Assessing the health of *Thalassia testudinum at the* landscape scale using chlorophyll fluorescence

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Center For Marine Science UNCW

America's Everglades

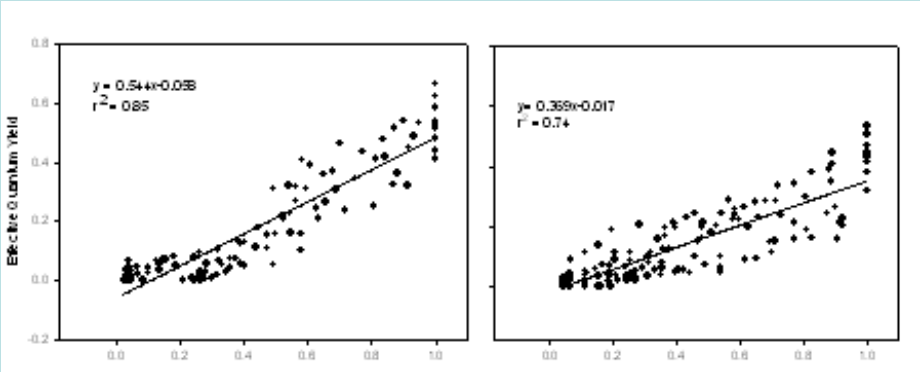
Funding Provided by sfwmd.gov

Why measure photosynthesis?

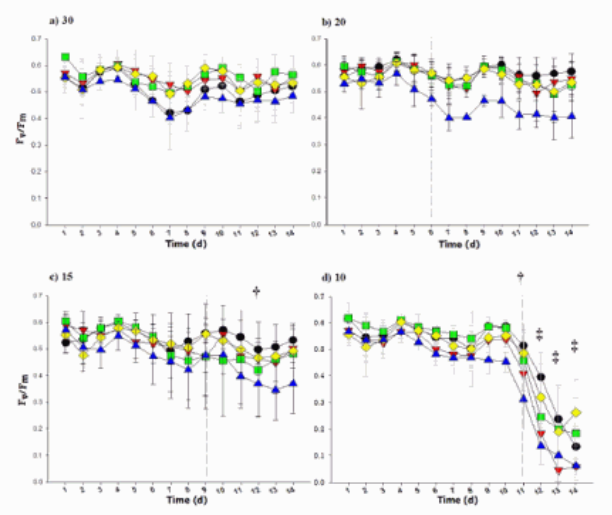
- Photosynthetic systems in plants are very **sensitive** to stress
- Photosynthetic changes will **precede** morphometric or density changes
- PAM fluorescence measures photosynthetic efficiency, capacity and acclimation in situ, **nondestructively**

PAM Fluorometry

- Physiological indicator of stress
  - UV radiation (Dawson & Dennison 1996)
  - Oxidative stress (Sampath-Wiley et al. 2008)
  - Pathogens & Herbicides (Durako & Kunzelman 2002, Ralph & Short 2002, Ralph & Macinnis 2003)
  - High/low irradiances (Ralph & Burchett 1995, Campbell et al. 2003, Beer et al. 2006)
  - Dessication (Björk et al. 1999, Shafer et al. 2007, Kahn & Durako 2008)
  - Salinity (Chartrand et al. 2009)

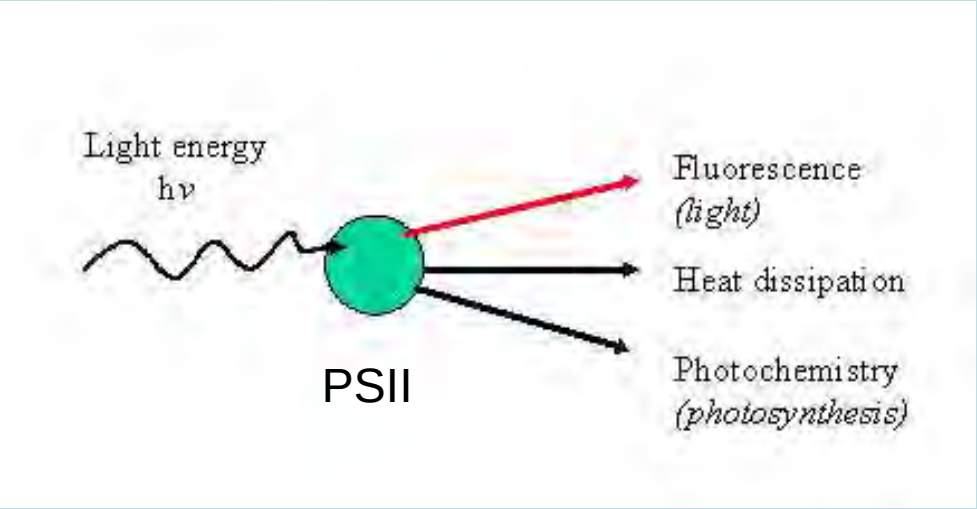


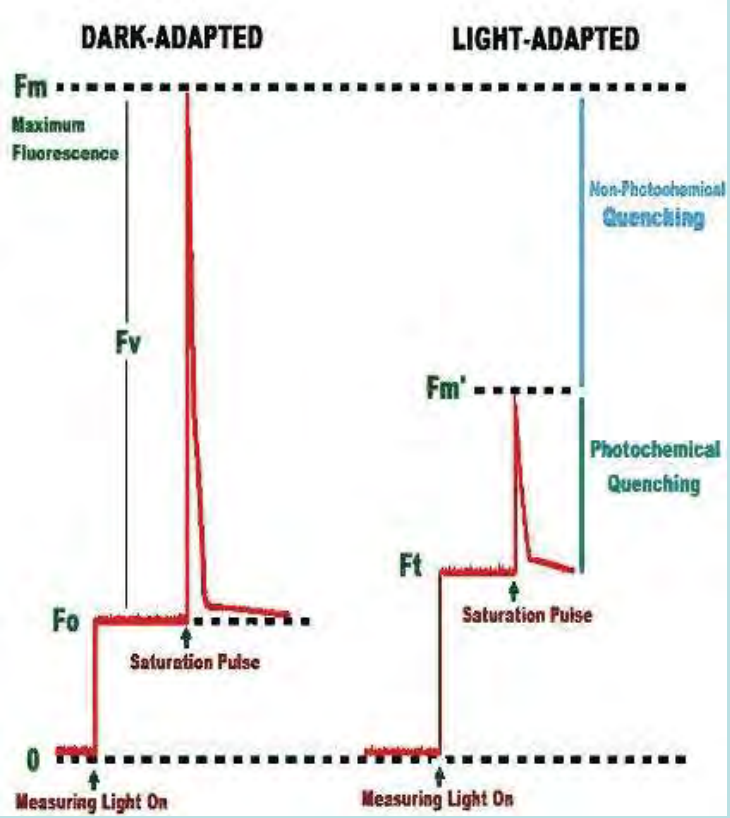
Dessication



Salinity

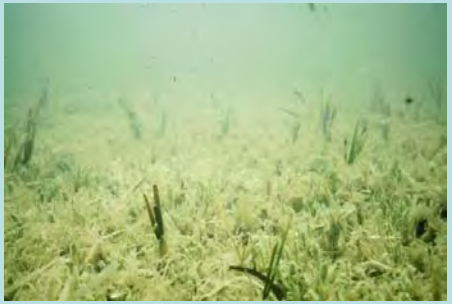
What PAMs measure

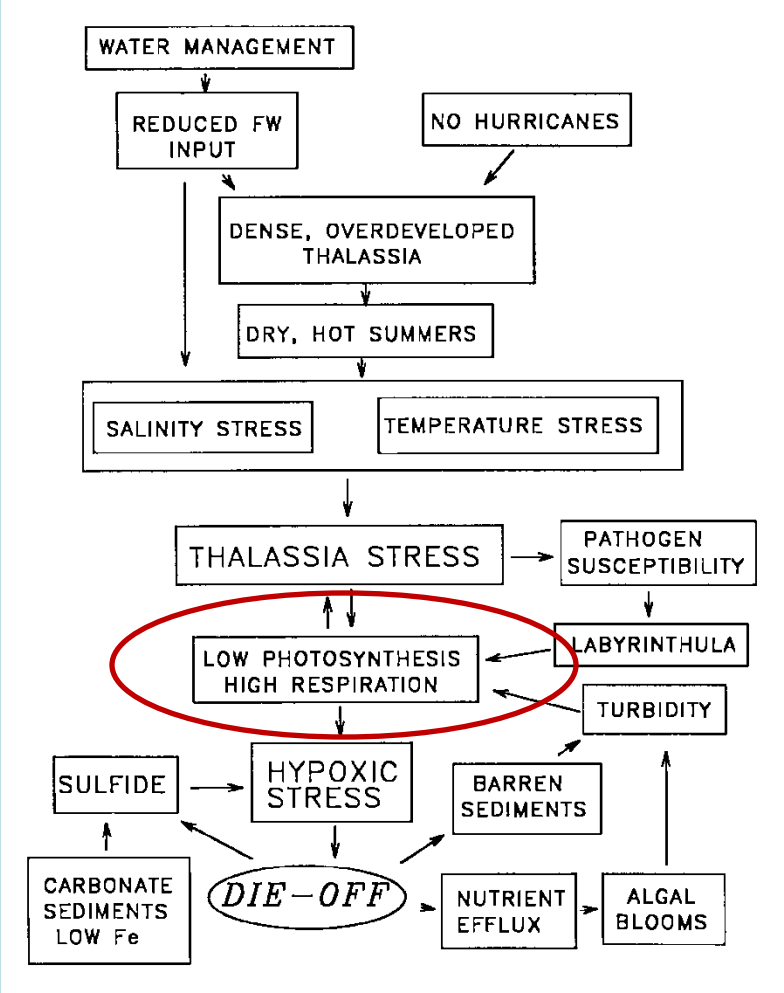




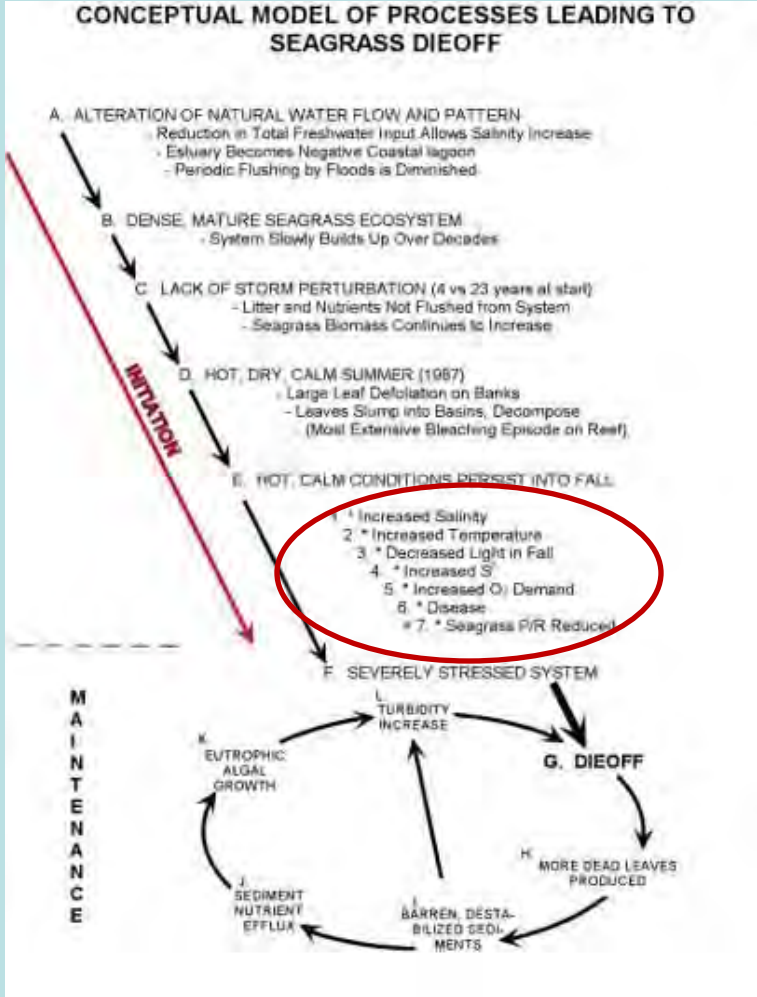
- Maximum Potential Yield:
- **(Fm-Fo/Fm) = Fv/Fm**
  - Requires 5-30 min dark acclimation
  - Potential quantum efficiency
- Effective Yield:
- **(Fm'-F)/Fm'= ΔF/Fm'**
  - Quantum efficiency under ambient light conditions
- Electron transport rate (ETR):
  - **ΔF/Fm' X PAR X AF(PAR) X 0.5**

Seagrass Die-Off 1988-1991



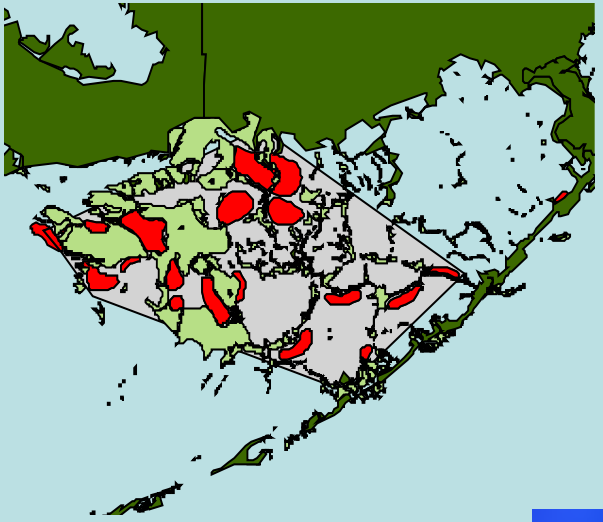


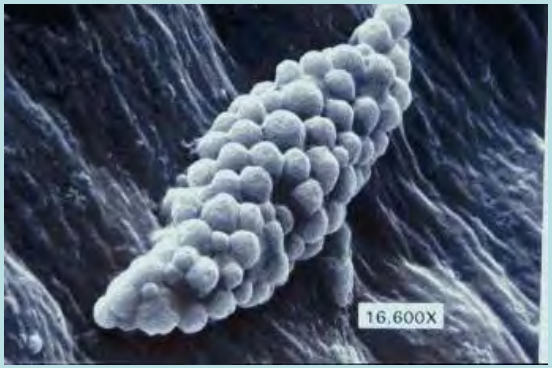
Carlson and Durako 1991

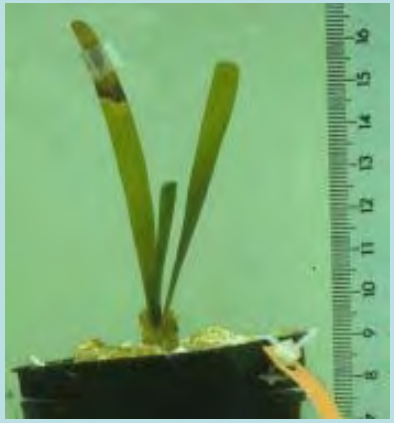


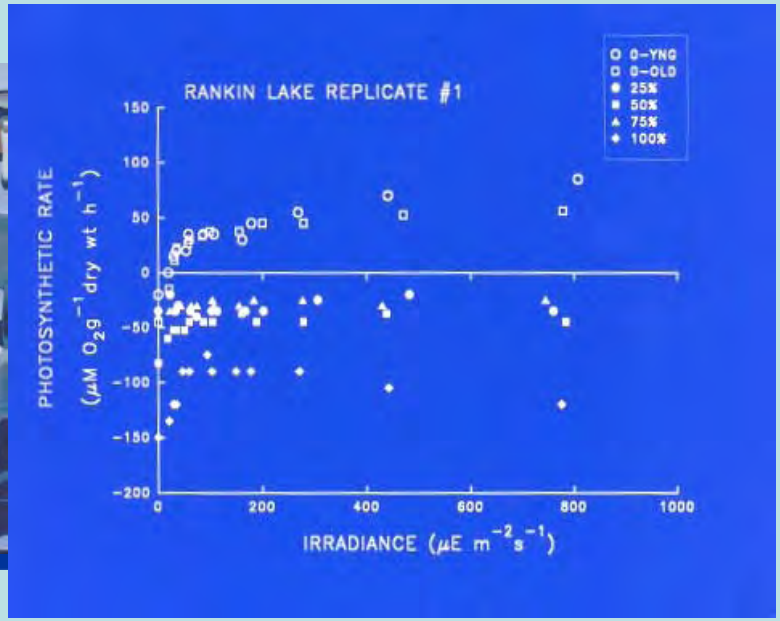
Zieman et al. 1991

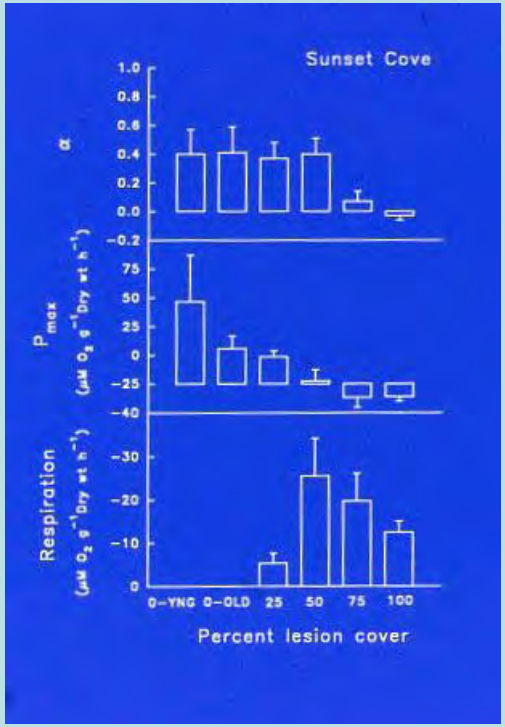
Photosynthesis (O_2) & Die-off





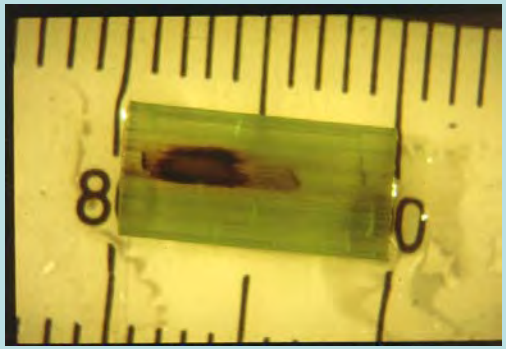


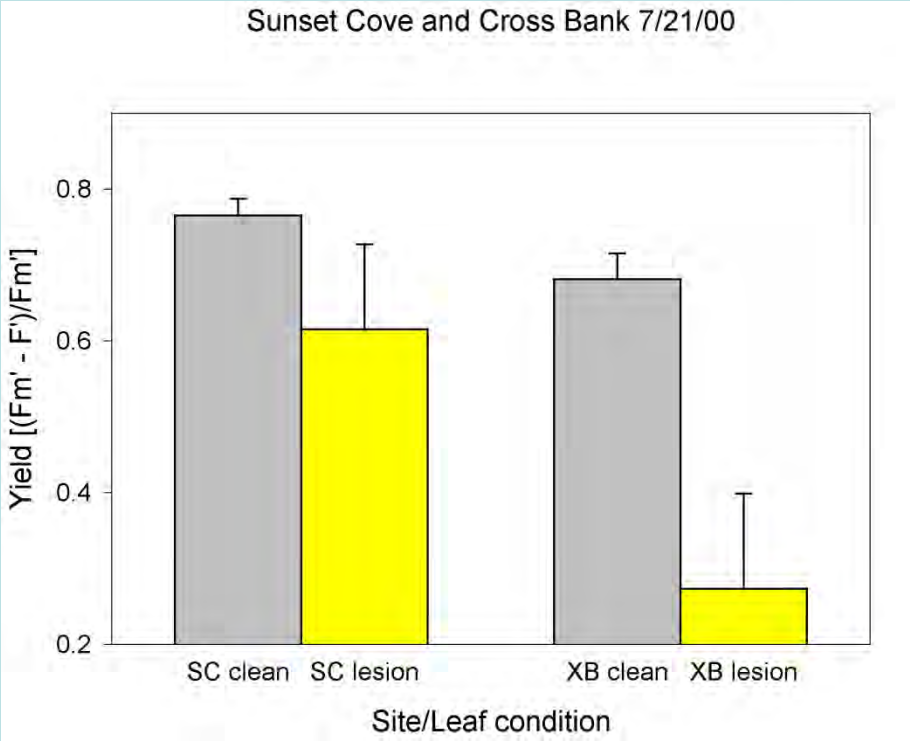


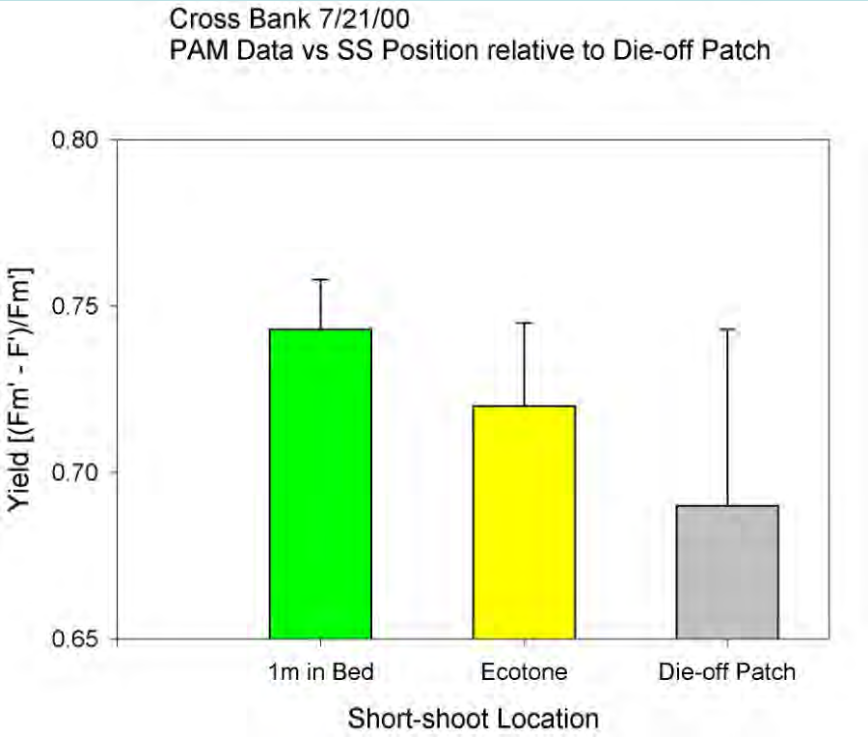


Durako & Kuss 1994

Fluorescence & Die-off (2000)

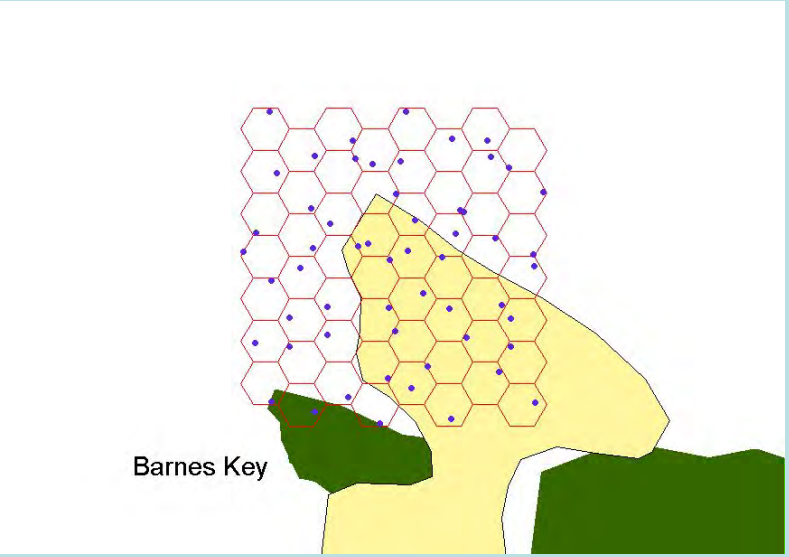


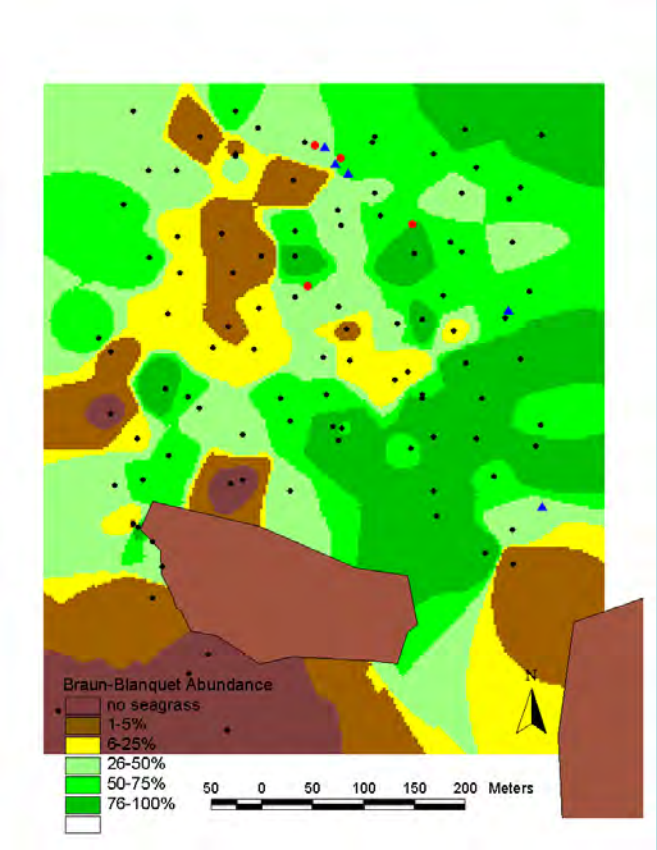


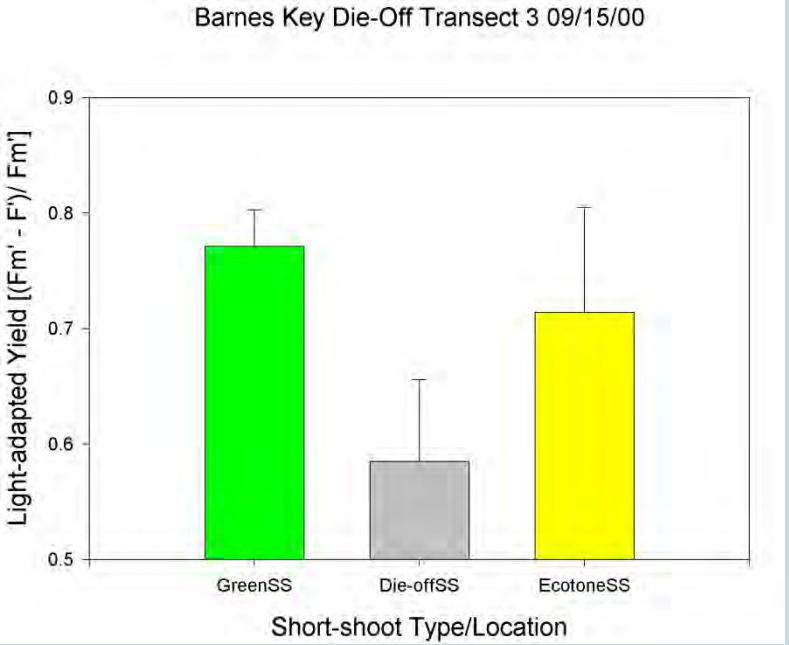


Durako & Kunzelman 2002

Fluorescence & Die-off (2000)

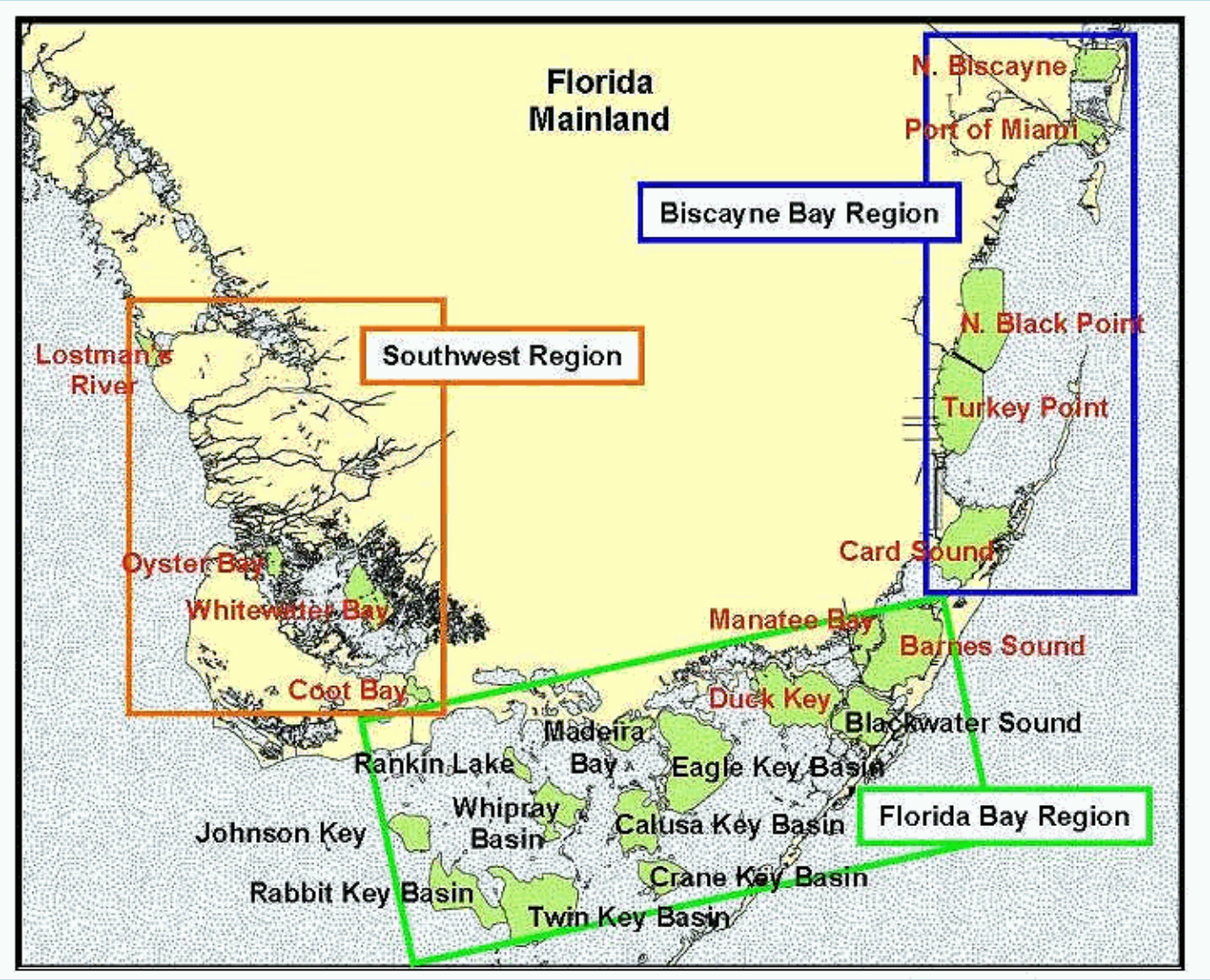






Durako & Kunzelman 2002

South Florida Fish Habitat Assessment Program
(FHAP-SF)



FHAP-SF SAMPLING DESIGN:

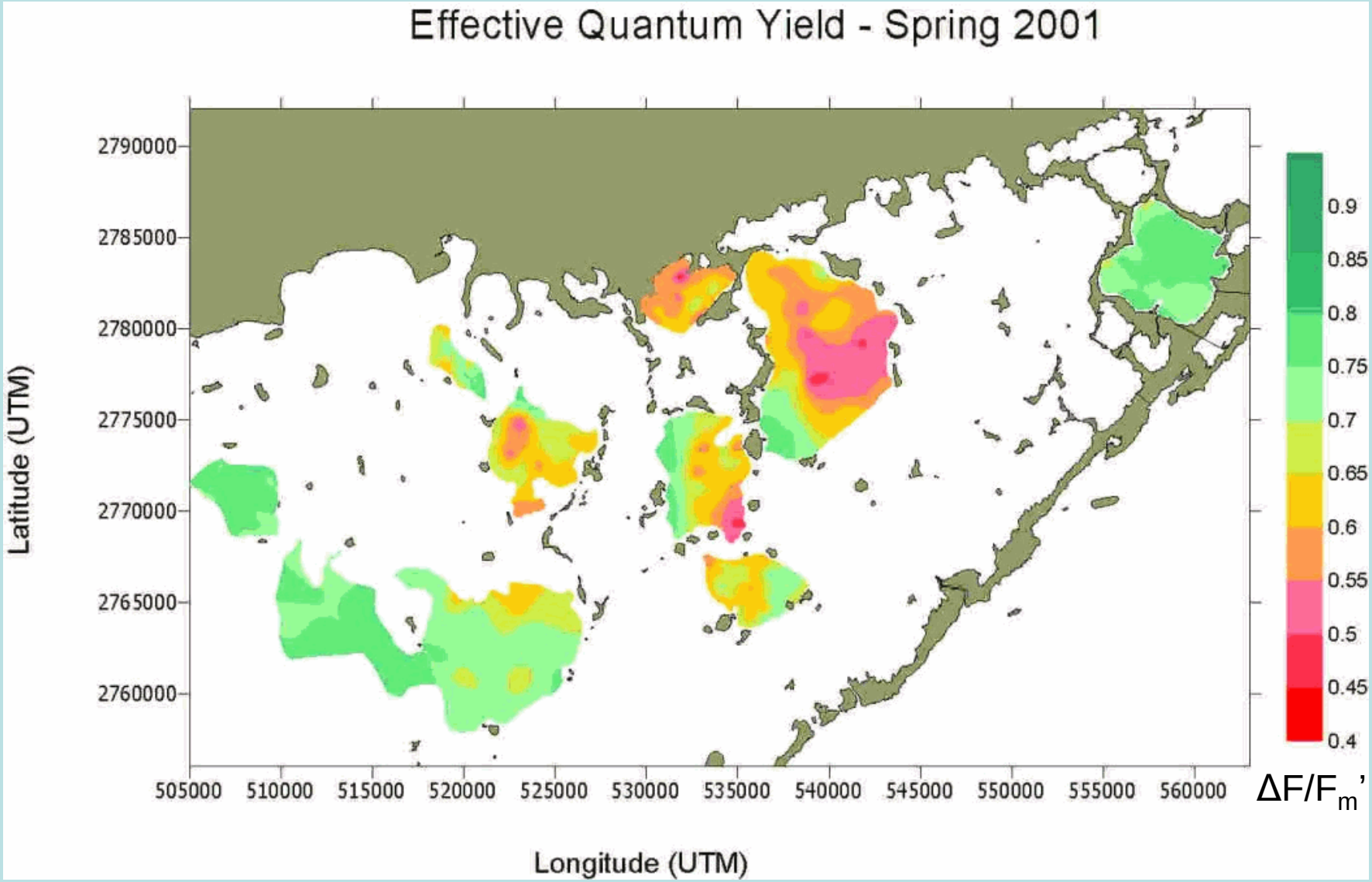
- **Each basin is divided into 30 tesselated hexagonal grid cells.**
- **A single sampling station is randomly selected from each grid cell.**
- **Systematic random-sampling design – good for interpolation**
- **Each basin is sampled in a day**
  - **≈ 15 min station^{-1}**
  - **Route minimizes travel time**

(no matter what the weather)

FHAP-SF PAM METHODS

Photobiology of *Thalassia testudinum*

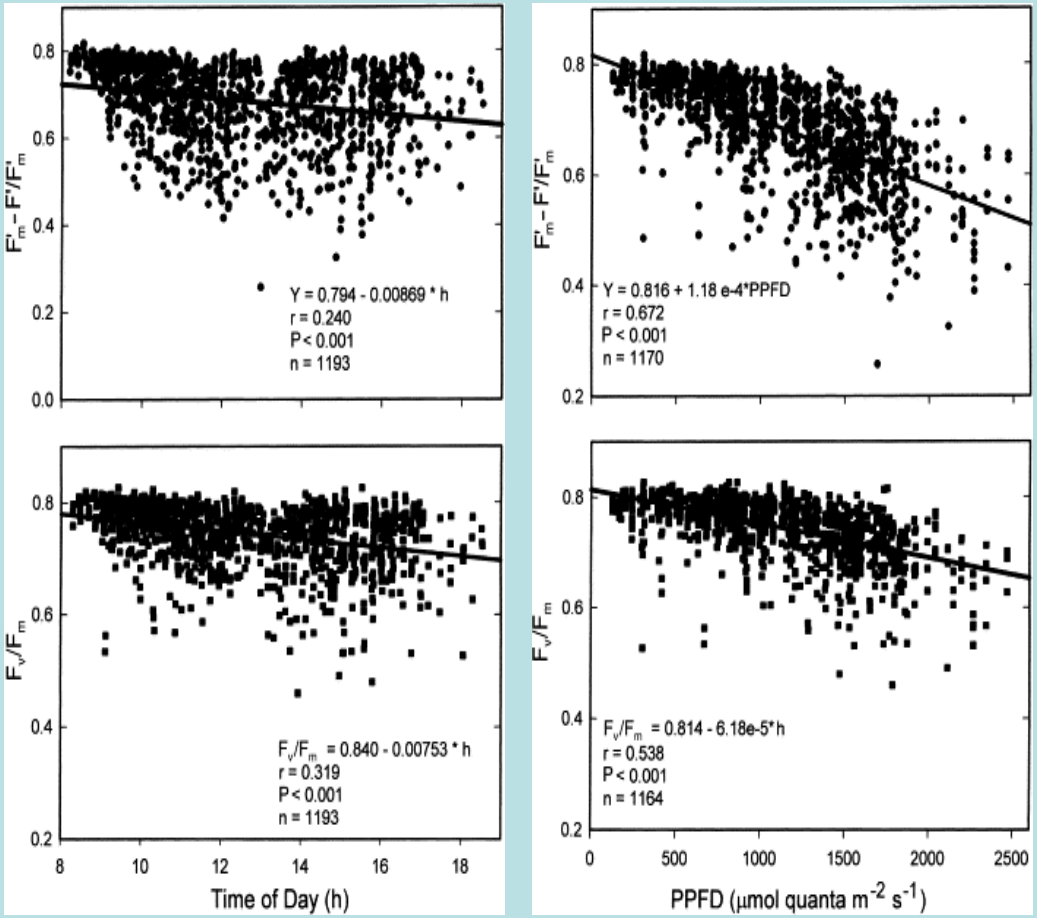
- **$\Delta F/F_m'$ measured at middle of 2nd rank blade**
- **Acrylic leaf clip**
- **Six short shoots per station**
  - **each shoot at least 2m apart**
- **Bottom PAR measured w/4π quantum sensor**


Effective Quantum Yield - Spring 2001
Latitude (UTM)
2790000
2785000
2780000
2775000
2770000
2765000
2760000
505000
510000
515000
520000
525000
530000
535000
540000
545000
550000
555000
560000
Longitude (UTM)
0.9
0.85
0.8
0.75
0.7
0.65
0.6
0.55
0.5
0.45
0.4
ΔF/Fm'

Diurnal variation

FHAP 2001

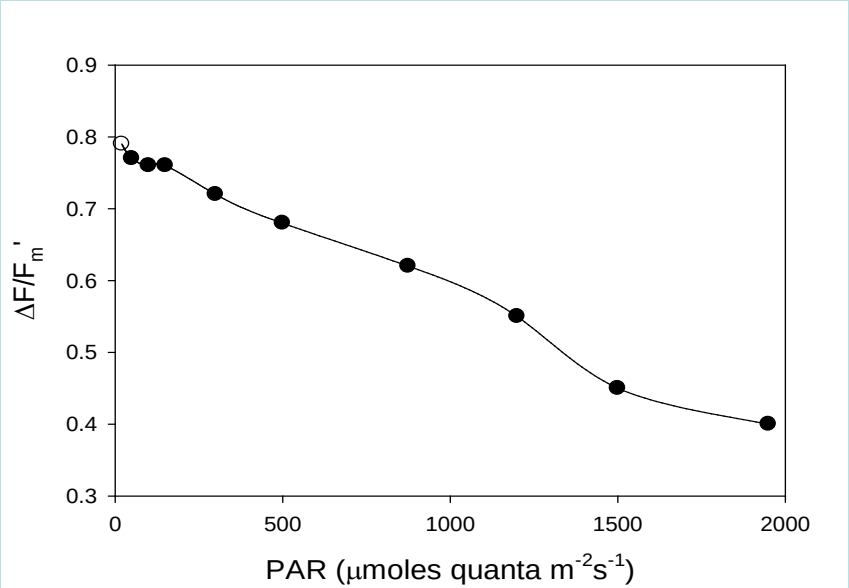
- Time of day effect
  - Irradiance effect
- Regressions for both effective and maximum (5-min dark) yields had significant negative slopes



Durako and Kunzelman 2002

Effective Yield (ΔF/Fm') decreases as irradiance increases

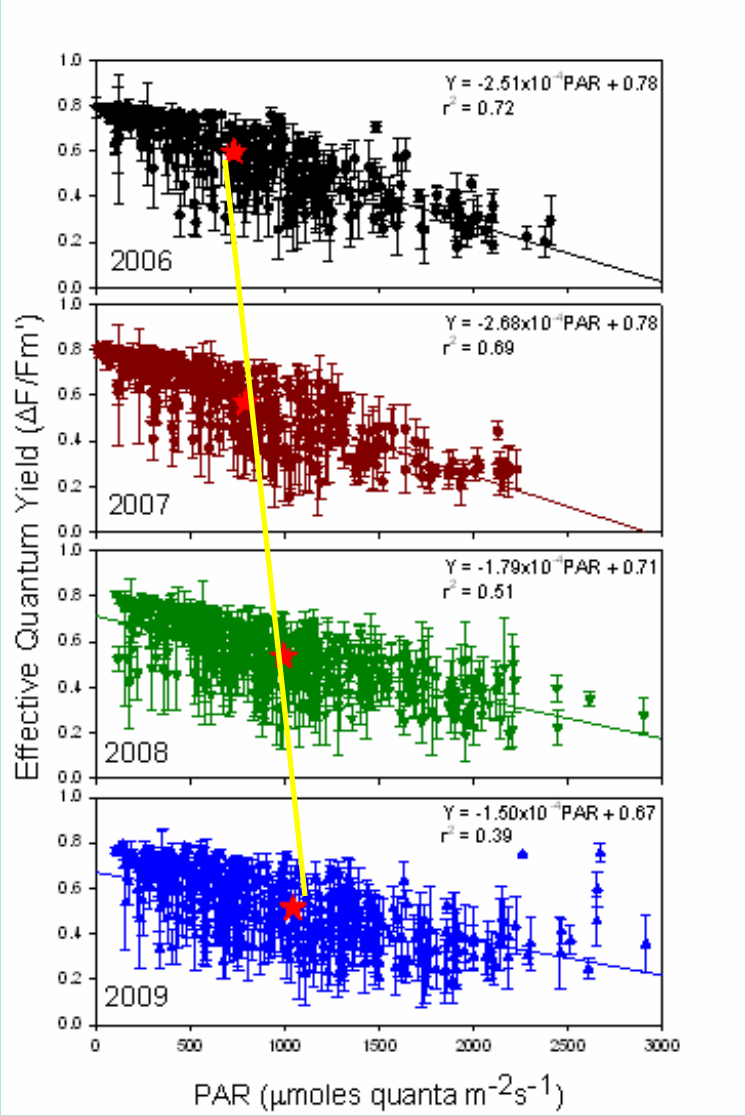
- increase in thermal energy dissipation (photoprotection)
- D1 protein damage (photoinhibition)



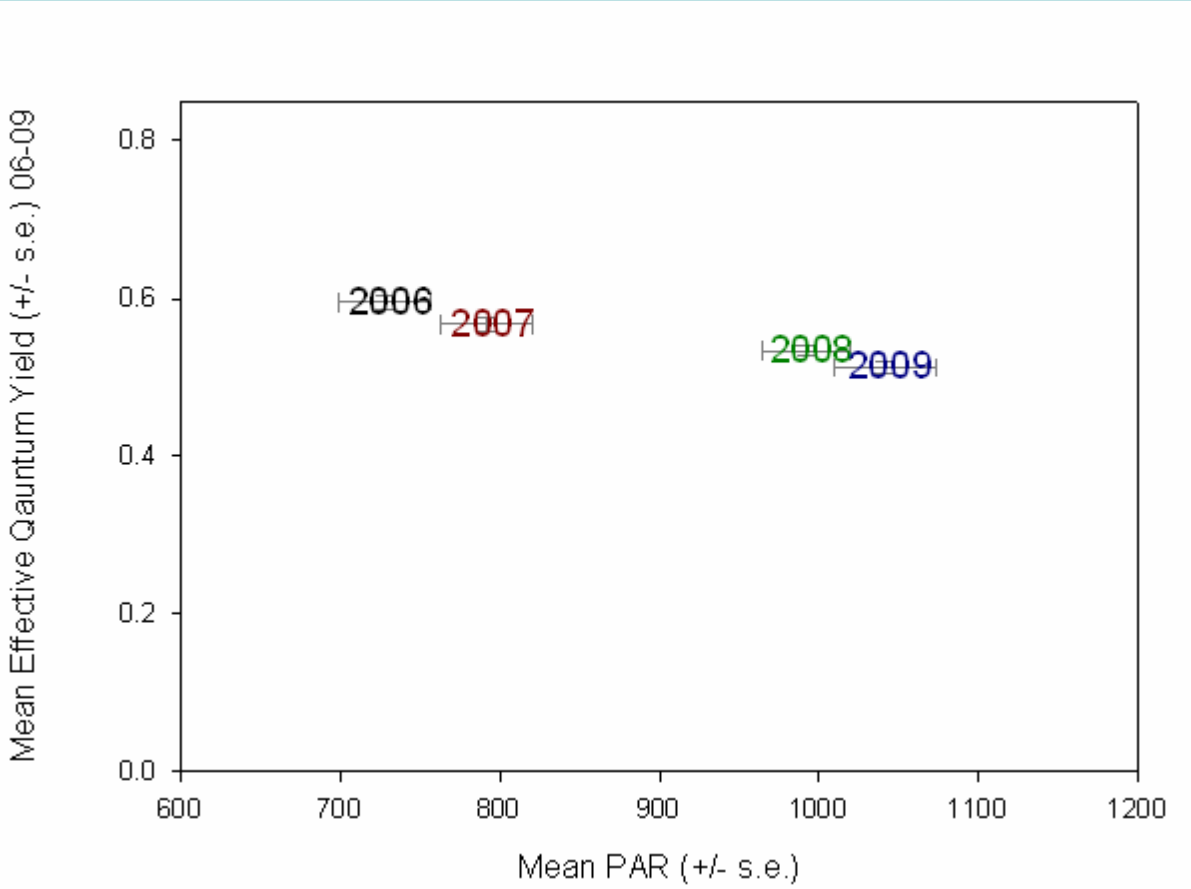
Efficiency of energy dissipation affected by photo-acclimation state or stress

So, what can the data it tell us?

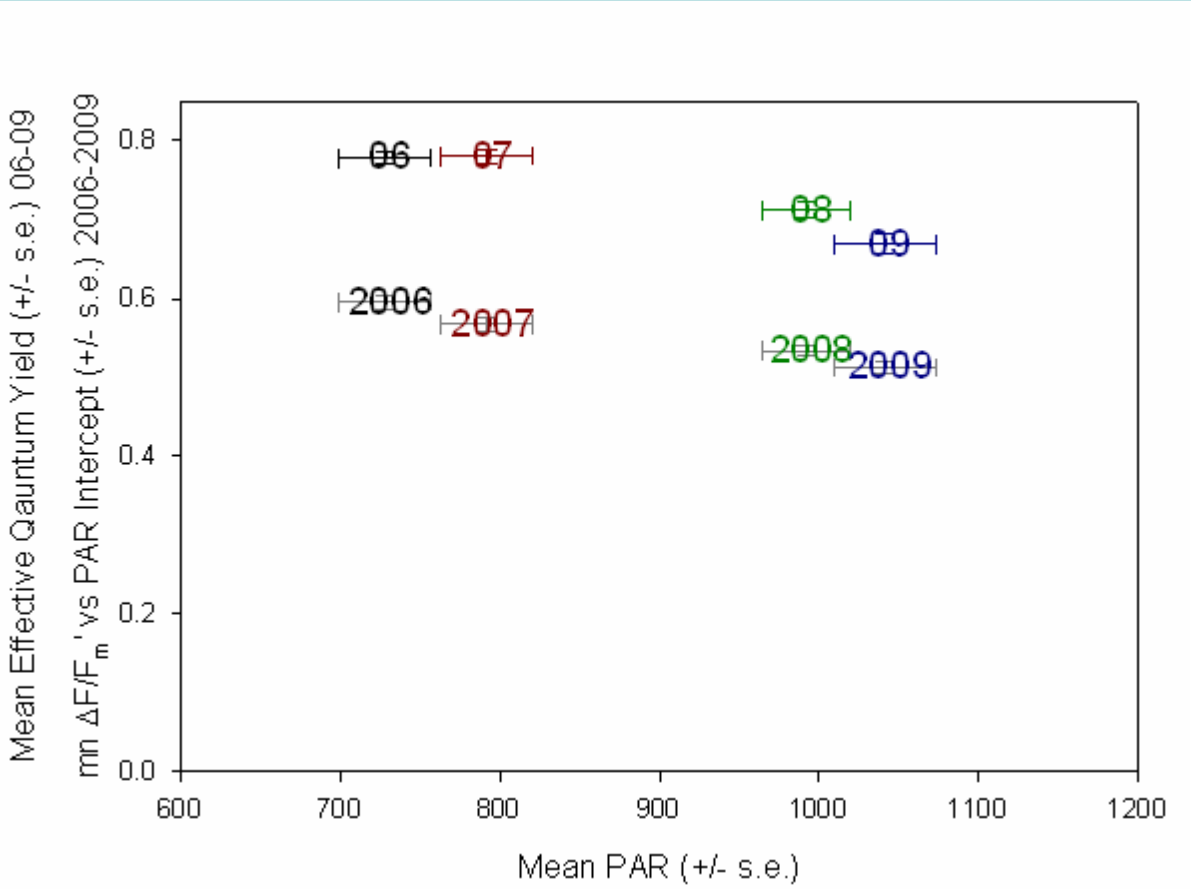
- @Bay scale 2006-2009
  - mean bottom irradiance has increased
  - mean $\Delta F/F_m$' has declined
- $\Delta F/F_m$' vs PAR regressions
  - slopes have declined
    - more efficient dissipation of excess E
      - sun acclimated
  - y-intercepts have declined
    - lower max quantum efficiencies
      - sun acclimated



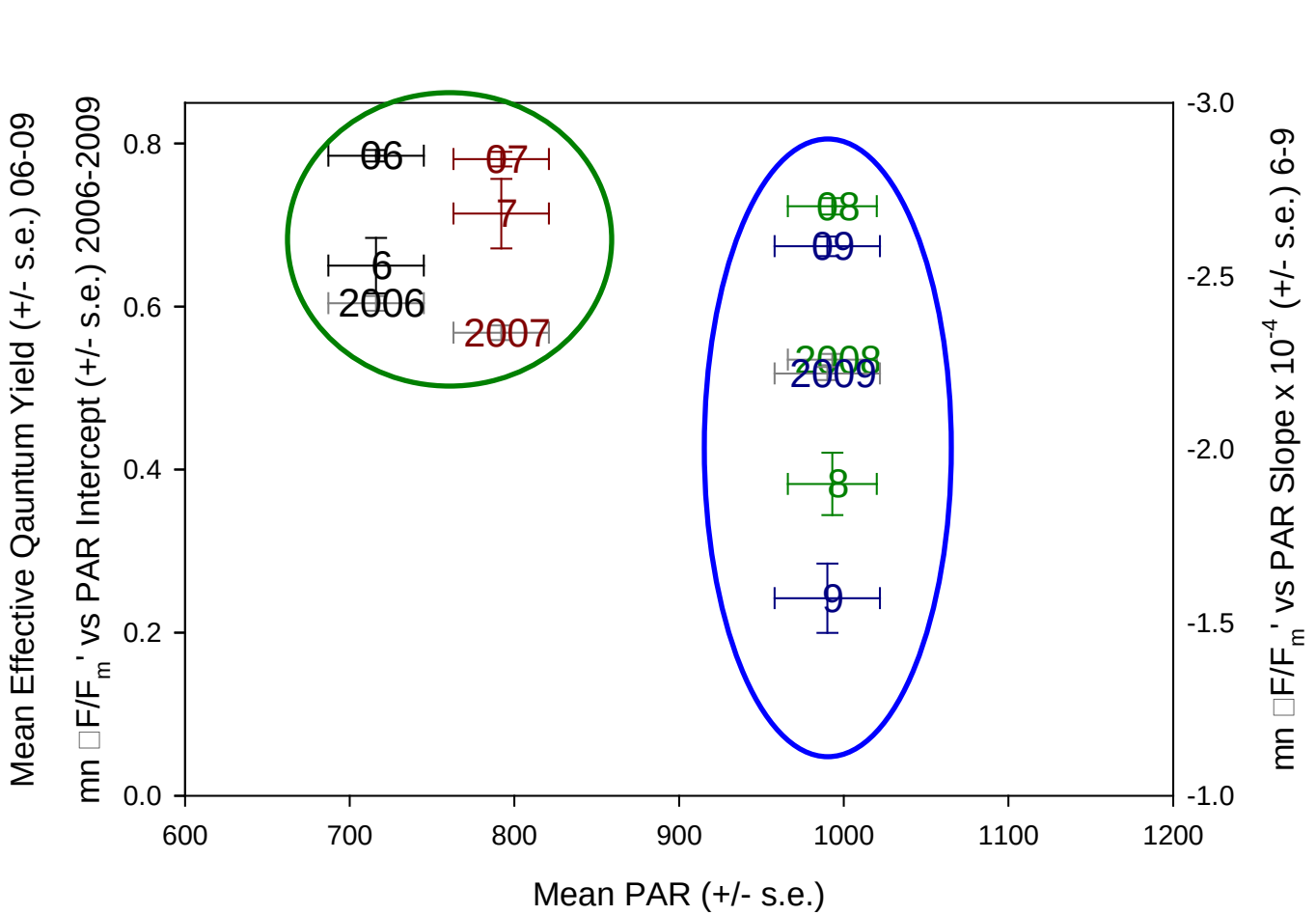
Bay Scale
mnΔF/F$_m$' vs mnPAR



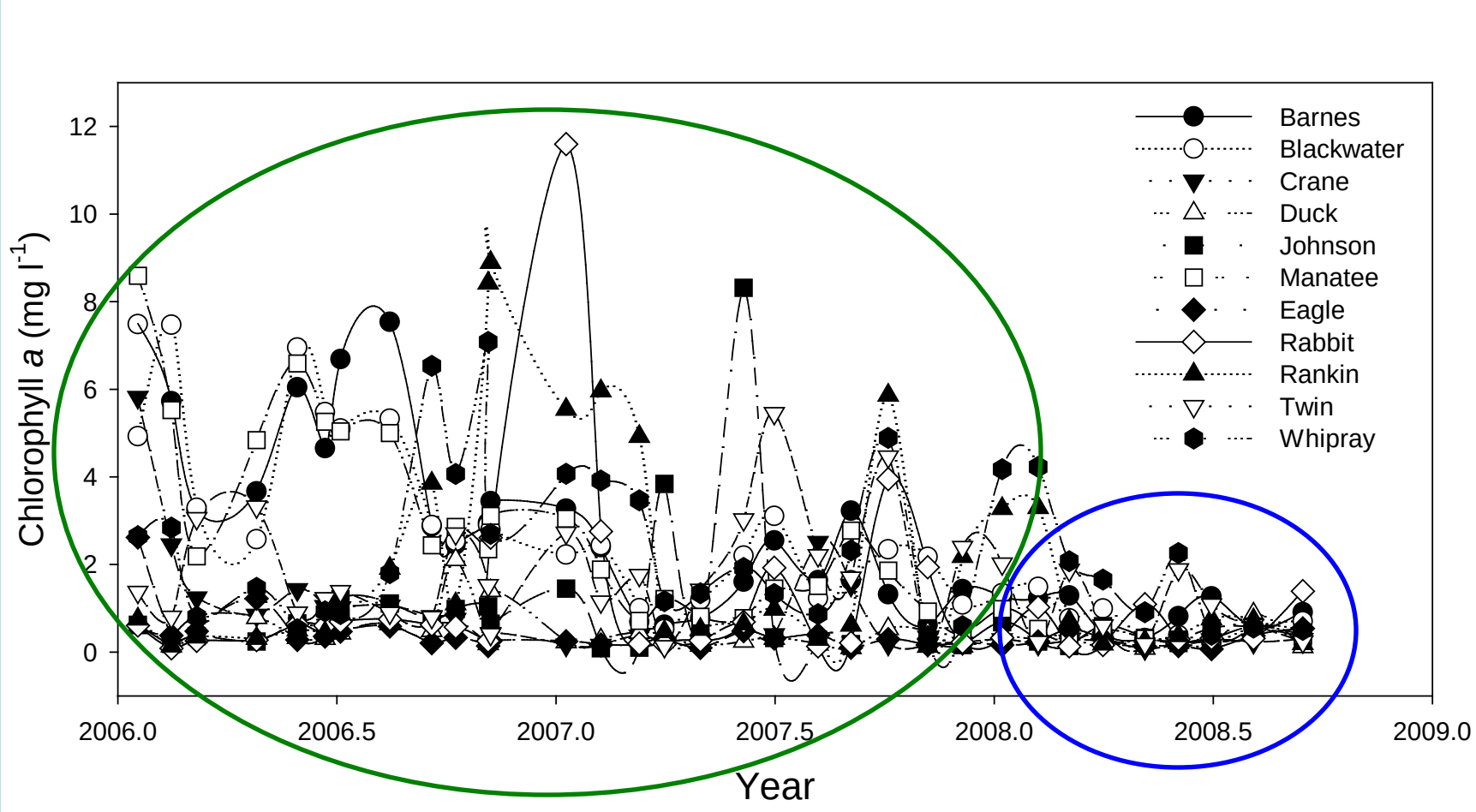
Bay Scale
$\Delta F/F_m$' vs PAR intercepts



Bay Scale
$\Delta F/F_m$' vs PAR slopes

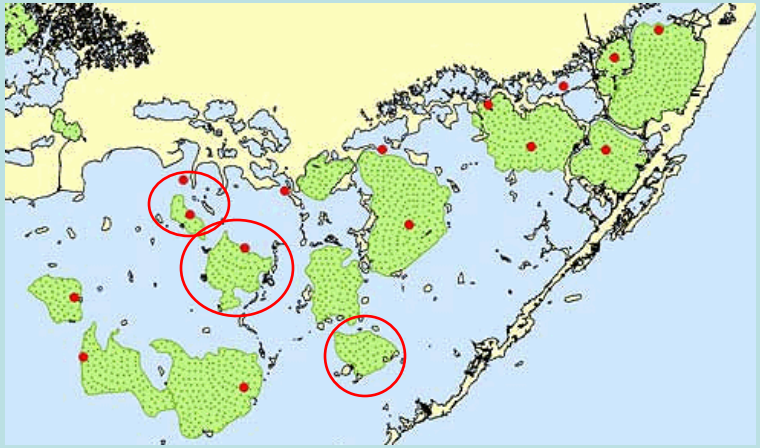


Bay-scale Chlorophyll

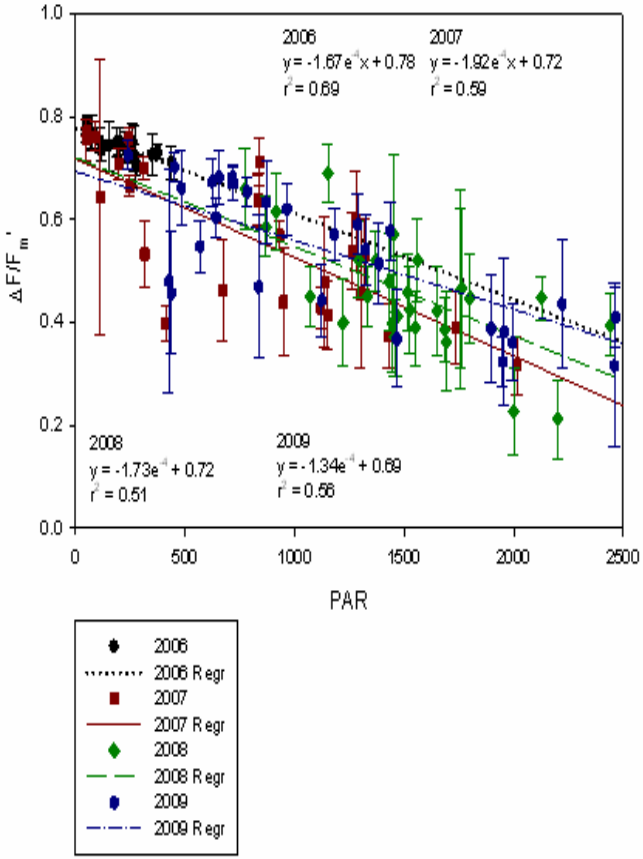


SERC-FIU Water Quality Monitoring Network

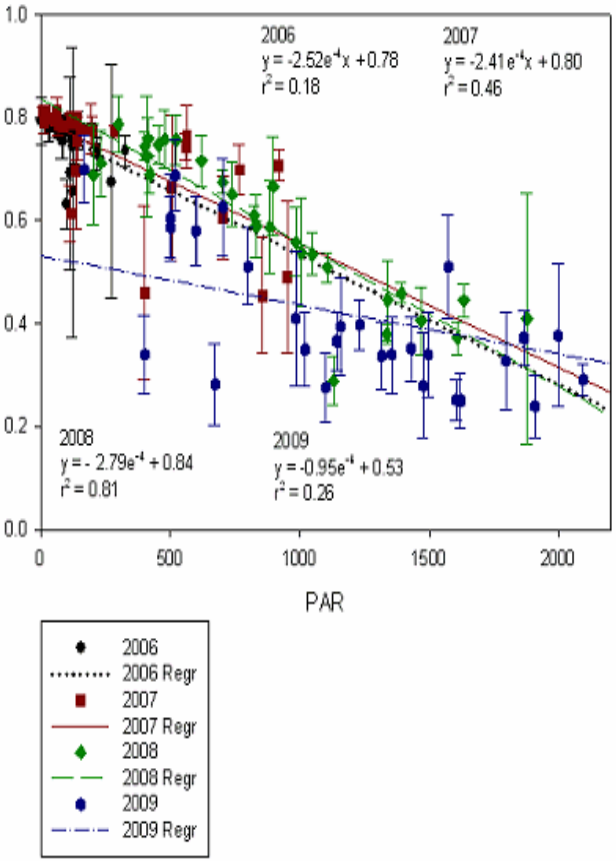
Basin Scale



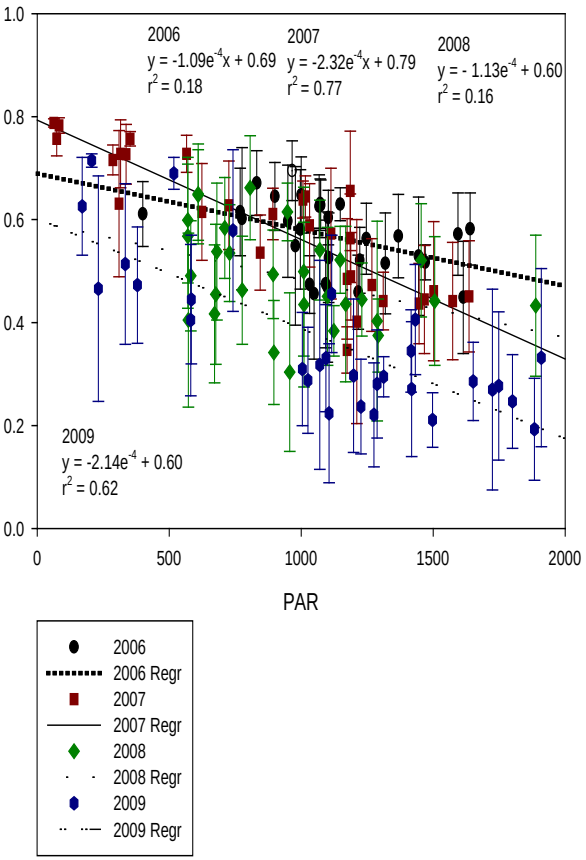
Crane Key Basin



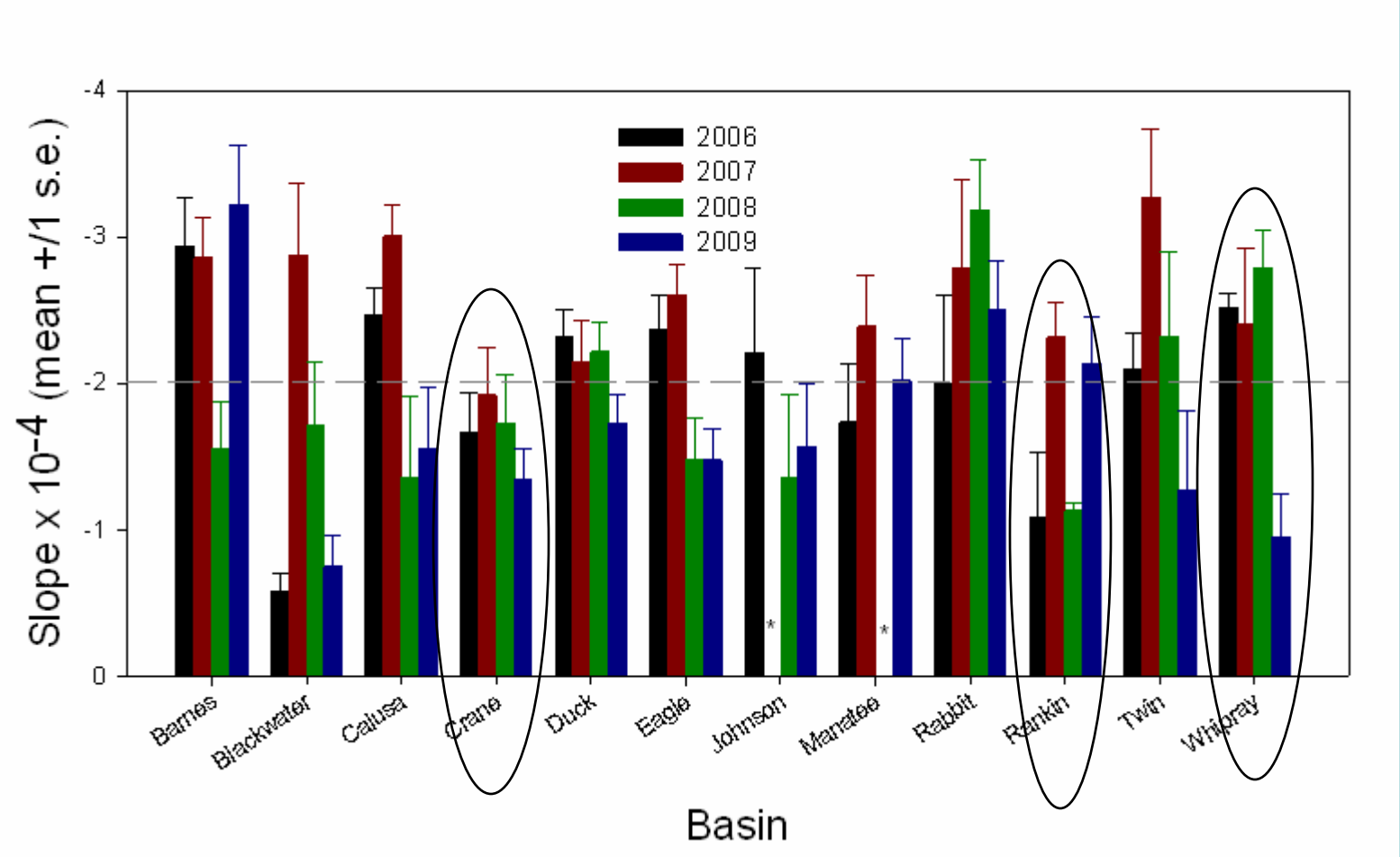
Whipray Bay



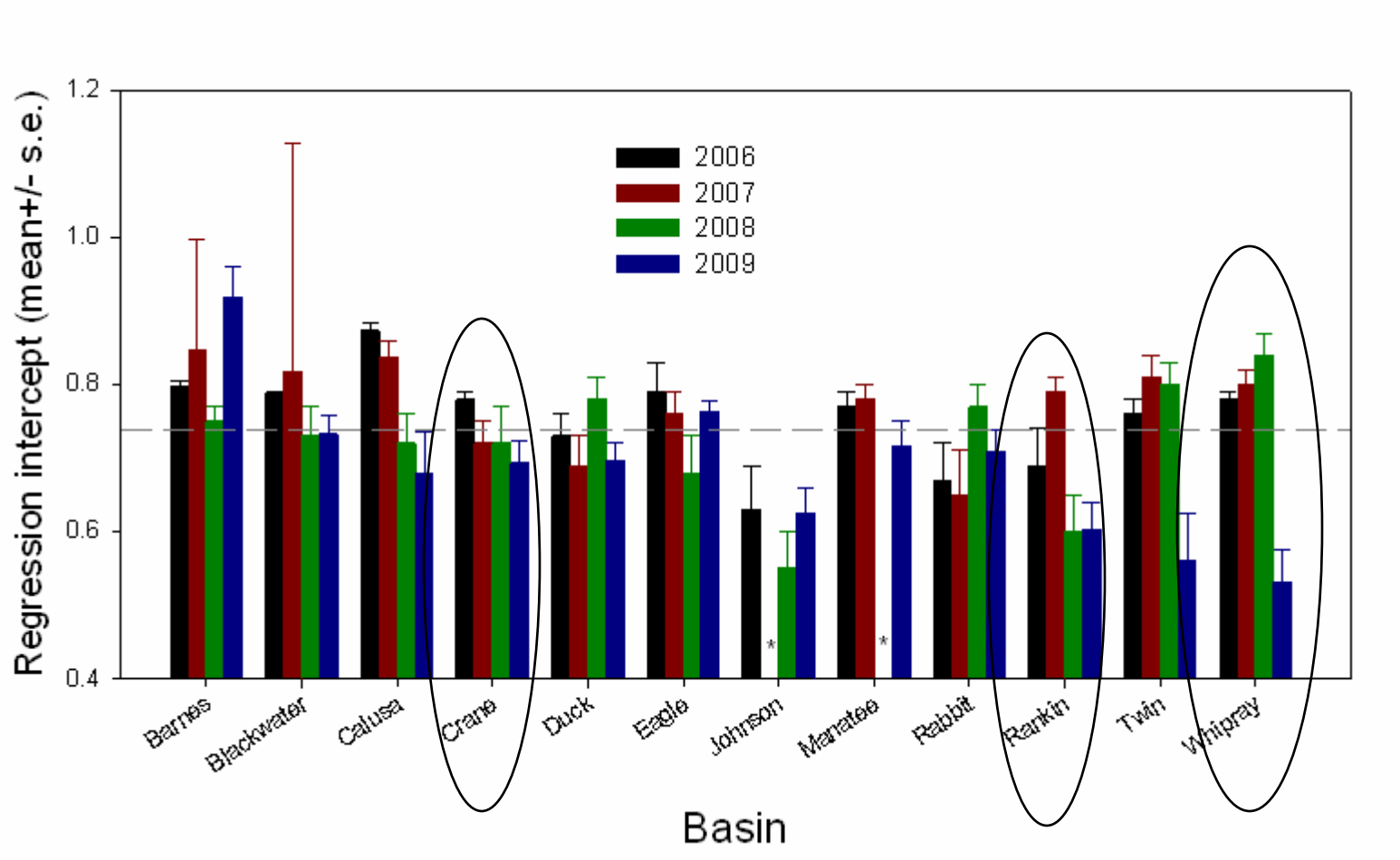
Rankin Lake



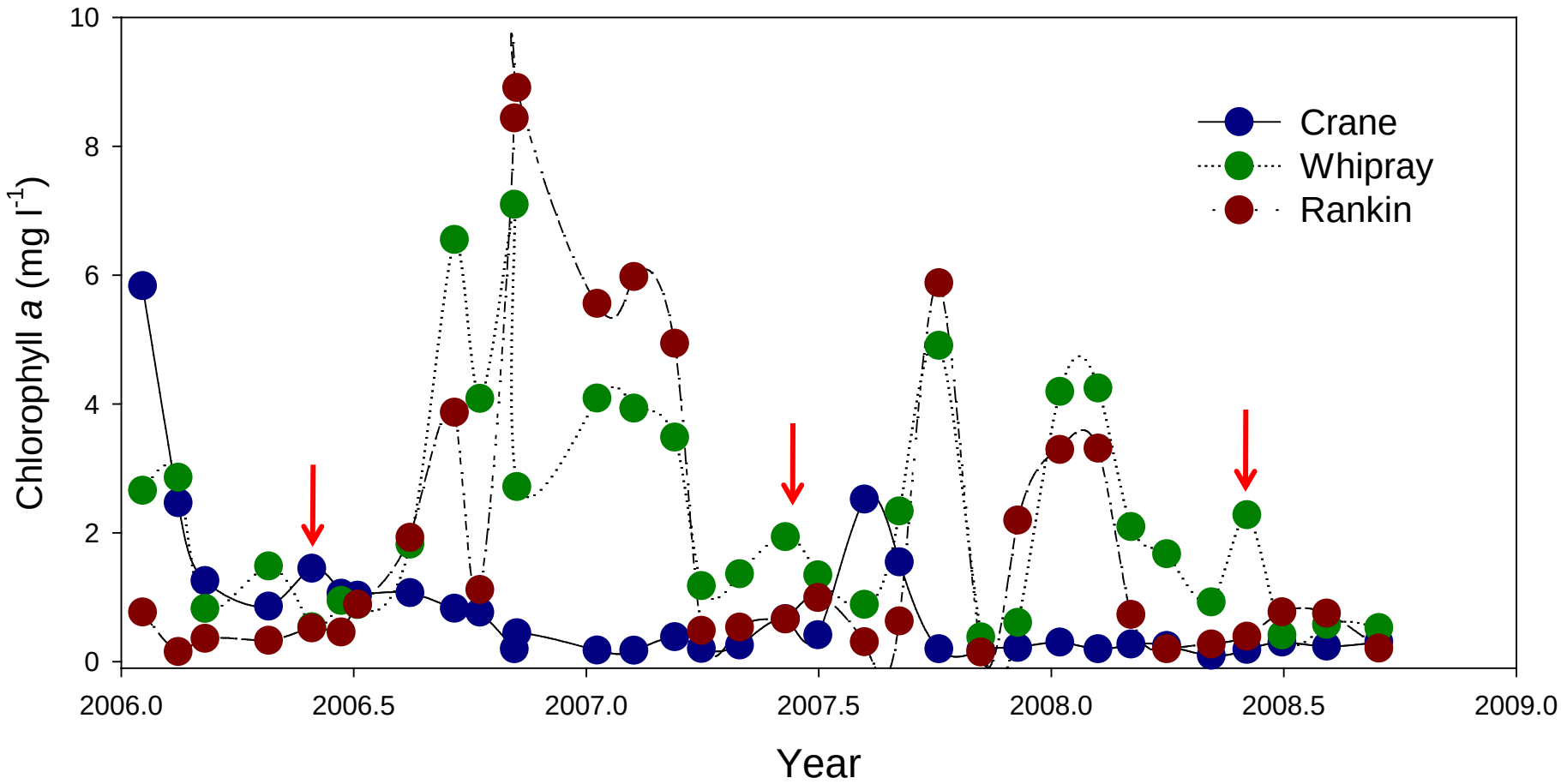
$\Delta F/F_m'$ vs PAR slopes



$\Delta F/F_m$' vs PAR intercepts



Basin-scale Chlorophyll

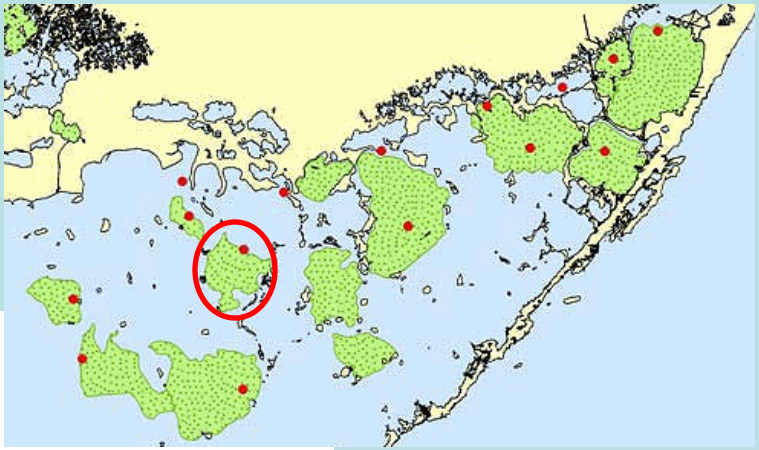


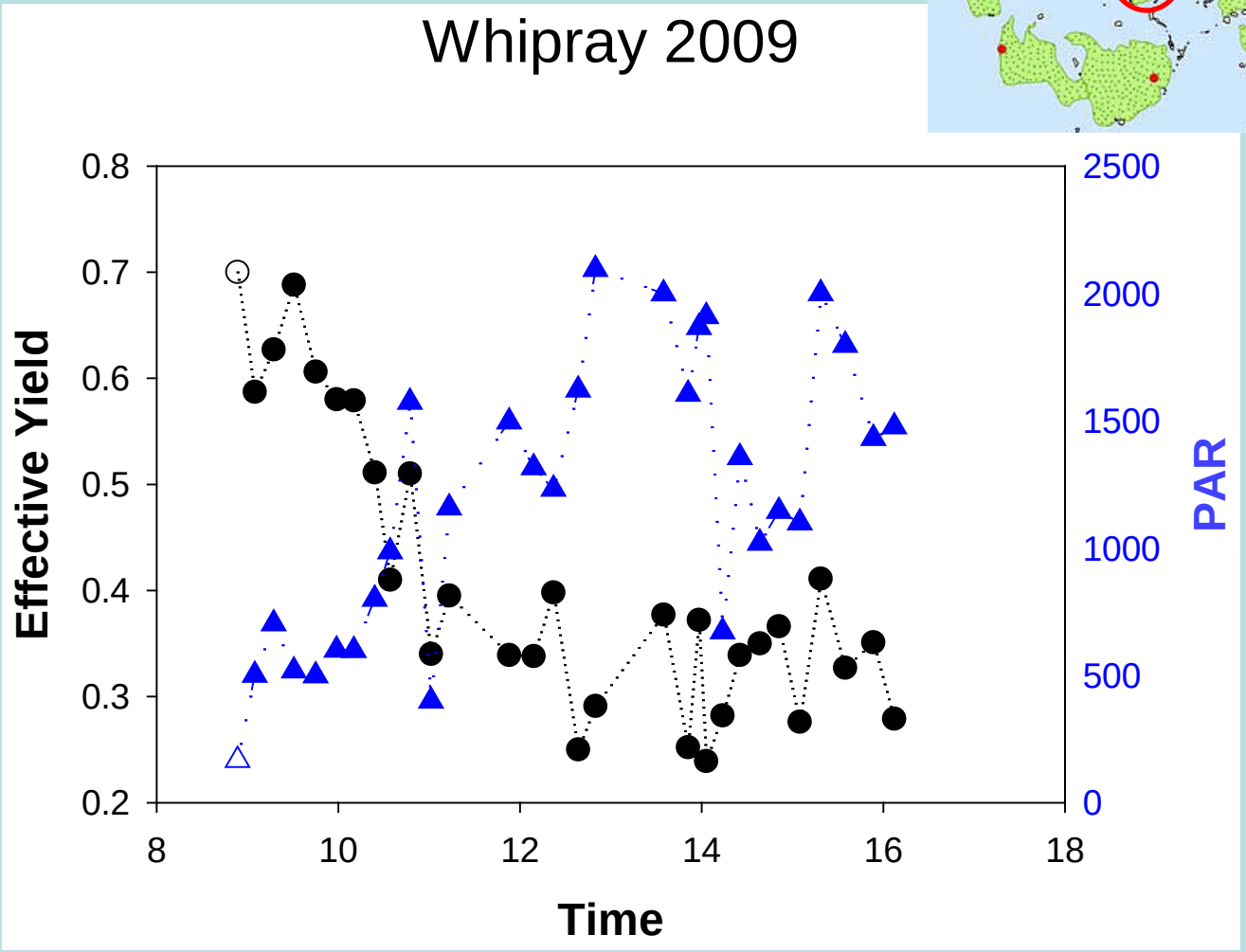
SERC-FIU Water Quality Monitoring Network

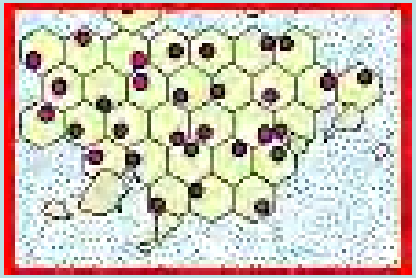
Conclusions

- As mean irradiance and water clarity has increased @ both bay and basin scales
  - $\Delta F/F_m'$ vs PAR slopes decreased
    - suggests more efficient dissipation of excess E
  - $\Delta F/F_m'$ vs PAR intercepts decreased
    - suggests decrease in maximum quantum efficiency

Source noise in the yield data

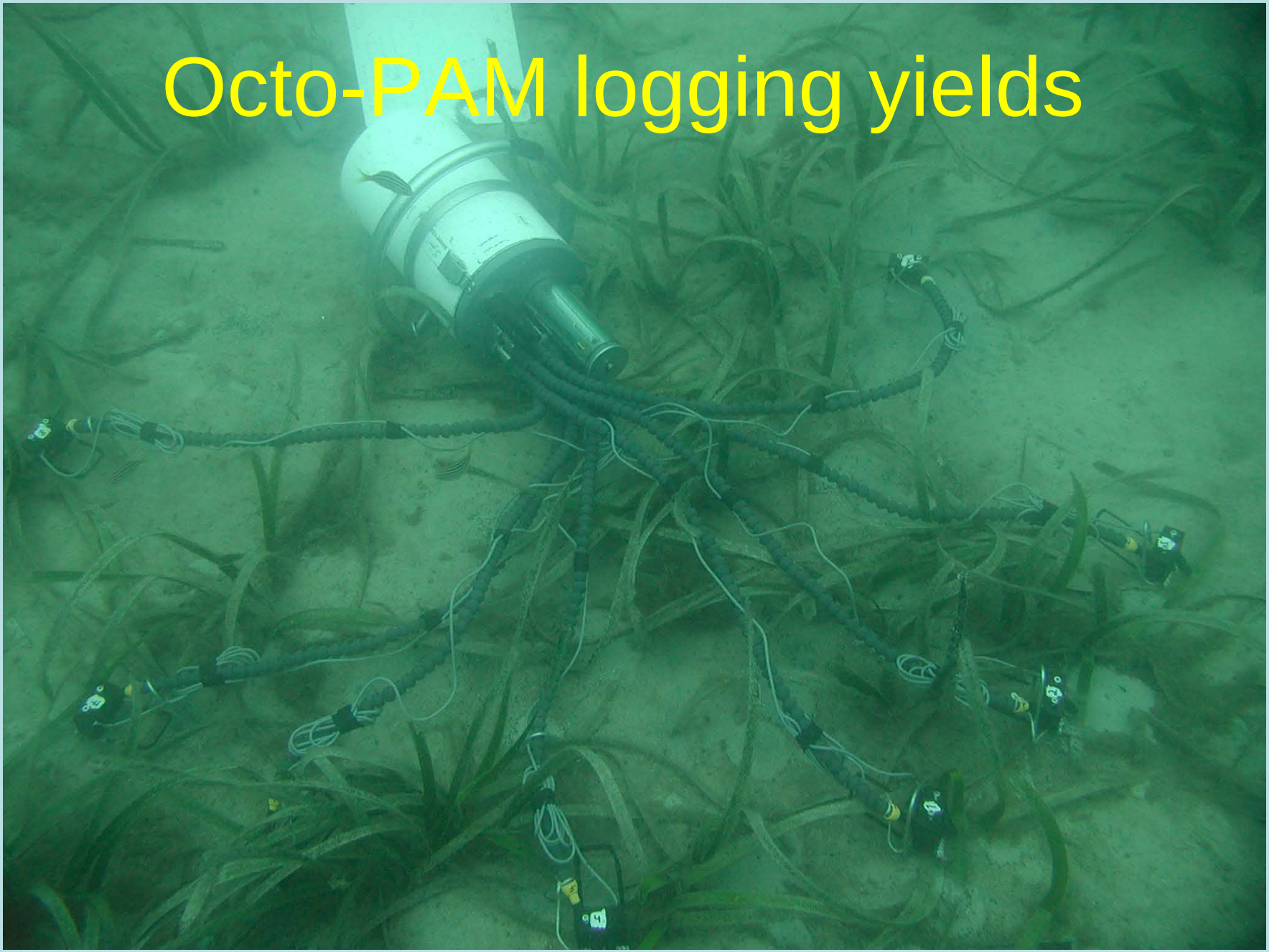


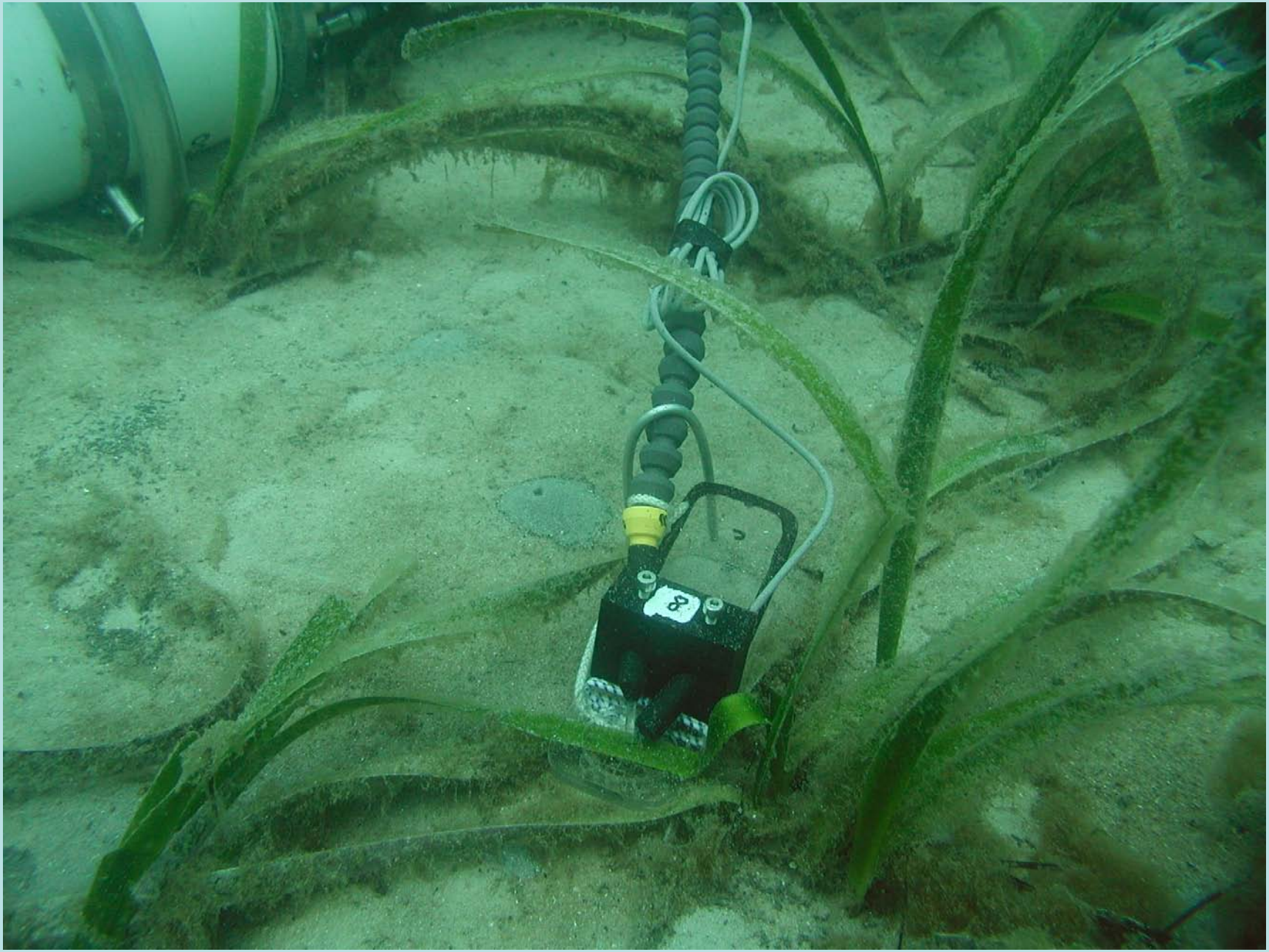




What we NEED!

Octo-PAM logging yields

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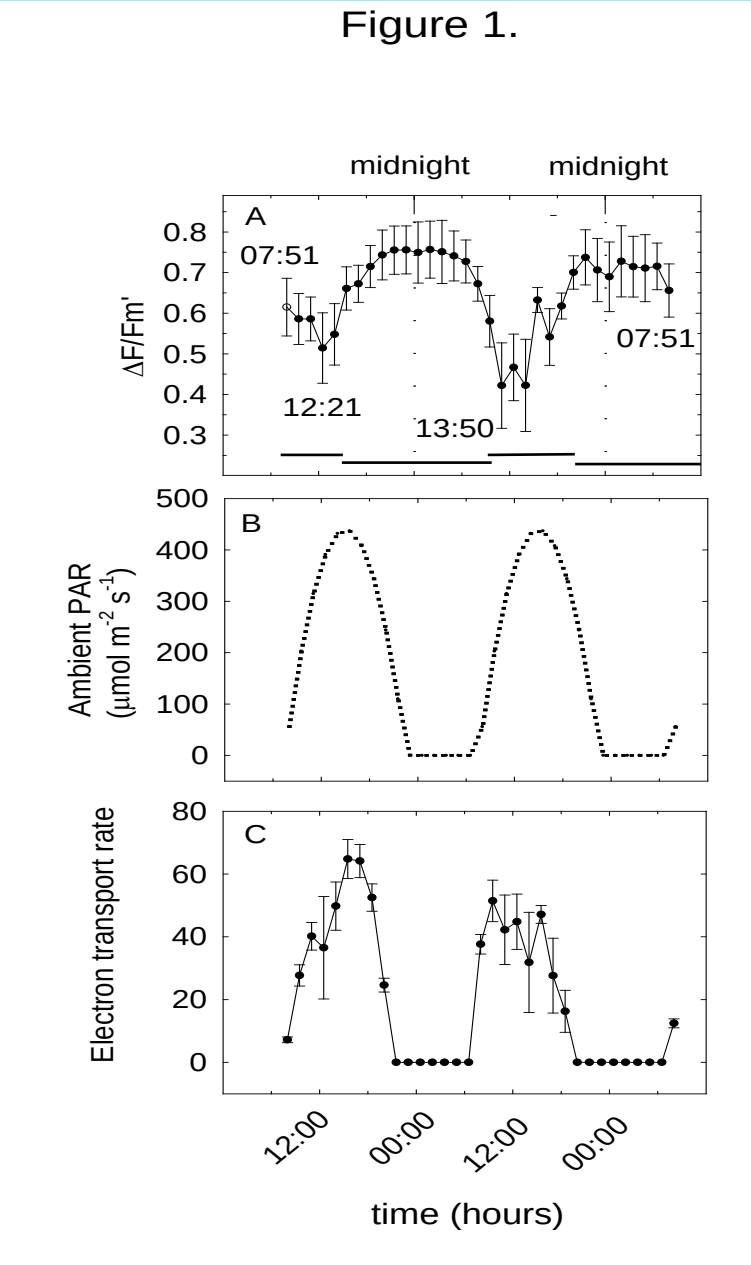


Runcie & Durako 2004

In situ PAM logging

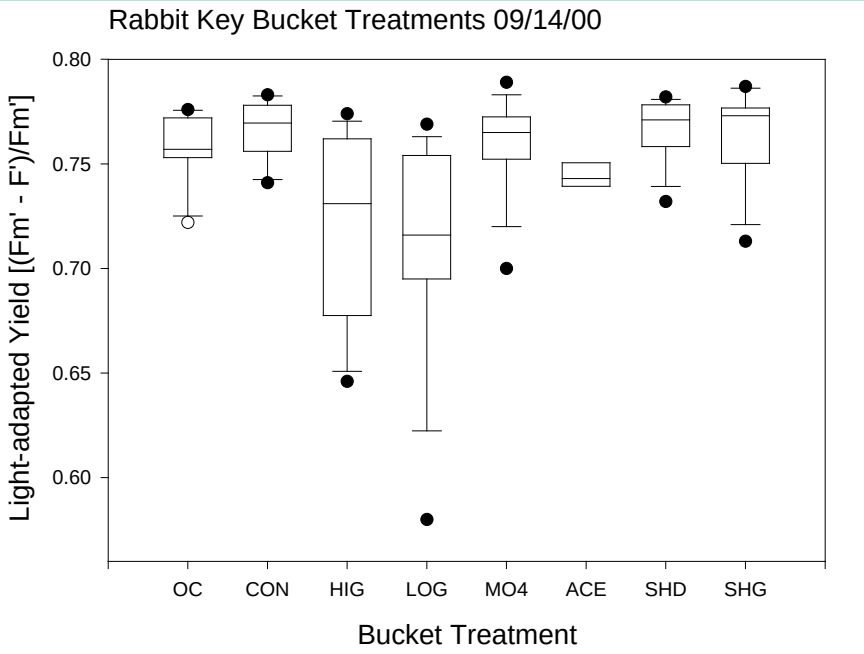
- **Allows direct measurement of maximum yield (to compare with diurnal regression intercept)**
- **Allows direct measurement of mid-day down regulation**
- **Can compare if hysteresis is present in am vs pm yields vs PAR (downregulation vs photoinhibition)**
- **Directly measures among-shoot variability in photophysiology**

Funding provided by sfwmd.gov

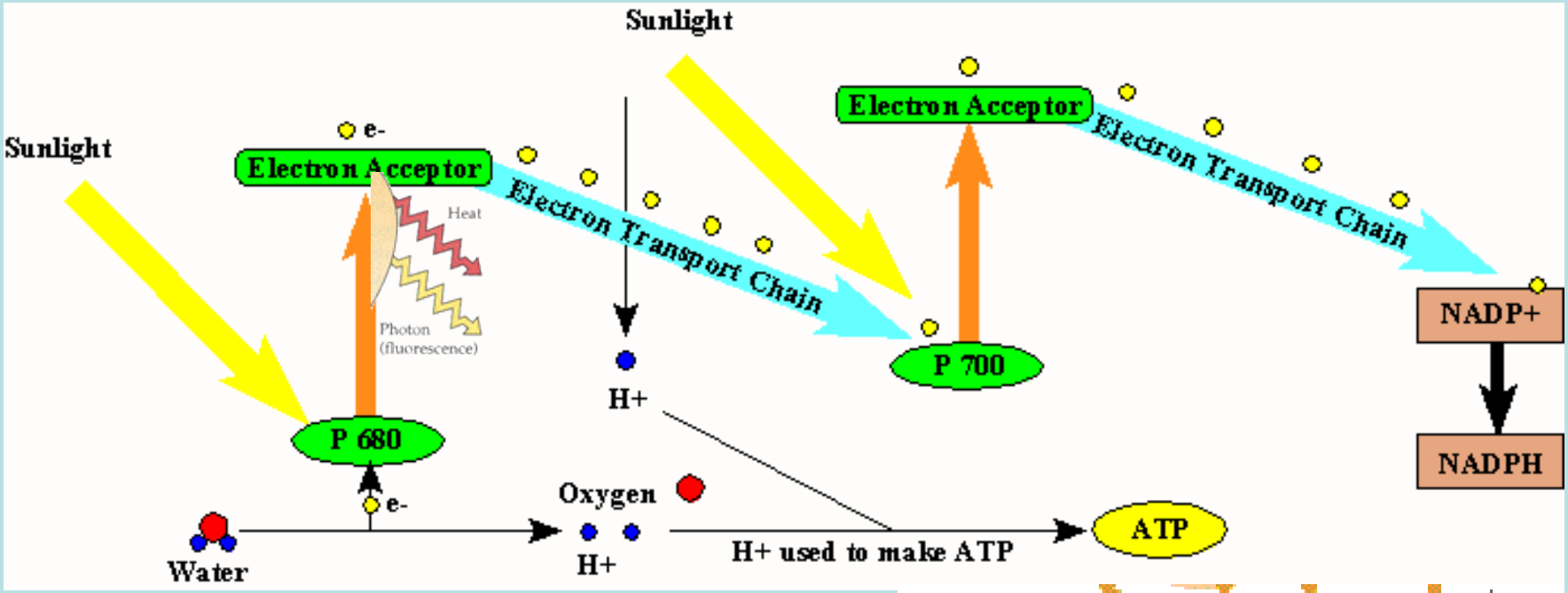
Photosynthetic yields (n=6) for *Thalassia* in sulfide treatment buckets at Rabbit Key Basin.

Treatment Key: OC= outside control; CON=control; HIG=high glucose; LOG=low glucose; MO4=molybdate; ACE=acetate; HD=shaded; SHG=shaded+glucose.

Photosynthetic yields are reduced with glucose and acetate additions to sediments; these treatments result in increased sulfide levels.



Photosynthesis

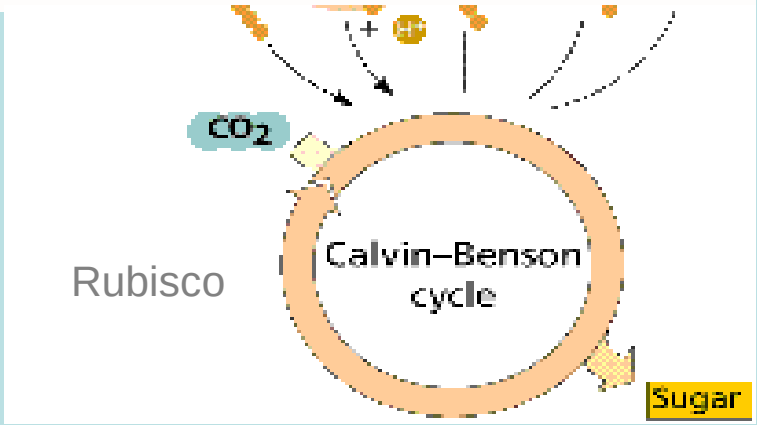


$6CO_2 + 6H_2O + \text{light} \rightarrow C_6H_{12}O_6 + 6O_2$

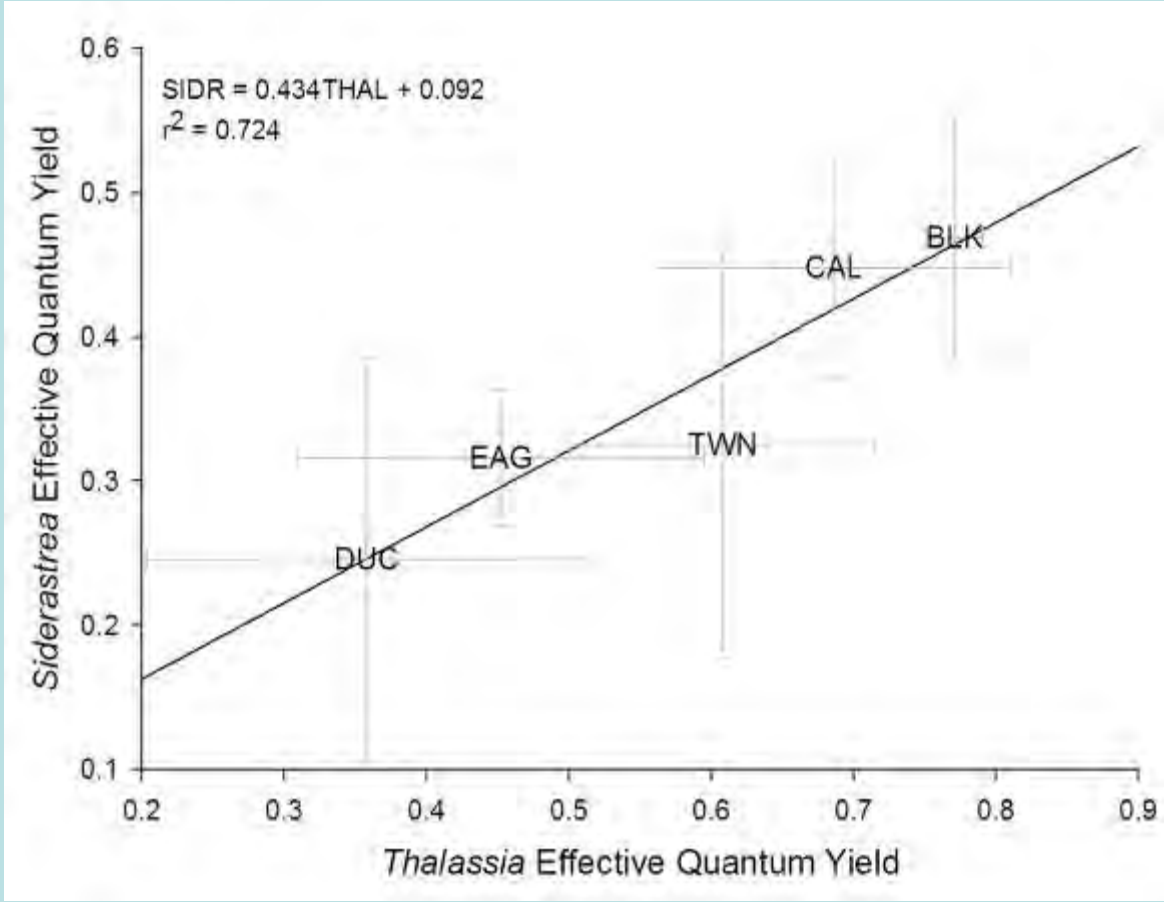
ETS provides energy (ATP) & reduction equivalents (NADPH), which are used to reduce CO_2 to sugar in the C-B cycle

O_2: CO_2 = 1.1-1.2 = Photosynthetic quotient

Chlorophyll fluorescence due to excess light energy @PSII



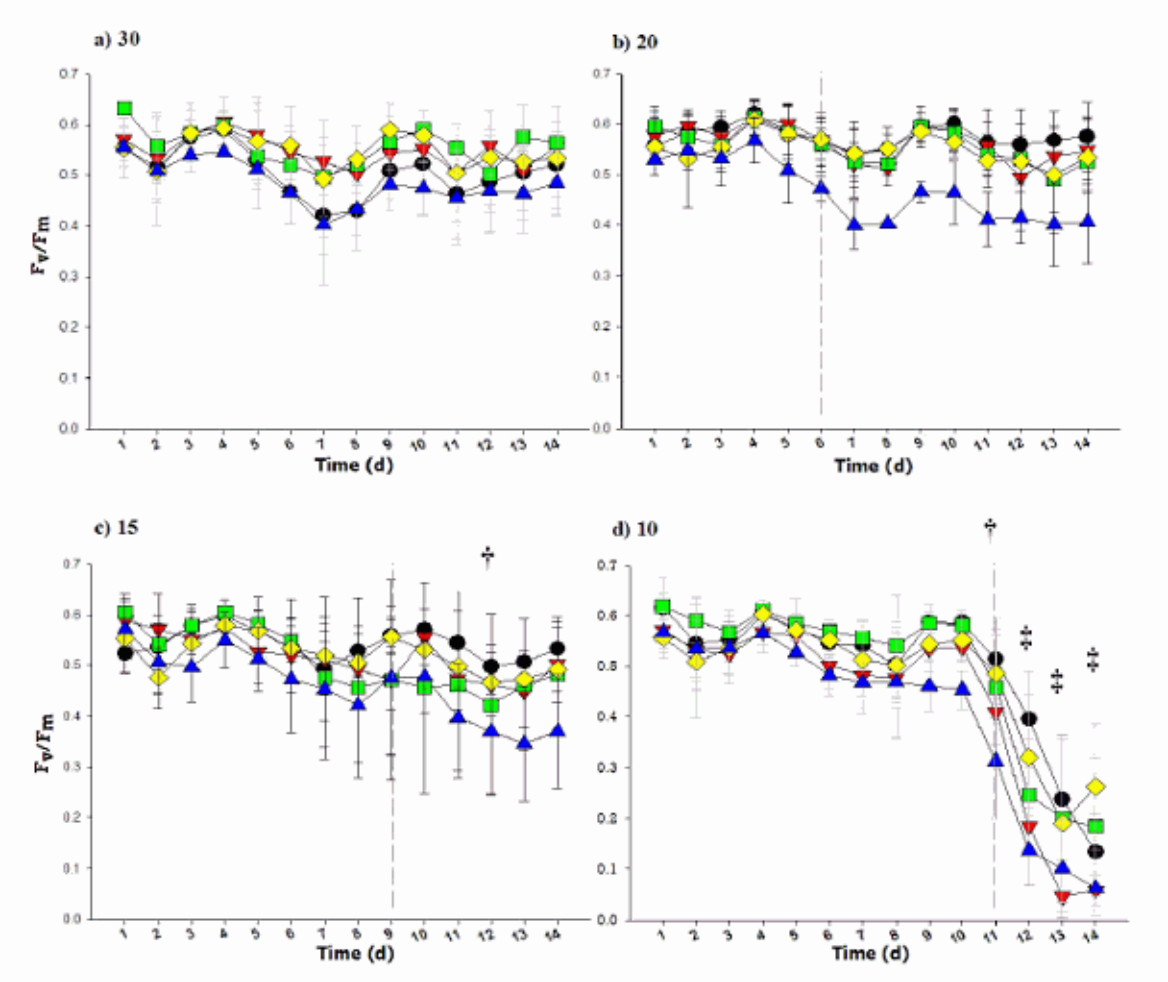
Seagrass vs Coral Photobiology

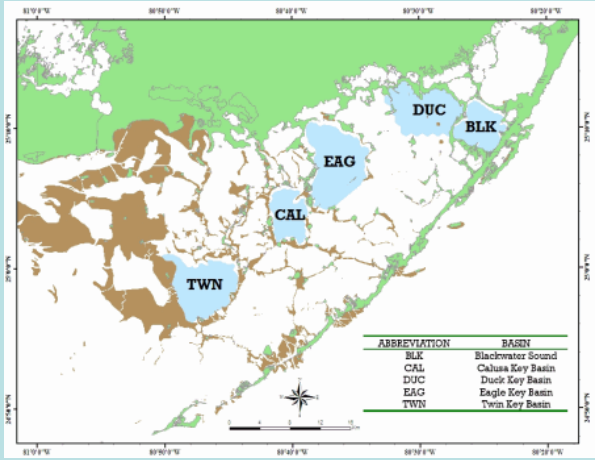


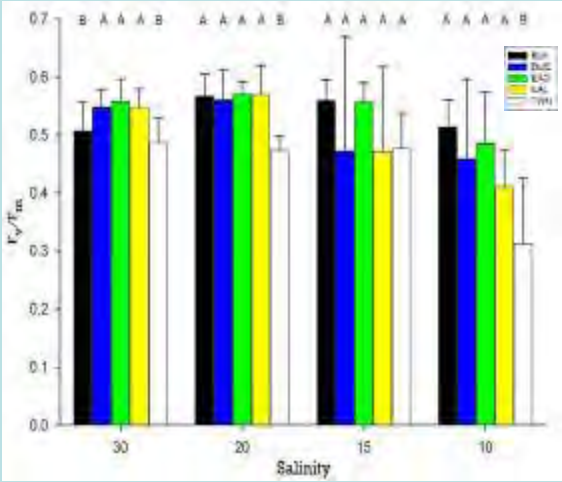
Chartrand and Durako 2009

***S. radians* & *T. testudinum* exhibit similar response to light environment**
May serve as an eco-indicator where *T. testudinum* is absent

Hypo-Salinity Effects on *S. radians*





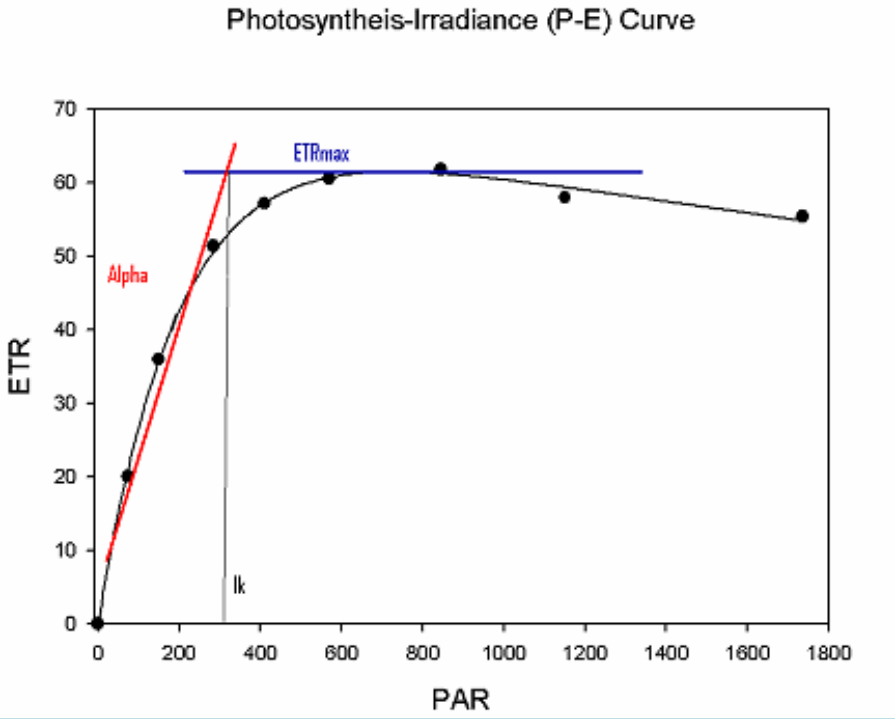


<u>Twin population</u>
Lowest Fv/Fm
Lowest S tolerance

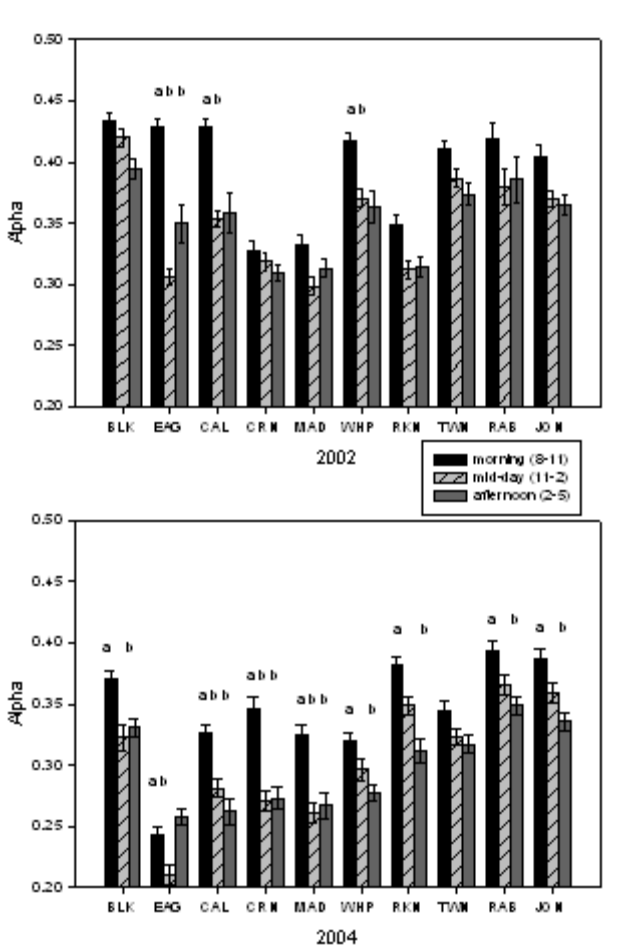
Rapid Light Curves (RLC)

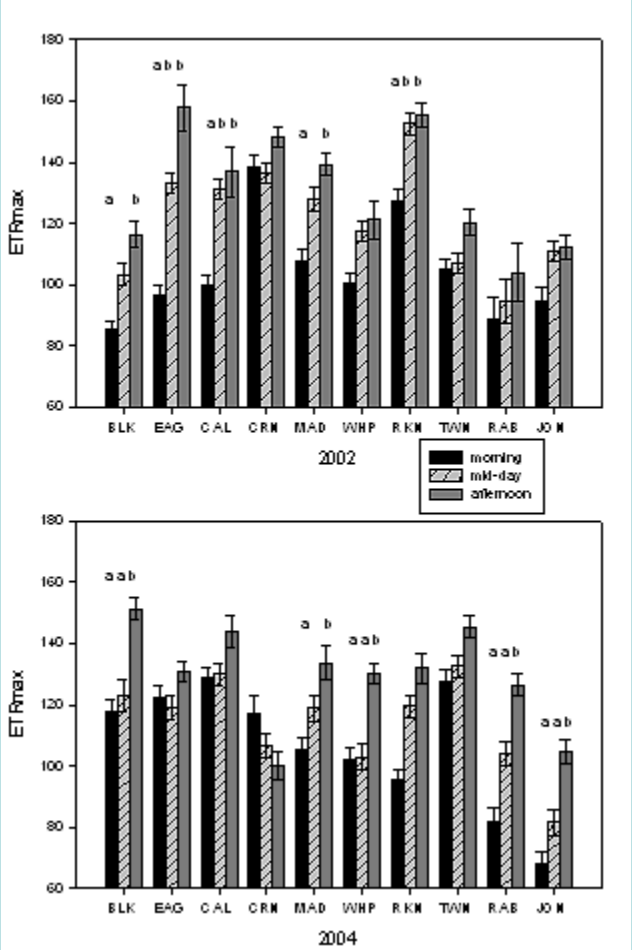
FHAP 2002 (& 2004)

- $\Delta F/F_m'$ at eight irradiance levels – 5s @each level
  - Non-steady state
- **Calculated rETR @ each irradiance**
- Fit curves to a double exponential decay function using a non-linear regression procedure in SAS
- Quantify RLC parameters
  - α (Efficiency, pigments)
  - $rETR_{max}$ (Capacity, Rubisco)
  - I_k (sun vs shade acclimated)



RLC Results





- α (Efficiency)
- Similar diurnal trends to yield

Belshe et al. 2007

- $rETR_{max}$ (Capacity)
- Opposite diurnal trends to yield

Diurnal Yield vs Diurnal RLC

Longstaff et al. 2002

Diurnal Yield

Calculate rETR from $\Delta F/F_m$' and ambient PAR during sampling

1 curve per basin

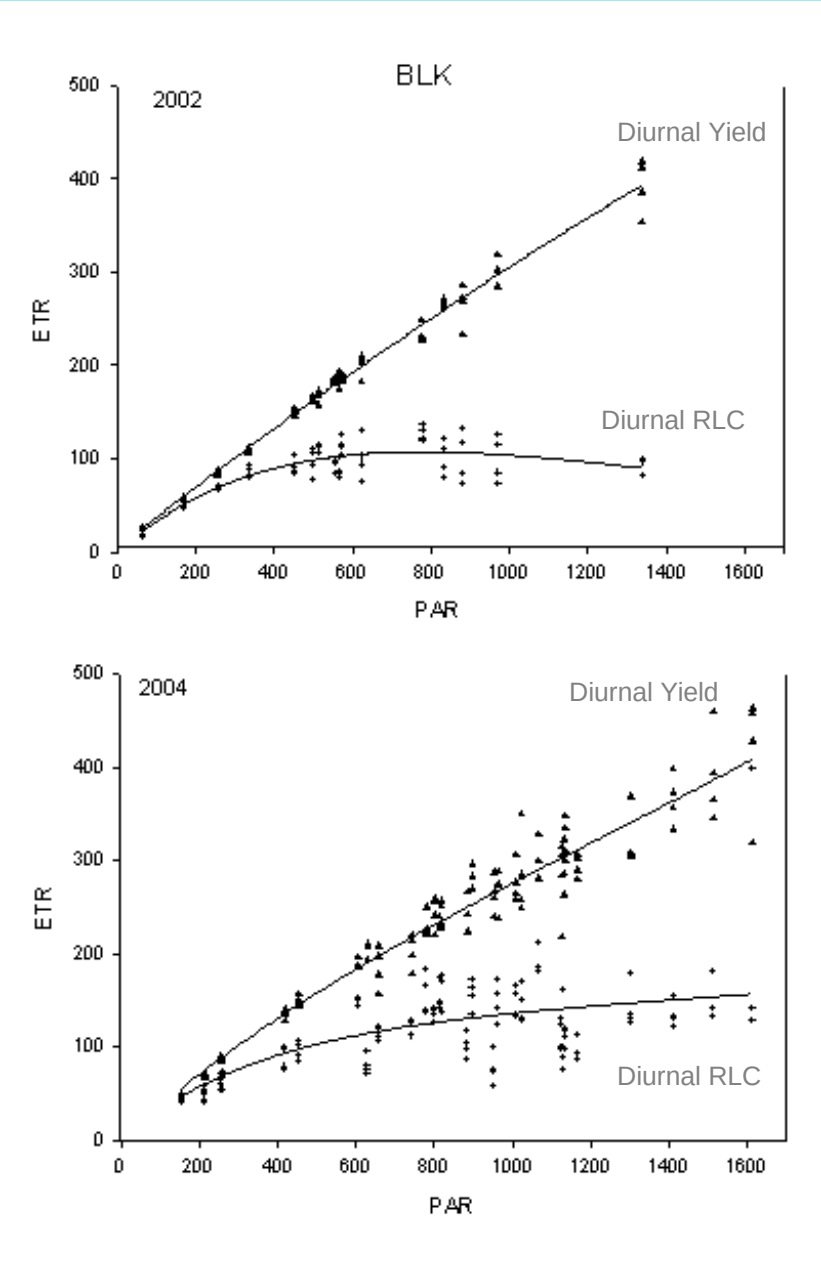
Does not reach asymptote – unrealistic $rETR_{max}$ and I_k

Diurnal RLC

Interpolate rETR for the ambient irradiance at the time of the RLC measurement using rETR vs. PAR values from the RLC

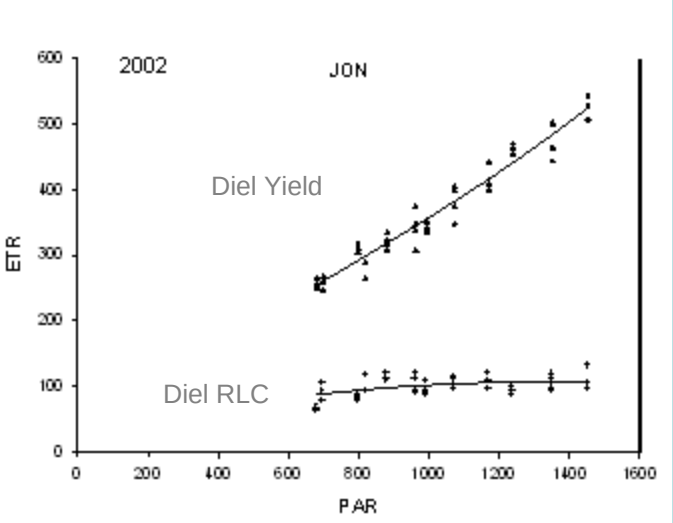
Does asymptote at higher PAR

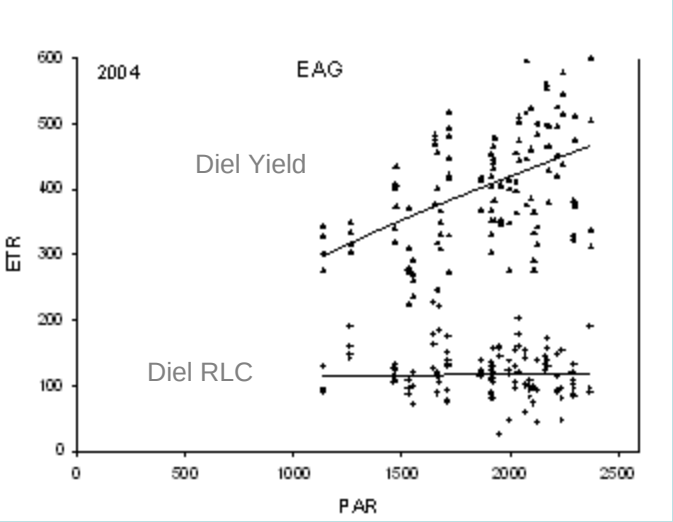
Belshe et al. 2008



However!

- Under high irradiance conditions (PAR$\geq I_k$) unable to predict initial slope (α)





Belshe et al. 2008

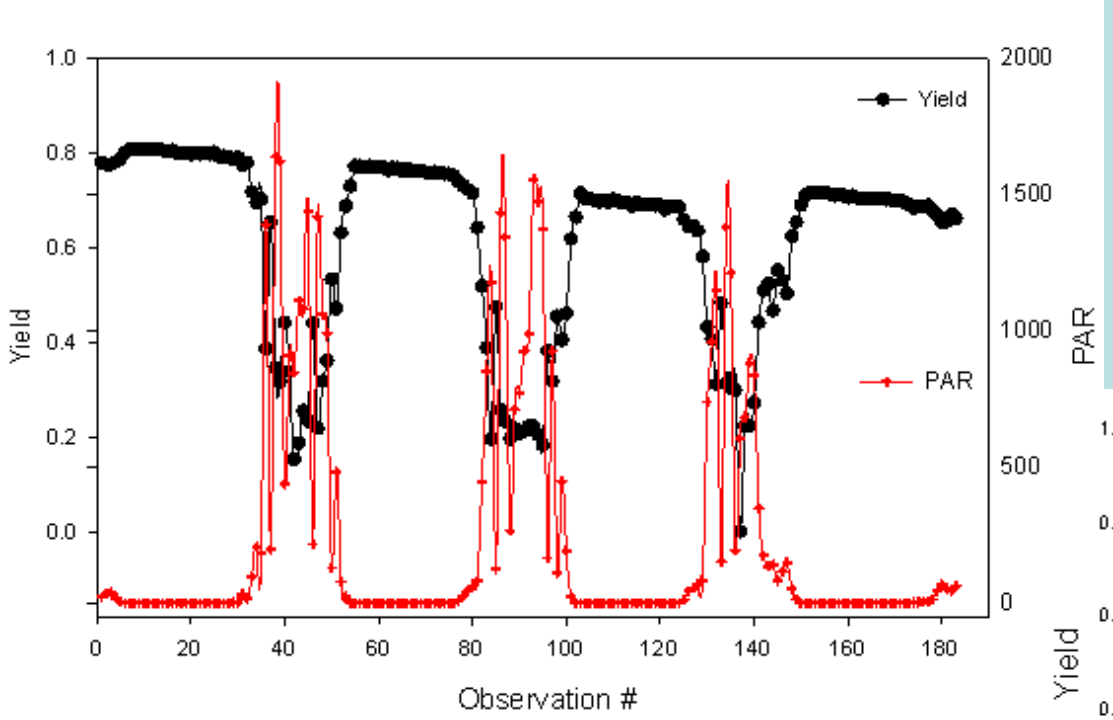
Terrible Table

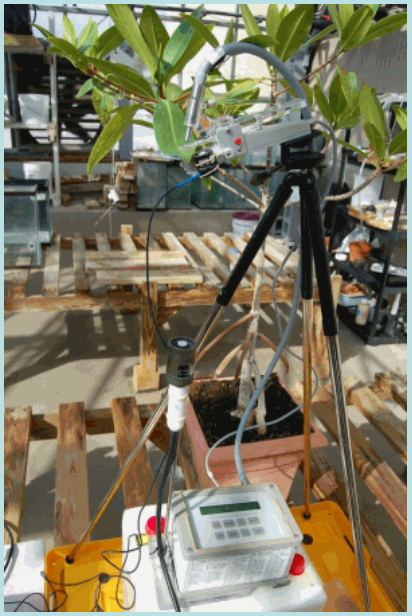
Table 1. ANCOVA results comparing 2006 to 2009 ΔF/Fm' versus PAR regression coefficients for *Thalassia testudinum* in Florida Bay.

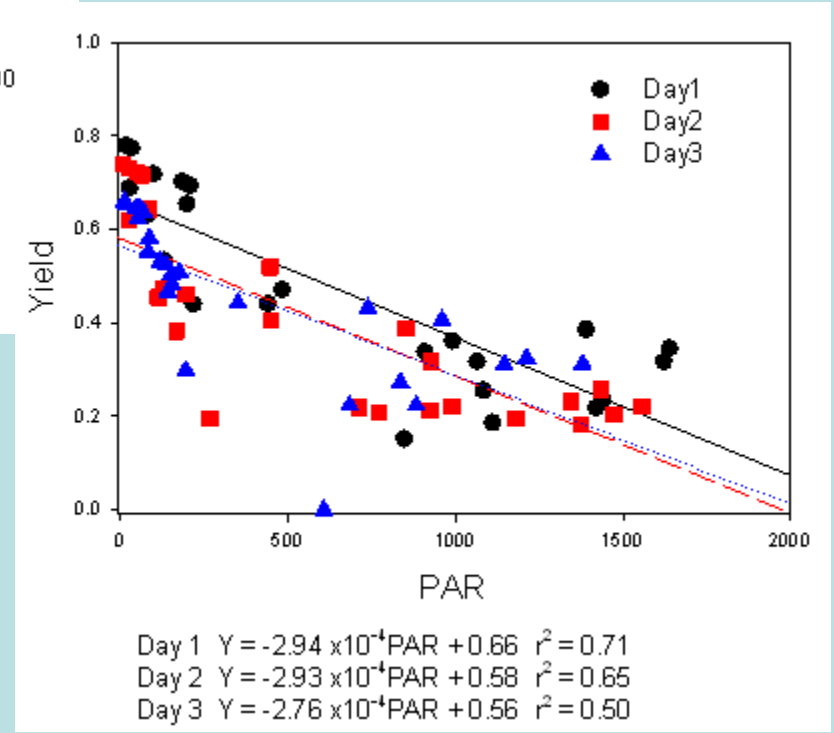
Basin	2006 vs 2007		2006 to 2008		2006 to 2009		2008 vs 2009	
	slopes[1]	intercepts[1]	slopes[1]	intercepts[1]	slopes[1]	intercepts[1]	slopes[1]	intercepts[1]
Barnes	n.s.	***	***	-	***	-	***	-
Blackwater	**	-	***	-	***	-	***	-
Calusa	n.s.	n.s.	**	-	**	-	n.s.	n.s.
Crane	**n.s.**	*****	**n.s.**	*****	**n.s.**	*****	**n.s.**	**n.s.**
Duck	n.s.	n.s.	n.s.	***	n.s.	***	n.s.	n.s.
Eagle	n.s.	***	*	-	***	-	n.s.	**
Johnson	n.d.[2]	n.d.[2]	n.s.[3]	n.s.[3]	n.s.[4]	n.s.[4]	n.s.	n.s.
Manatee	n.s.	n.s.	n.d.[5]	n.d.[5]	n.s.[6]	*[6]	n.d.[5]	n.d.[5]
Rabbit	n.s.	***	n.s	***	n.s.	***	n.s.	n.s.
Rankin	*****	**-**	*******	**-**	*****	**–**	**n.s.**	*******
Twin	***	-	***	-	***	–	n.s.	***
Whipray	**n.s.**	**n.s.**	**n.s.**	**n.s.**	*******	**-**	*******	**-**

[1]n.s.= not significantly different at P<0.05, significant at P<0.05, ** significant at P< 0.01, *** significant at P<0.001;

Does the y-intercept estimate F_v/F_m?

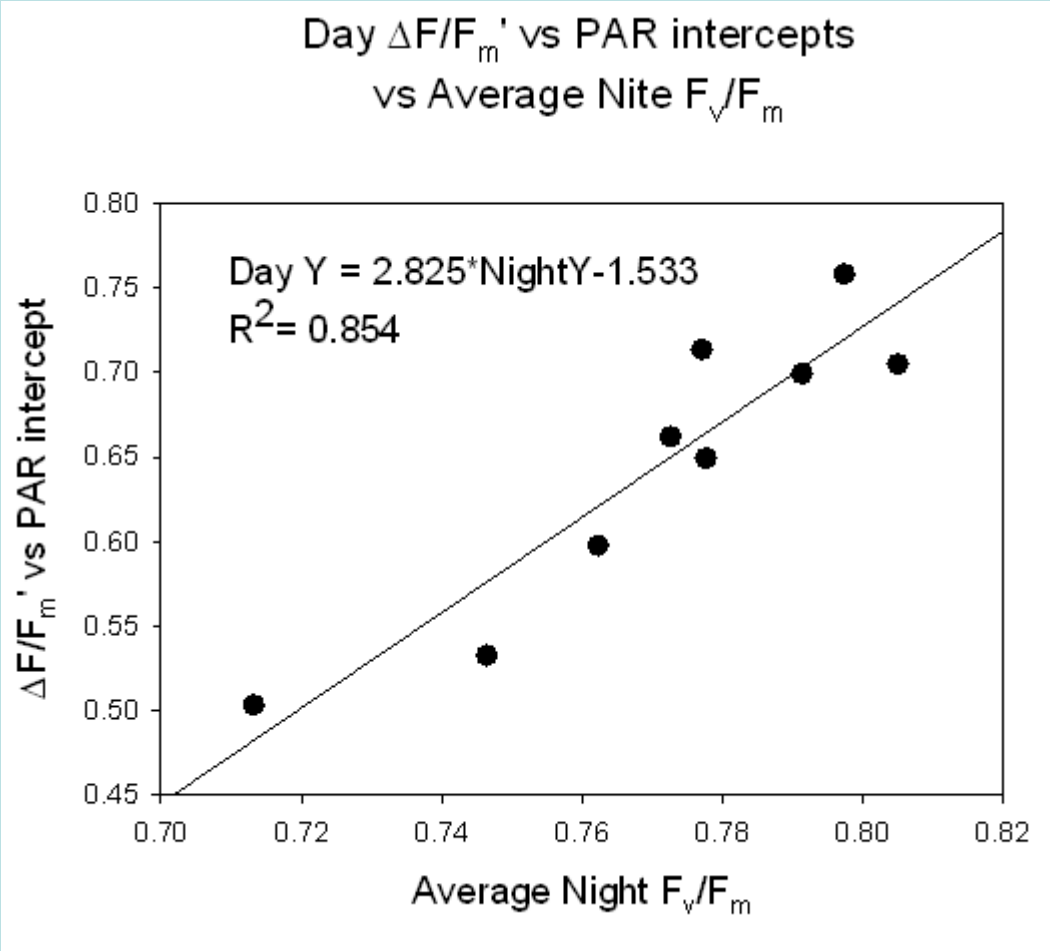




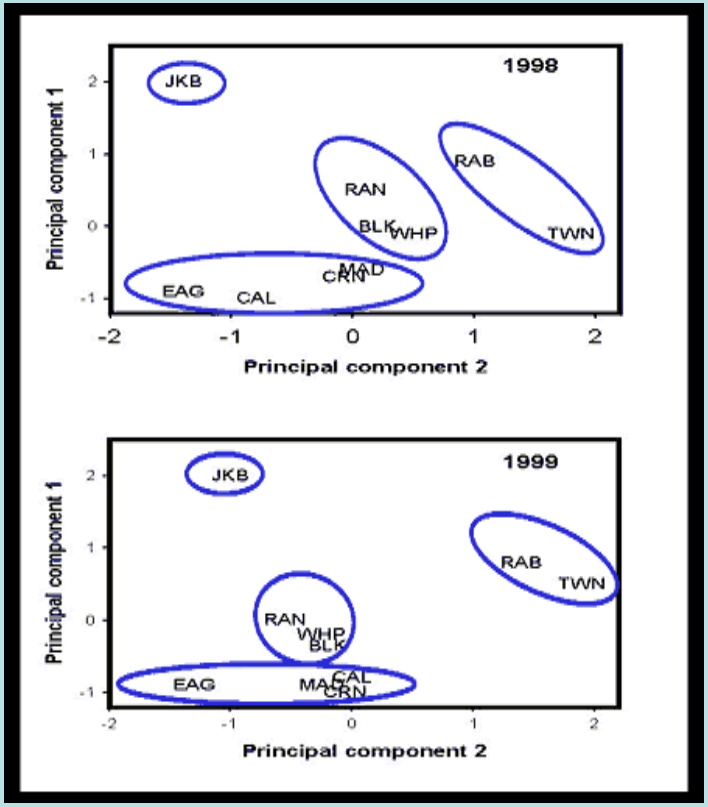


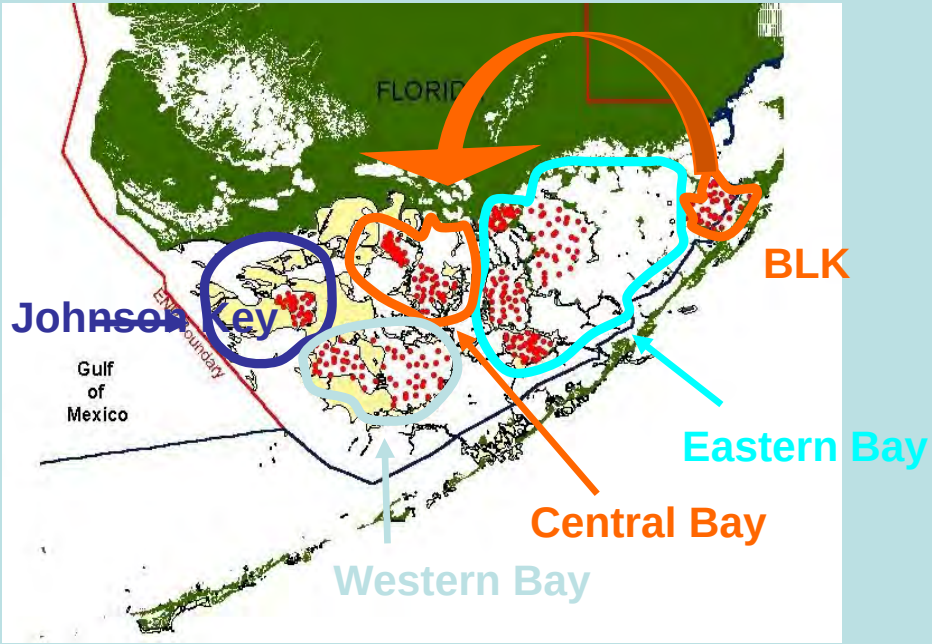
Airell Swanson
DIS

Seems to


Day ΔF/Fm' vs PAR intercepts
vs Average Nite Fv/Fm
Day Y = 2.825*NightY-1.533
R2= 0.854
ΔF/Fm' vs PAR intercept
Average Night Fv/Fm
0.80
0.75
0.70
0.65
0.60
0.55
0.50
0.45
0.70
0.72
0.74
0.76
0.78
0.80
0.82

FHAP Morphometrics

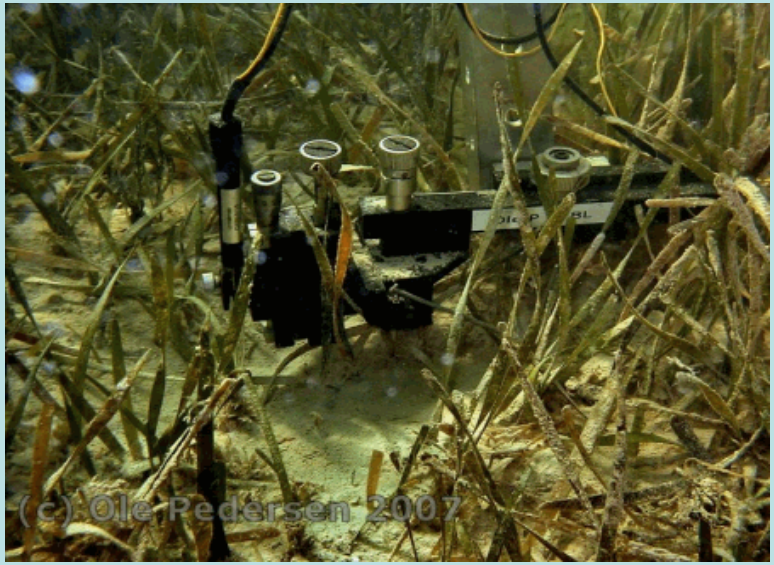


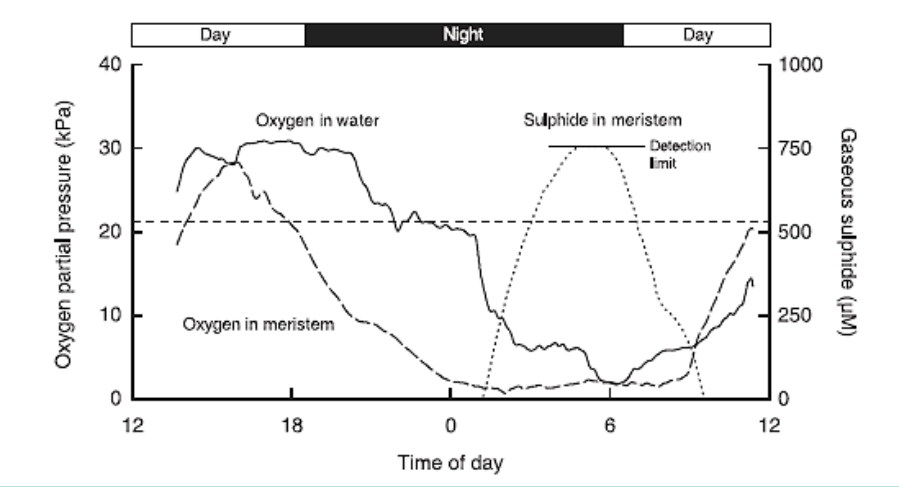


Ecological Zones based on *T. testudinum* morphometrics correspond to light availability (Phleps et al. 1995) and WQ zones (Boyer et al. 1999)

Hackney and Durako 2005, 2006

Oxygen & Sulfide





Borum et al. 2004