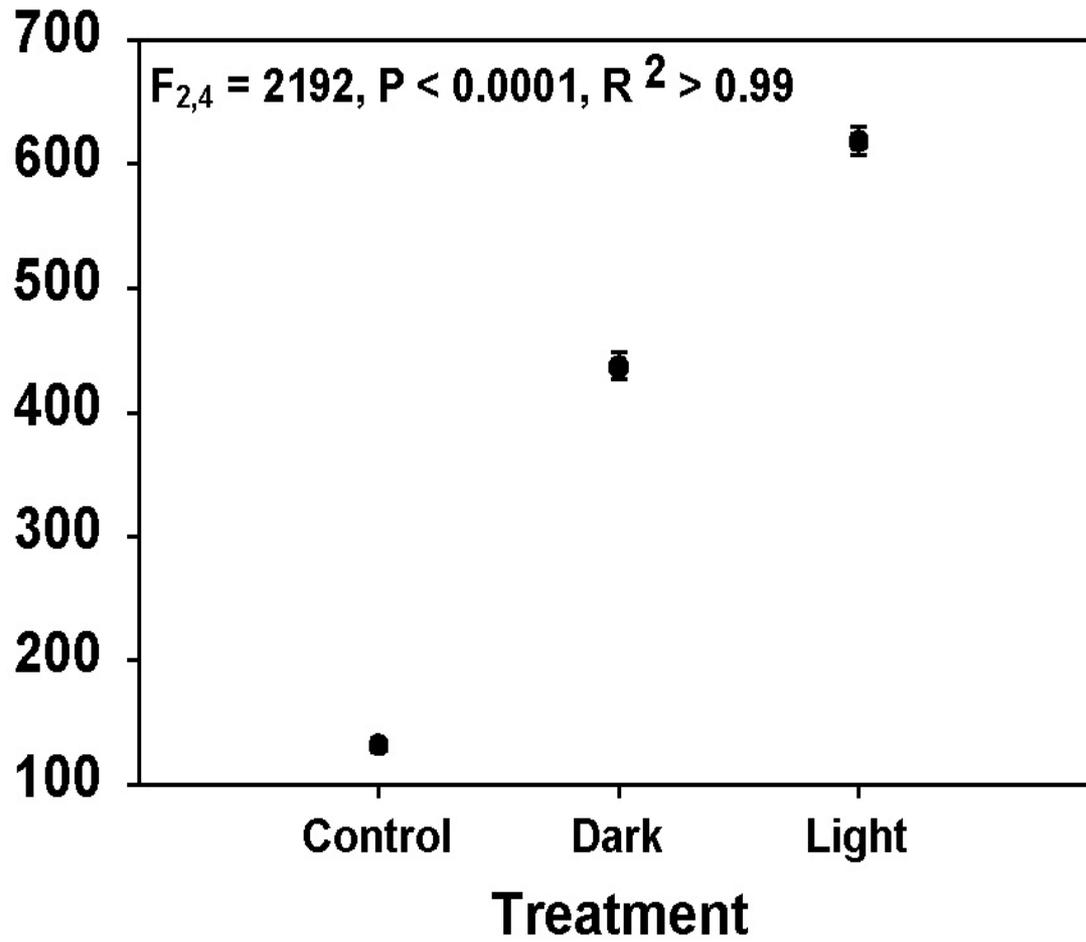
# Lipid Peroxide

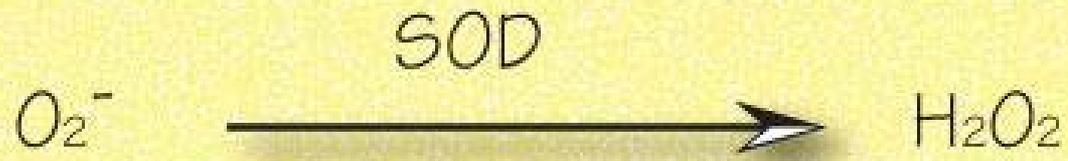


# Ubiquitin

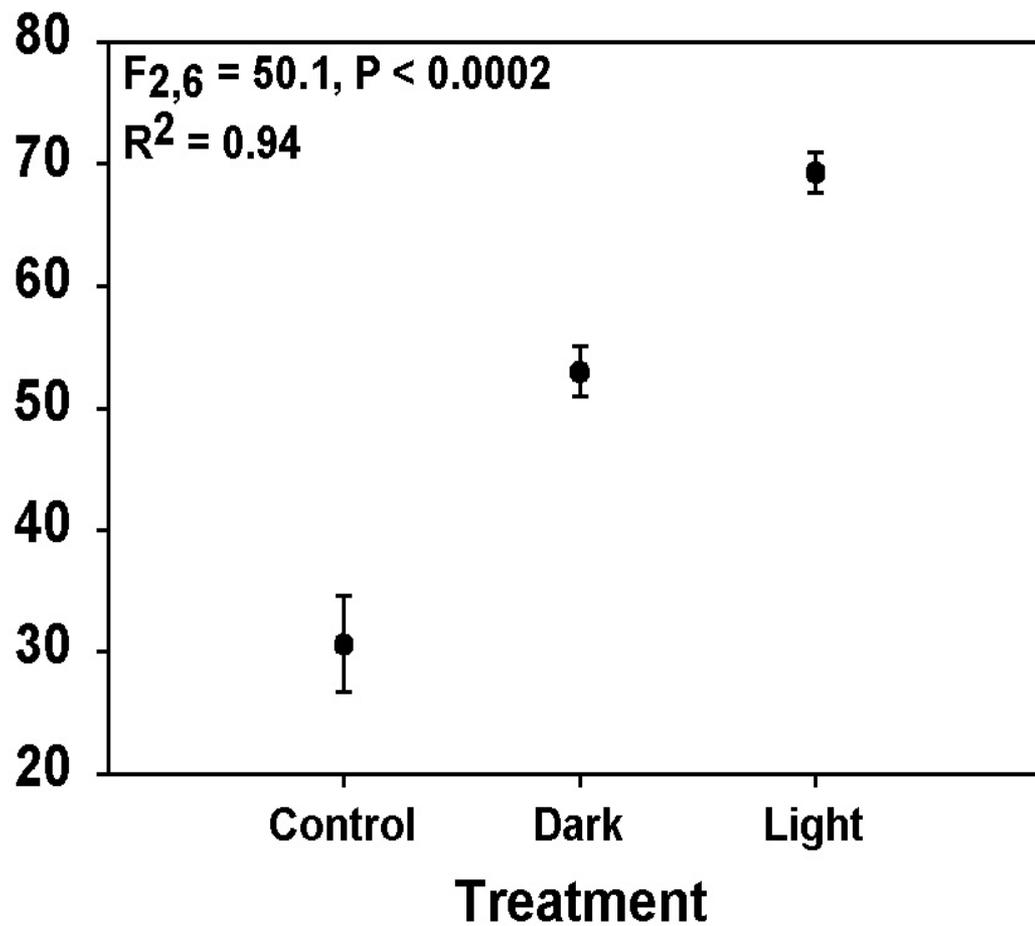


# ubiquitin

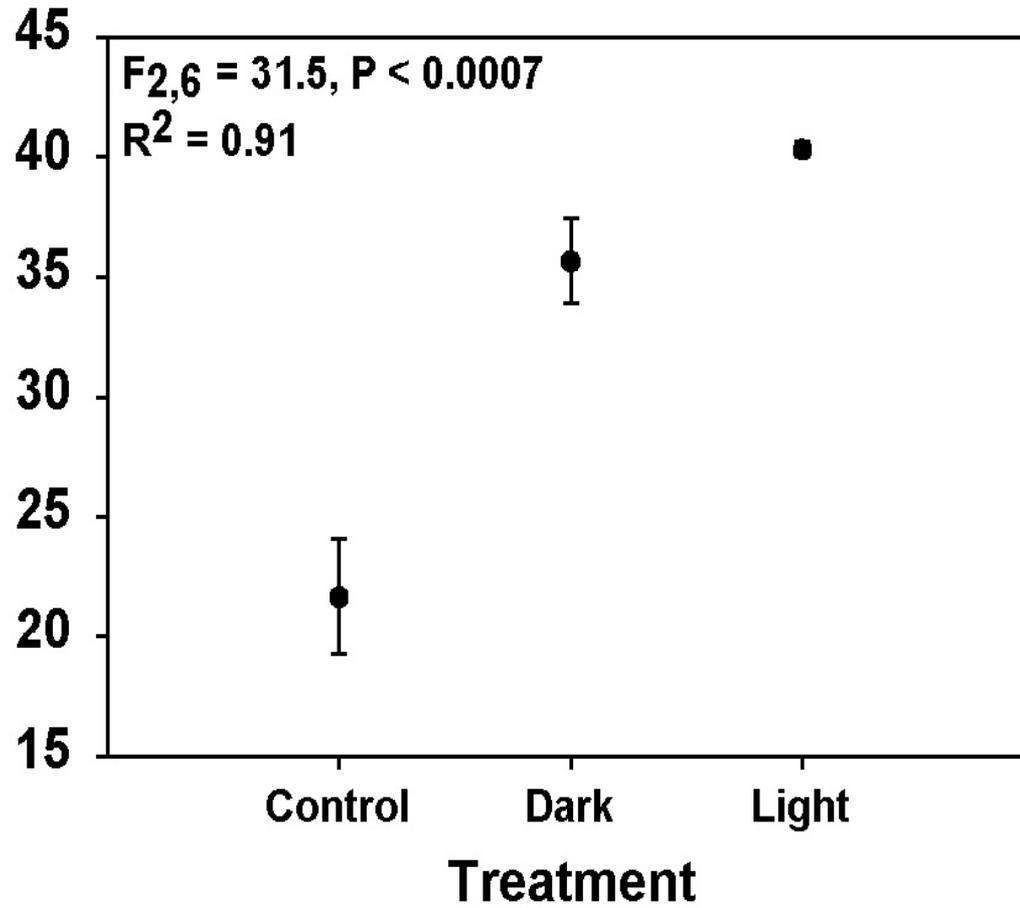




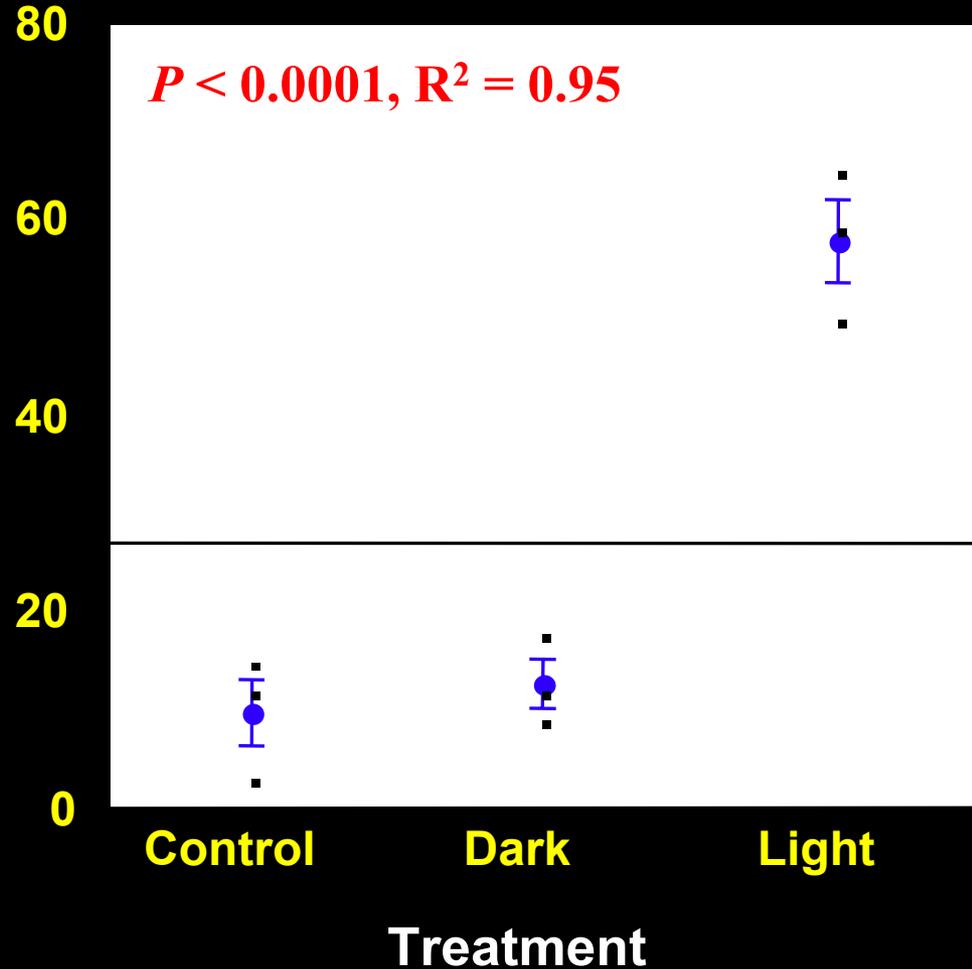
### Cu/Zn SOD



## Mn SOD



# Chloroplast small heat-shock protein



# *Summary - Laboratory*

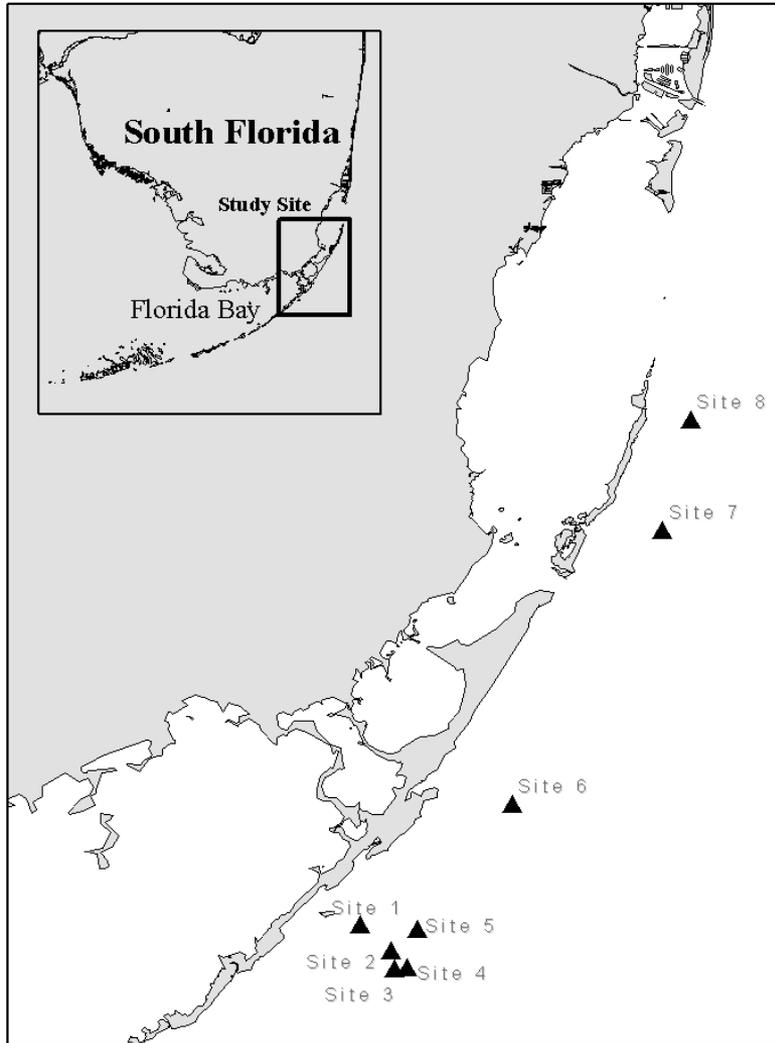
Assaying 10 cellular parameters ....

- **Demonstrated significant differences in physiological status and an ability to distinguish separate and combined effects of heat and light on the coral and its symbiont**
- **Data suggests a light-dependent generation of active oxygen species via photosystem II**
- **We provide strong evidence for an oxidative stress/photosystem II mechanism for coral bleaching**

# Cellular Diagnostics in the Field

- Can the cellular diagnostics determine if corals during a growing season are experiencing oxidative stress?
- Is there a correlation between oxidative stress and coral bleaching in the field?
- Can the cellular diagnostics determine if any of its biomarkers may be responsible for an adaptive response against bleaching?

# Sampling Locations



Map by Mike Callahan

	Site Name	Depth
1	Rodriguez Key	3 m
2	SW Three Sisters	6 m
3	Between Molasses and Pickels	10 m
4	SW Molasses	18 m
5	White Bank	6 m
6	Algae reef	6 m
7	Alina's reef	6 m
8	East Bache Shoal	6m

20

Ft

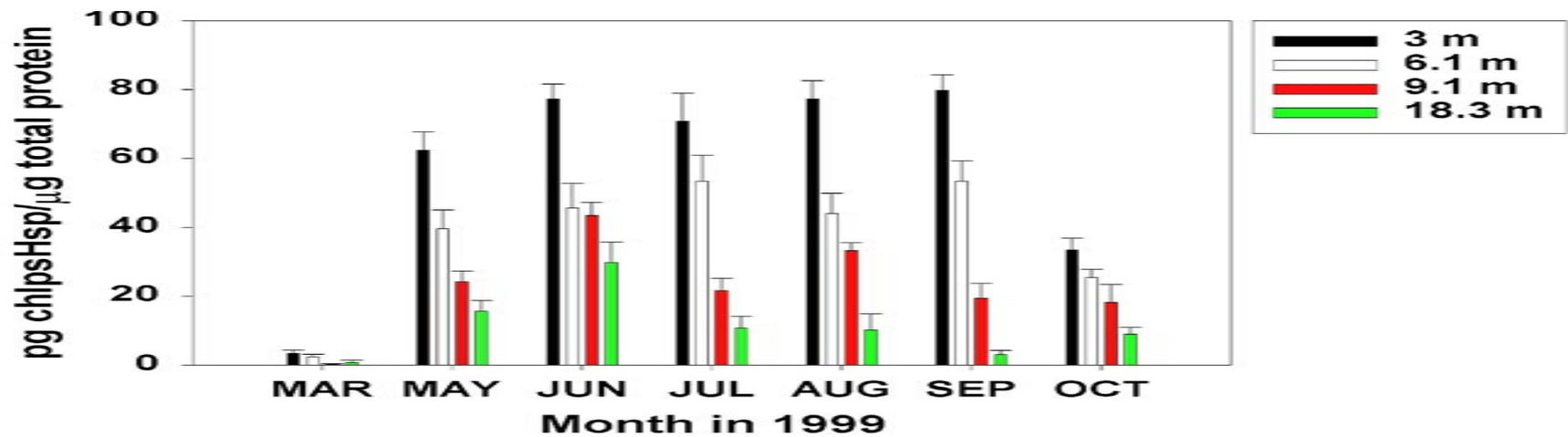
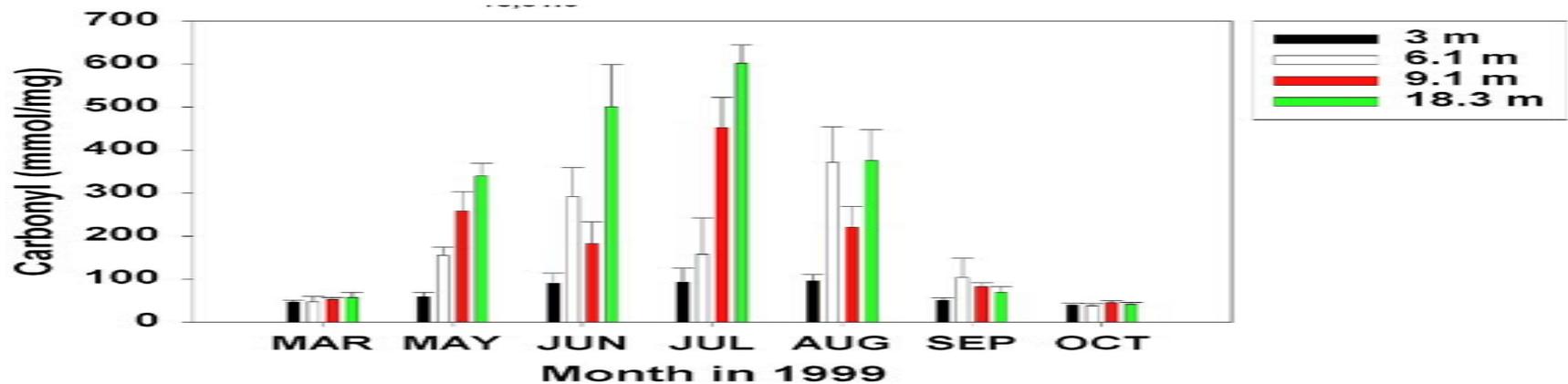
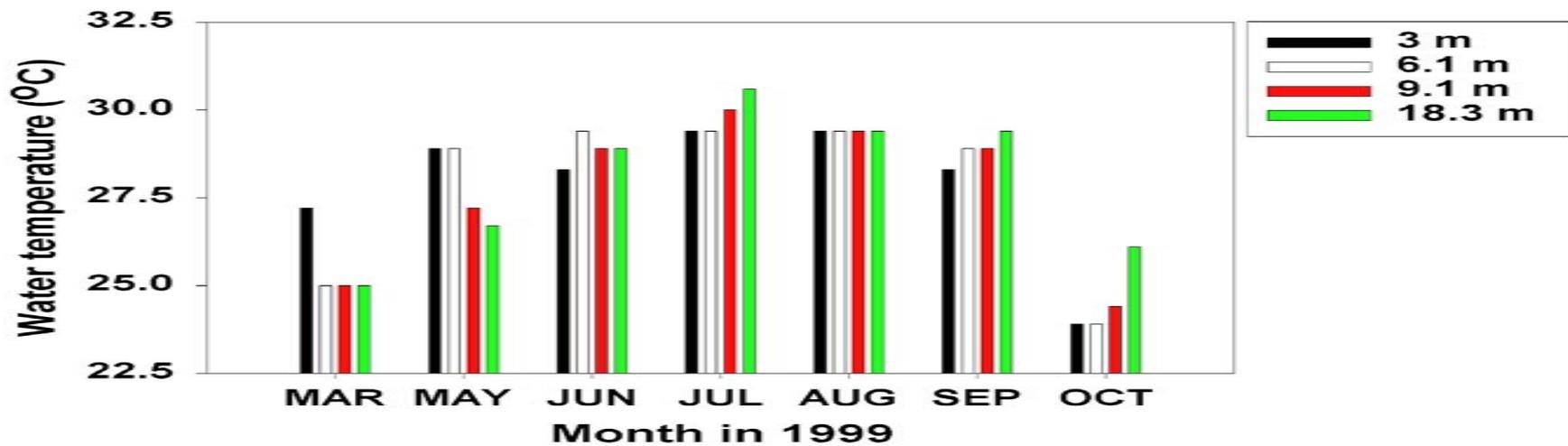


30 Ft



60 Ft

10 Ft



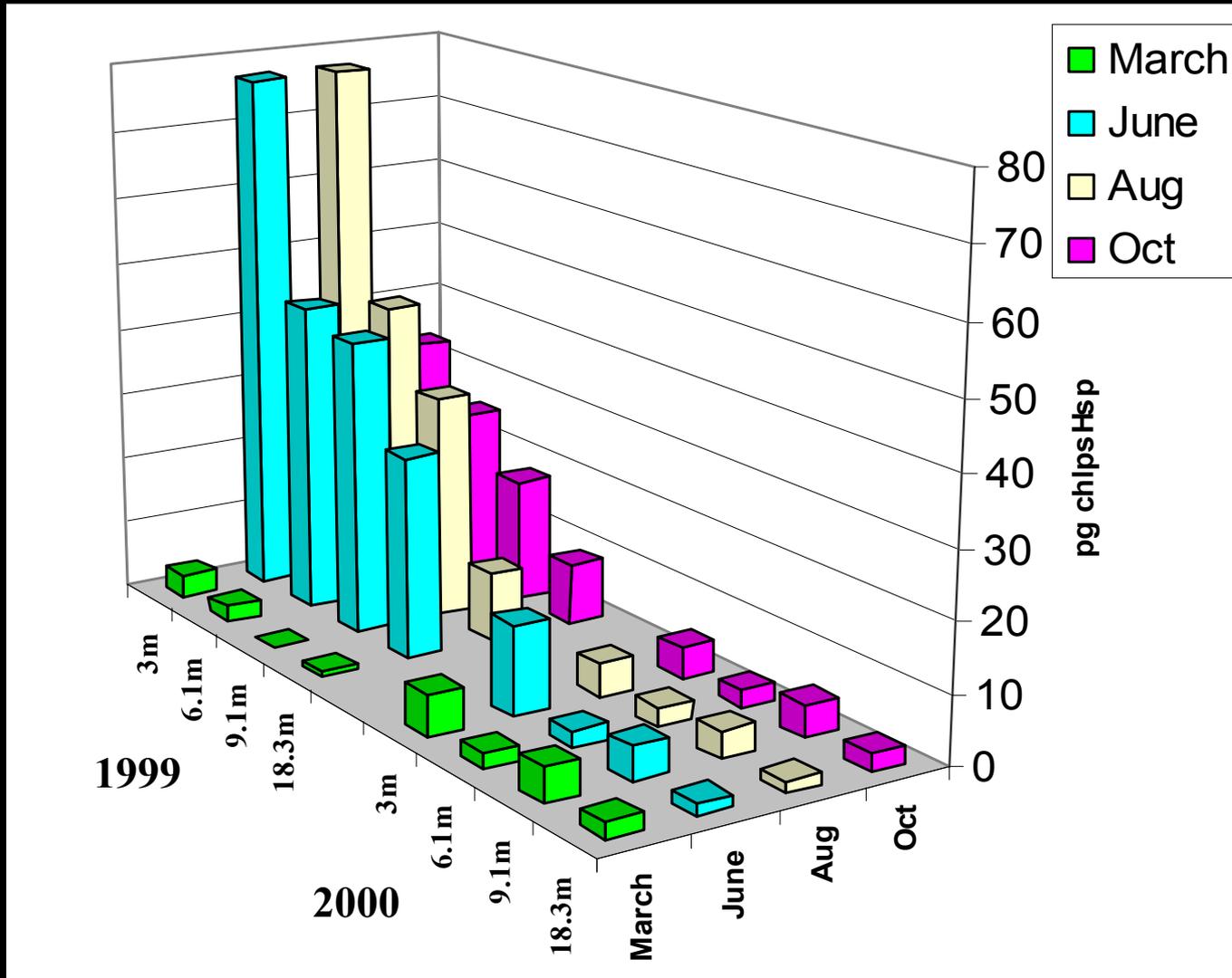
# SUMMARY

- **Bleaching was associated with significant accumulation of oxidative damage products**
- **The anti-oxidant/stress response capacity of the coral (anthozoan) plays a significant role in PROTECTING against coral bleaching....**
- **The chloroplast sHsp is a major adaptation of the algal symbiont in PREVENTING the production of reactive oxygen species**

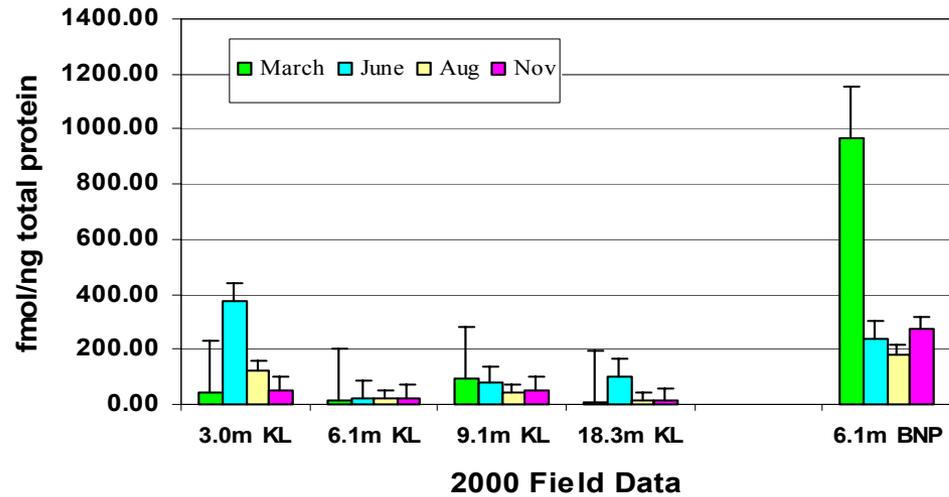
# SUMMARY

- Several parameters could *PREDICT coral bleaching 3-6 months in advance* of any visual signs in the field (eg carbonyl, chlpsHsp)
- Both laboratory and field data support the hypothesis that Photosystem II may be a principal component in generating the oxidative stress associated with coral bleaching

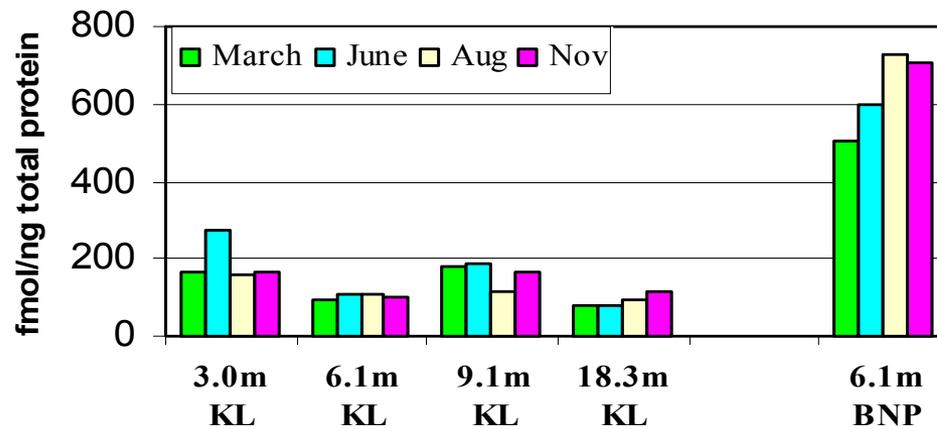
# Chloroplast small Heat-shock Protein



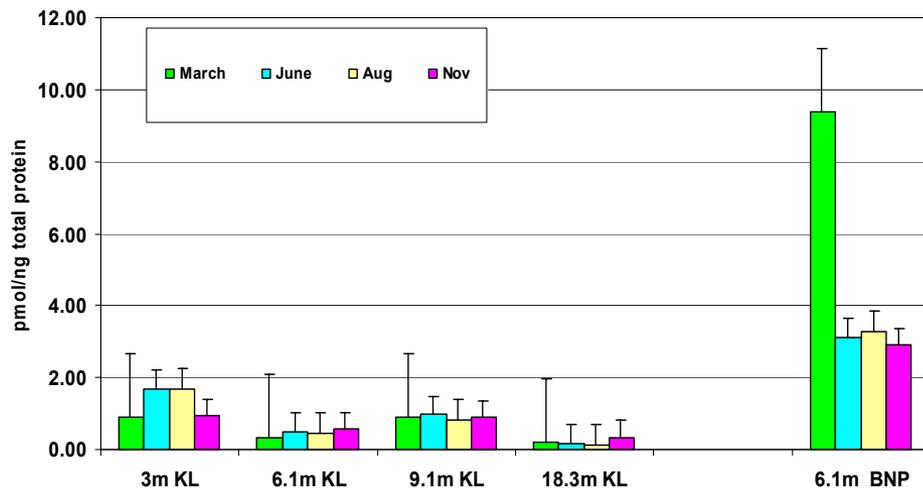
## Ubiquitin



## Hsp 70 Cnidarian

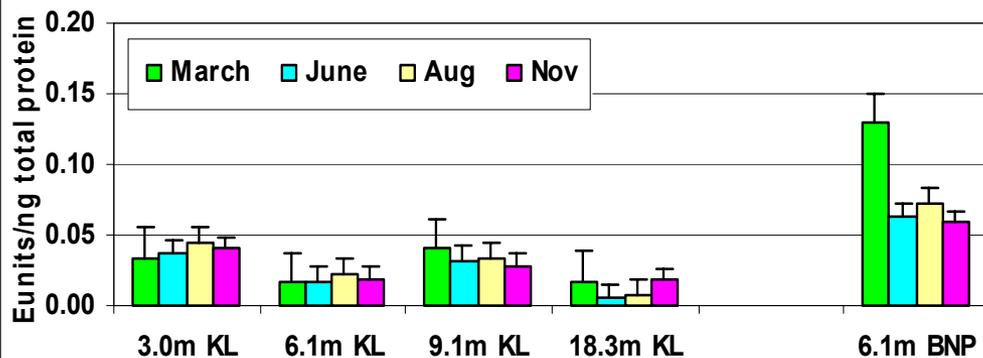


### GST Invertebrate



2000 Field Data

### Multi-Drug Resistance (MDR)



2000 Field Data

# Summary

- **Cellular markers can diagnose whether an organism is stressed and the type of stress.**
- **Differentiate between global impacts and local stressors.**
- **Cellular markers can assist in tracking stressor sources.**

# Summary

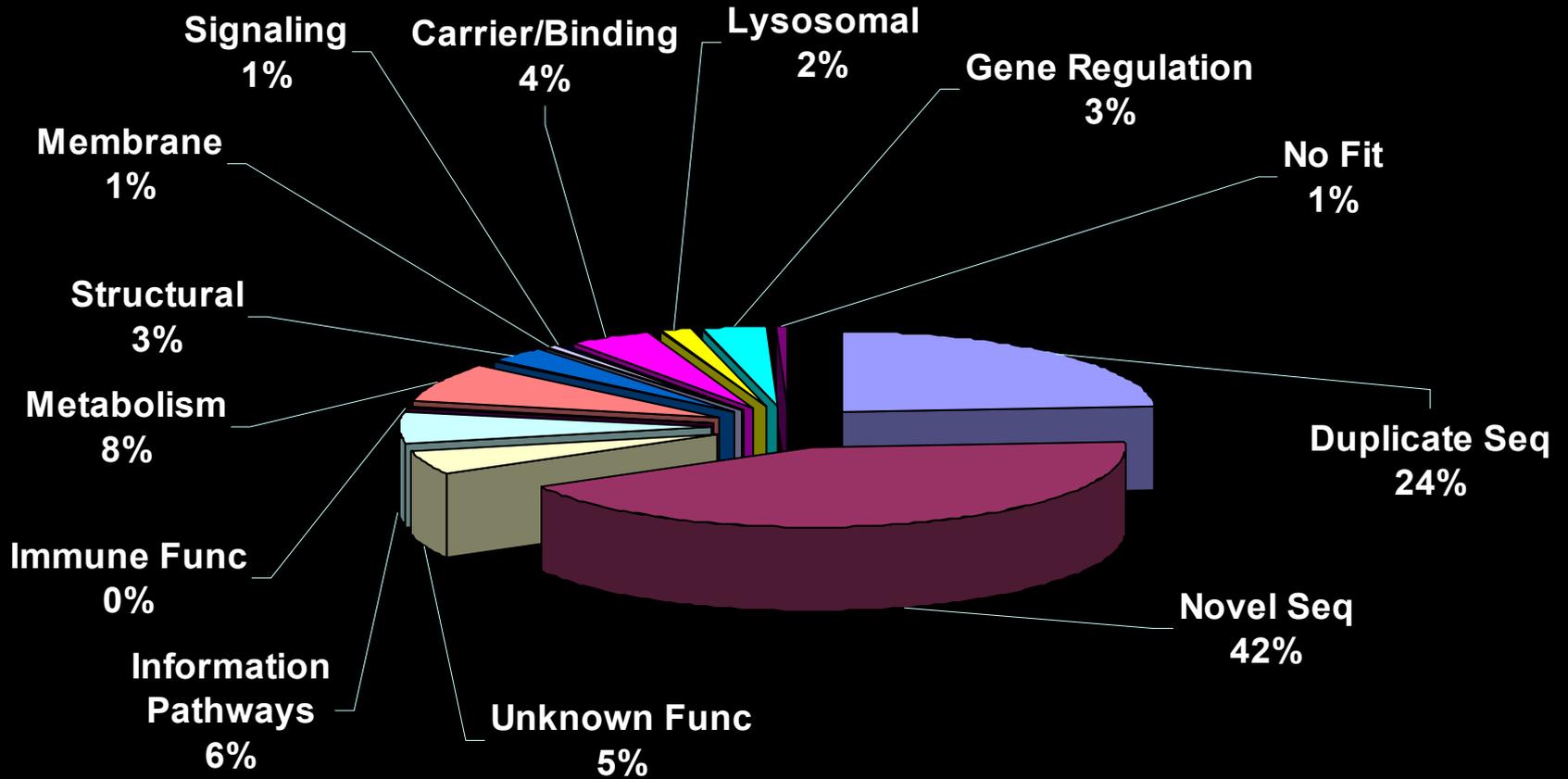
- **Because they monitor fundamental processes at the lowest levels of biological organization, Cellular markers can predict higher-order responses – ecological forecasting.**
- **Method can be linked to remote sensing (i.e., temporal texture analysis) for rapid response capabilities.**

**What are our future  
directions?**

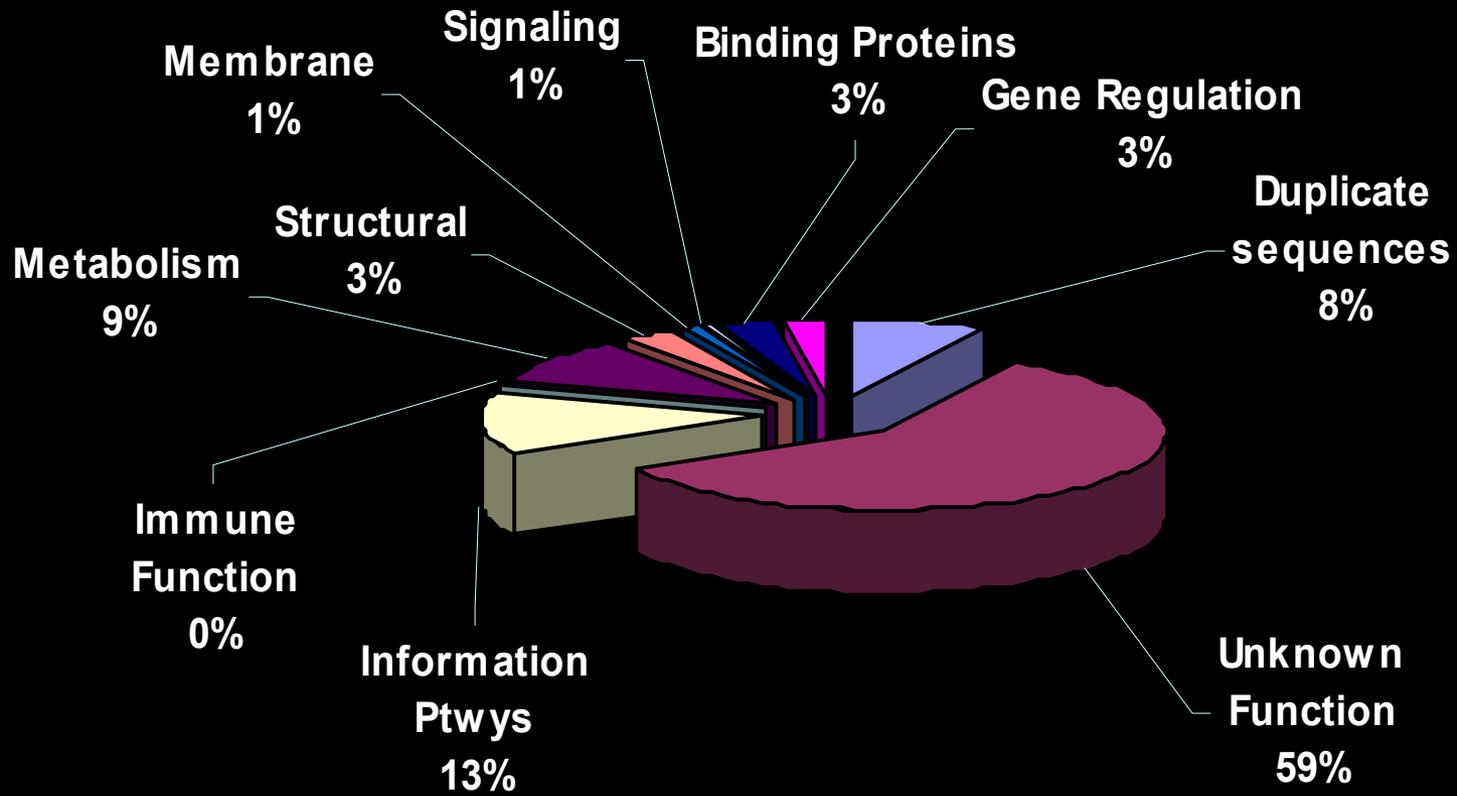
# *Functional Genomics*

# EST Libraries

# Montastraea EST Library



# Oculina EST Library



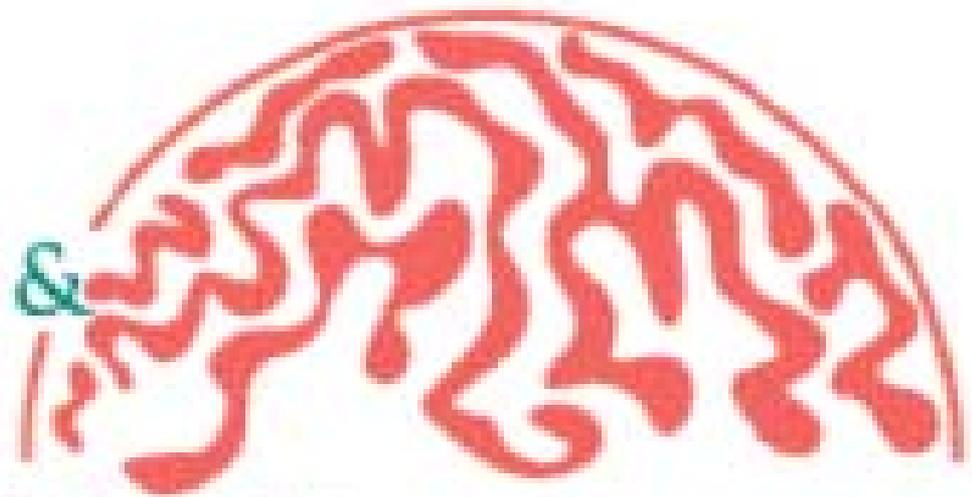
# Proteomics

.....*Characterization of gene expression patterns at a protein level*



- **Do Environmental Stressors increase coral's susceptibility to disease?**
- **Can we identify new diagnostic markers of cellular physiological status?**

CORAL  
DISEASE &  
HEALTH  
CONSORTIUM



SOLUTIONS TODAY  
FOR REEFS TOMORROW.

# CDHC Goals

- **Provide early warning of disease & disease outbreaks**
- **Identify causative factors and possible mitigation or prevention measures**
- **Offer viable risk management options**
- **Encourage a new generation of coral researchers through education and outreach**

# CDHC at Work...

- **Nomenclature**
- **Model System(s)**
- **Field Assessment of Coral Reef Condition**
- **Microbiology**
- **Toxicology**
- **Histopathology**
- **Molecular**
- **Bioinformatics**
- **Education and Outreach**

# **An Integrated Database for Coral Health and Disease**

## **Focus of Coral Disease & Health Consortium (CDHC)**

- medical diagnostics approach
- partnership of experts
- standardization of nomenclature, field assessment, collection and transport, diagnostics, etc...
- synthesize information across disciplines

# *Bioinformatics*

1. Data storage infrastructure
2. Exploratory analysis -  
artificial neural networking  
dimensionality (identify non-linear correlations)
3. Inference analysis

# Bioinformatics Approach

**Interactions of molecular and cellular level processes within the organism in response to environmental change (Chapman 2001).**

**Requires data from the molecular to the ecosystem level, as well as theoretical models of how these variables interact.**

**Building such a system (and research community) involves use of integrated databases (web services) and application of algorithms to address problems of coral health and disease diagnosis.**

## **Integrated database design draws from:**

- current databases (SST, CREWS)**
- existing sources (guidelines, SOPs, manuals)**
- standardized methods (AGGRA, CARICOMP, etc.)**
- definition of process (Ex. histopathology/diagnosis)**
- relationships among variables (biomarker response)**

**Bioinformatics tries to makes sense of this by finding mathematical solutions to identify patterns and non-linear relationships inherent in the data.**

# Diagnostic Protocol

History / Environment

Gross Observation

Appropriate Sampling

**Histopathology**  
Routine  
Special

**Microbiology**  
Bacteriology  
Virology  
Mycology

**Molecular**  
Biomarkers  
PCR

**Toxicology**  
Contaminants  
Biotoxins

**Integration of data across disciplines to assess health of coral reefs and diagnose disease. Each discipline can be considered a component of an integrated database.**

- Please visit our website for continuing updates and new products from the ....

# Coral Disease and Health Consortium

[http://www.coral.noaa.gov/coral\\_disease/cdhc.html](http://www.coral.noaa.gov/coral_disease/cdhc.html)

# Acknowledgments

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# **Molecular Biomarker System**

- **Indicates whether structural integrity of the cell is challenged**
- **Indicates whether there is a response to oxidative stress**
- **Indicates whether metabolic processes received insult**
- **Provides evidence of stressor type**

**research  
laboratories**

**diagnostic  
resources**

**specialized  
resources**

**CDHC Office**  
1. Communication  
2. Coordination  
3. Data integration  
4. Recommendations

**environmental  
data resources**

**epizootiologic  
programs**

**education  
resources**

