Effects of Ocean Acidification on Primary Producers

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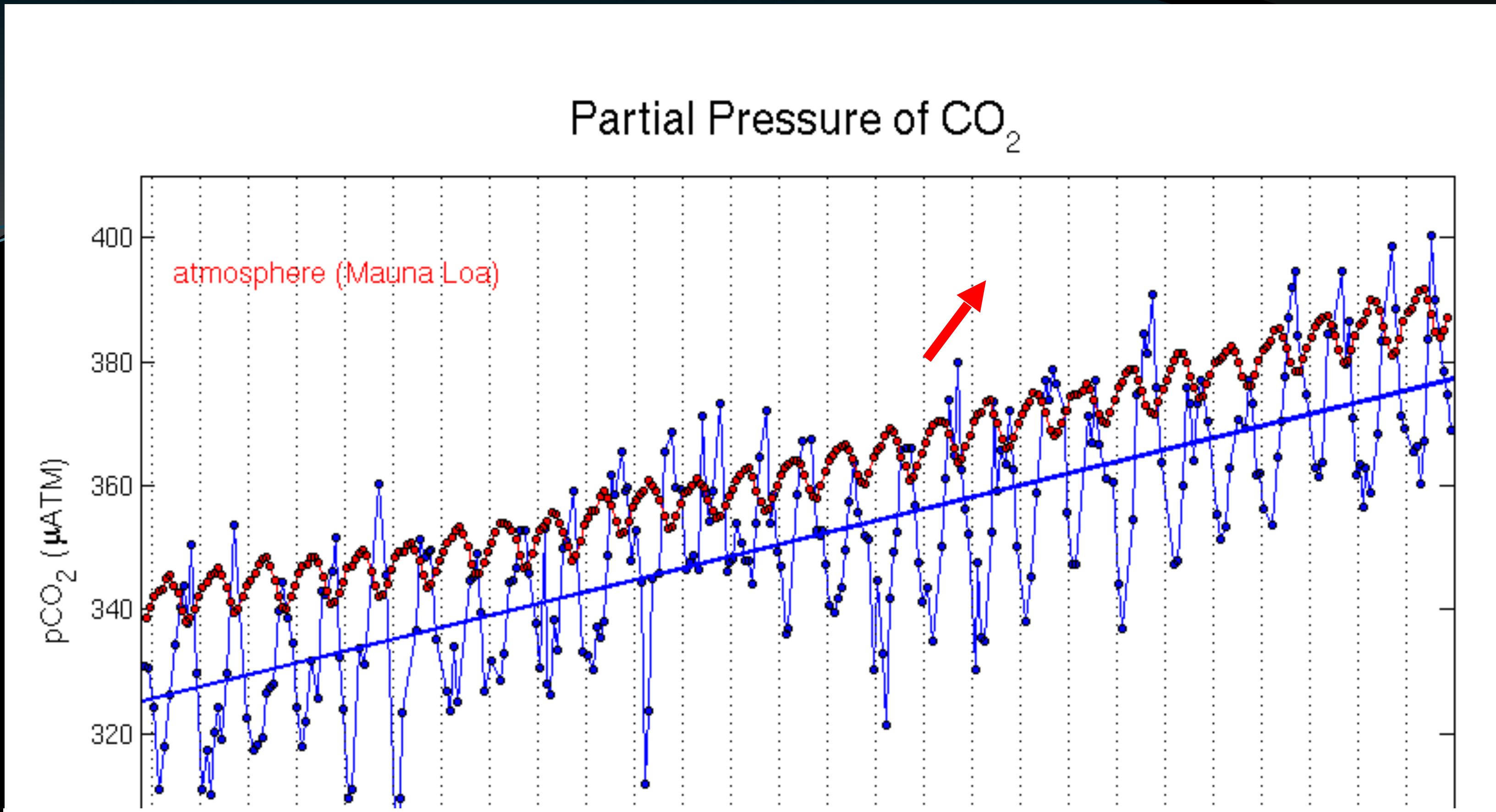
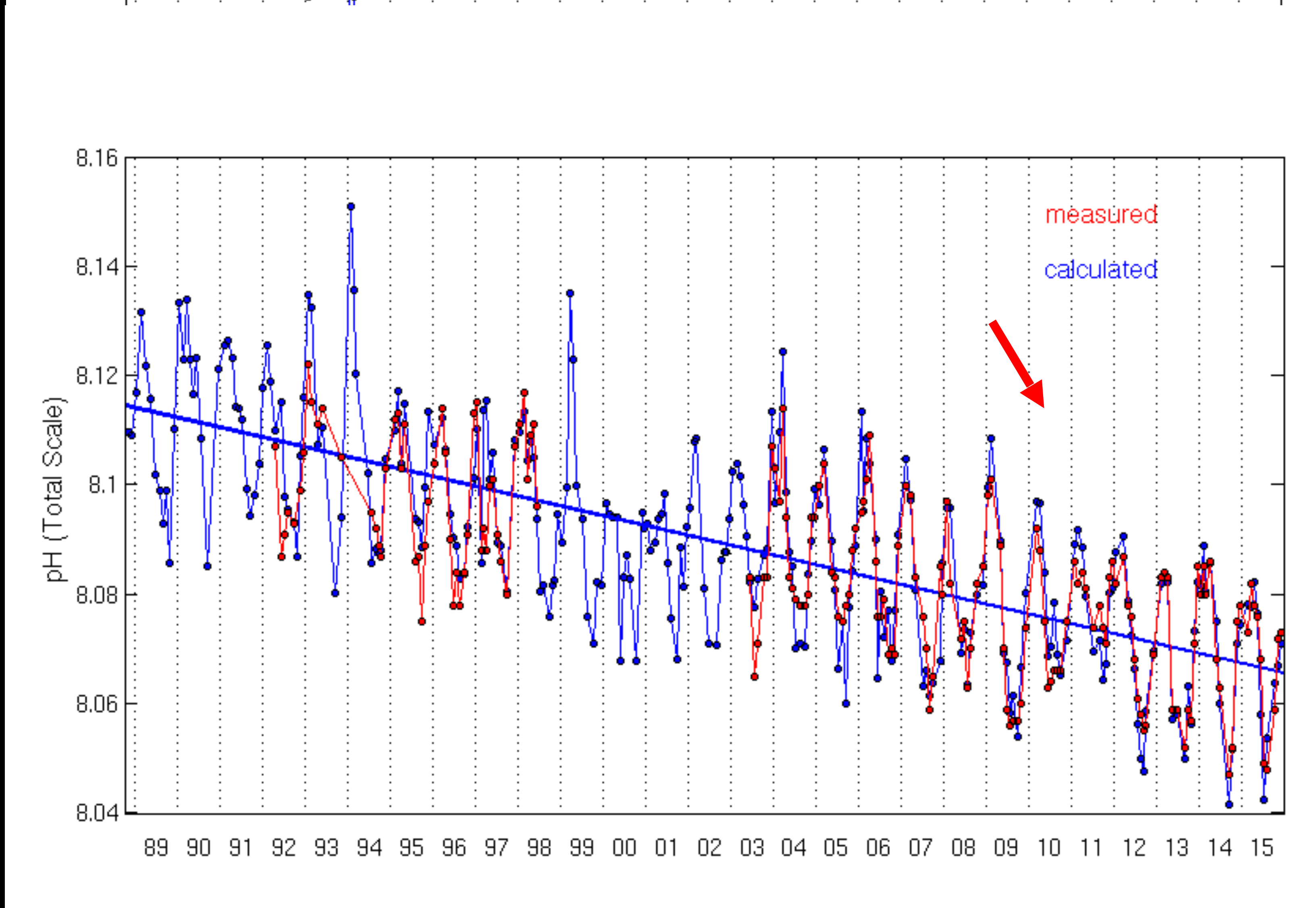
(Xiamen University)





CO_2 ↗

pH ↘

"Ocean Acidification"
***Nature* (2003)**

Hawaii Ocean Time Series
http://hahana.soest.hawaii.edu/hot/

Ocean acidification alters carbonate chemistry

CO_2 CO_2 CO_2

$$CO_2\,(g) \rightarrow CO_2\,(aq)$$

$$CO_2\,(aq) + H_2O \rightarrow H_2CO_3$$

$$H_2CO_3 \rightarrow HCO_3^- + H^+$$

$$CO_3^{2-} + H^+ \rightarrow HCO_3^-$$

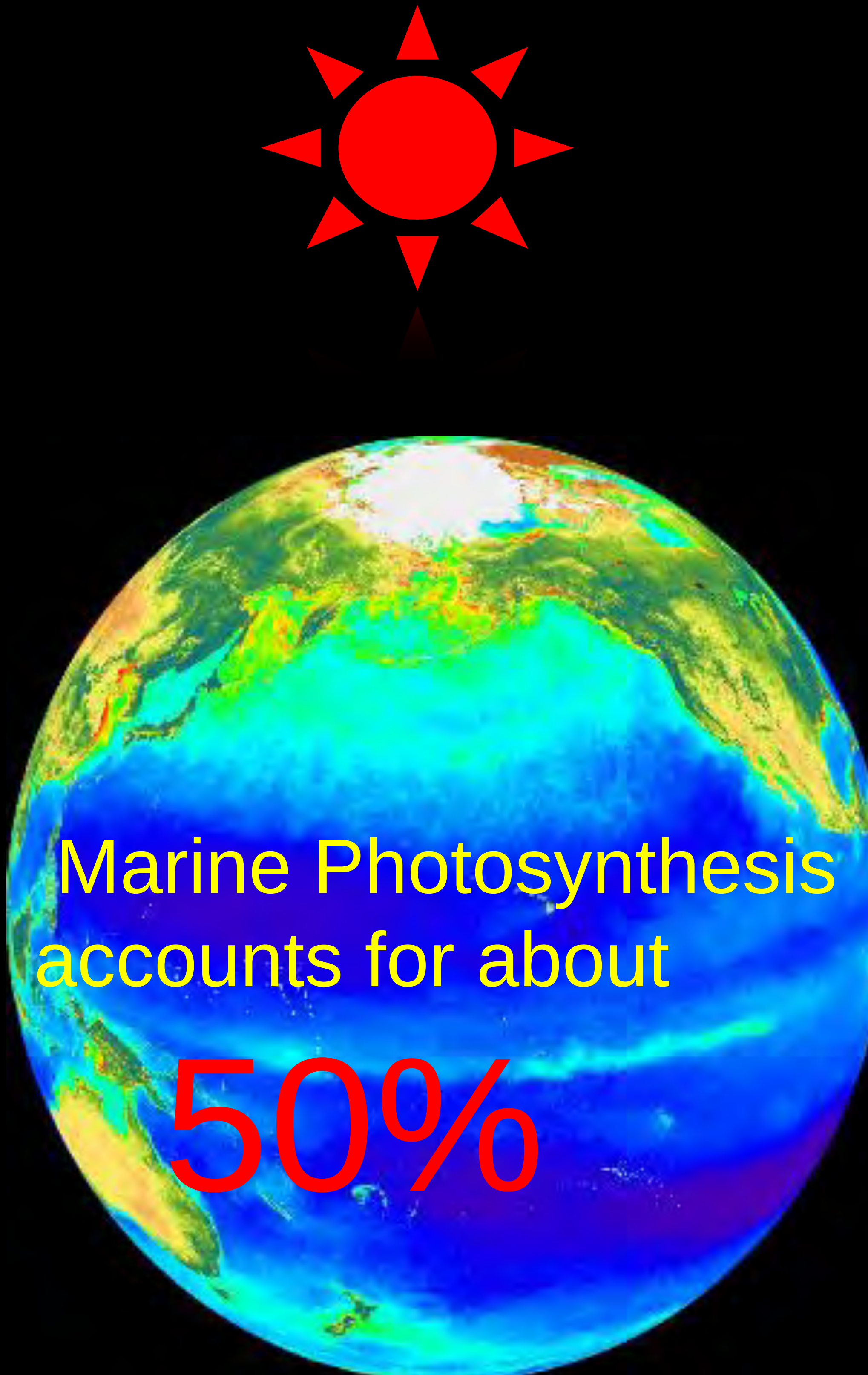
H^+ 150% (↑)

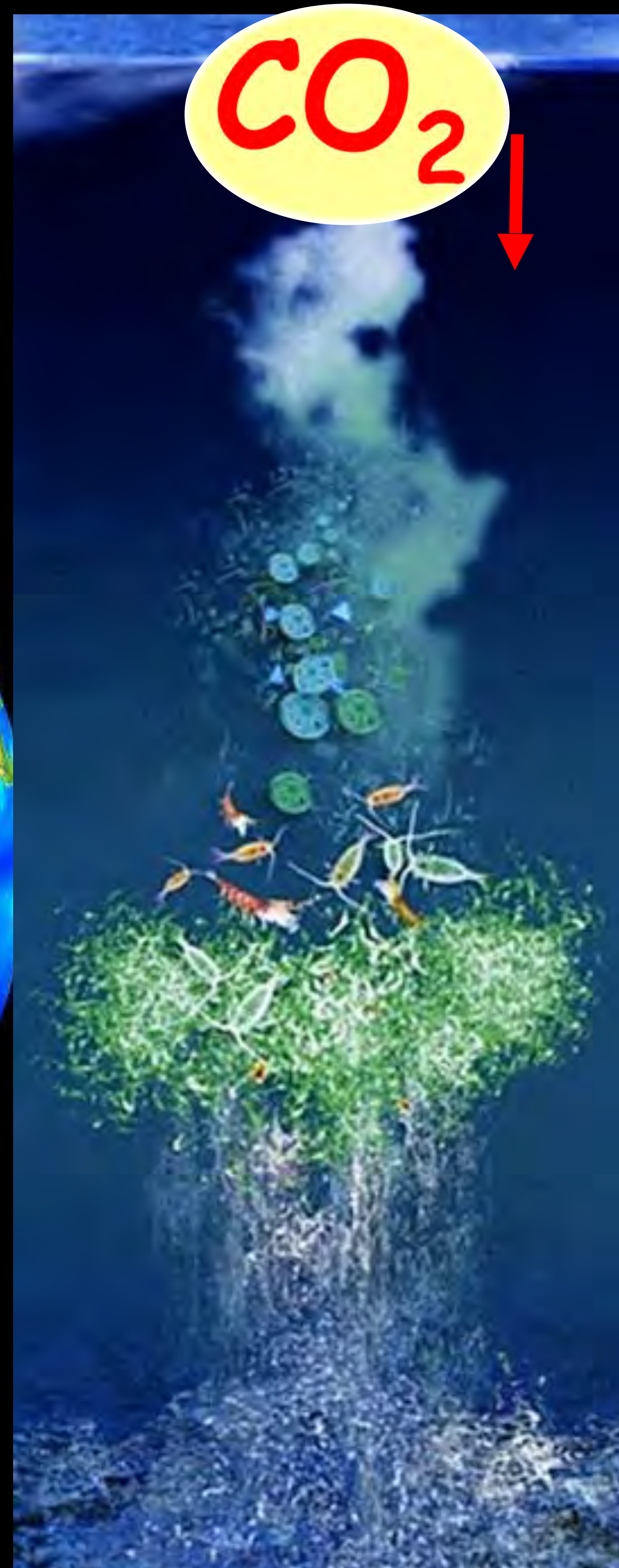
0.4, 40%, 10%

pH ↓ CO_3^{2-} ↓ HCO_3^- ↑

$\Omega = [CO_3^{2-}]_{MEAS} / [CO_3^{2-}]_{CAL}$ ↓ **40%**

Gattuso et al. 2015 *Science*





Marine Photosynthesis drives oceanic biological CO2 pump that takes up (per hr) over 100 million tons of fossil fuel CO_2

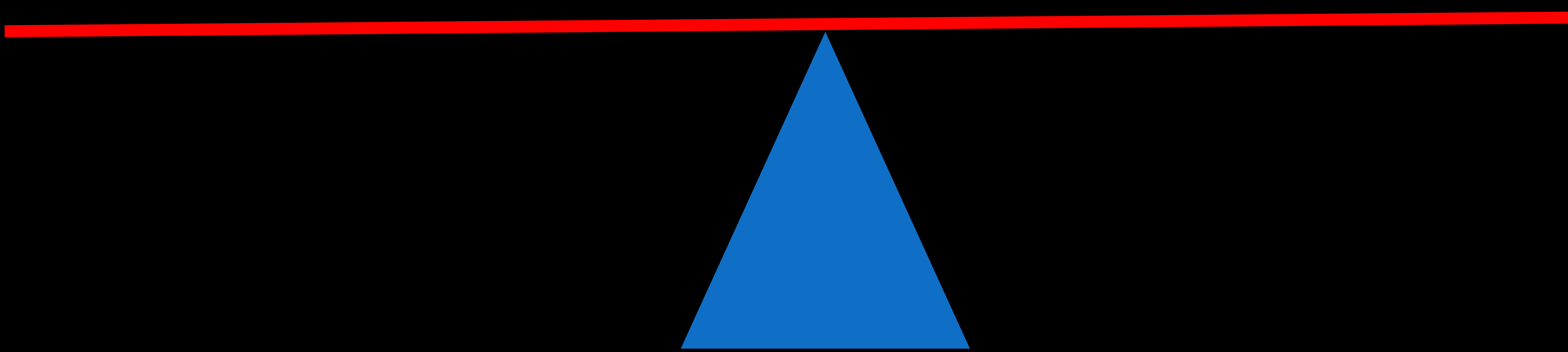
Gattuso JP et al. 2015. *Science*

About 1272 papers on responses of marine photosynthetic organisms to OA till Jul. 1, 2018 (OA-ICC bibliographic database)

Nature 1997,
Science 2008
PNAS 2016…….

Nature 2000, 2011;
Nat Clim Change 2012
Science 2017……..

- Stimulating Neutral Inhibitive



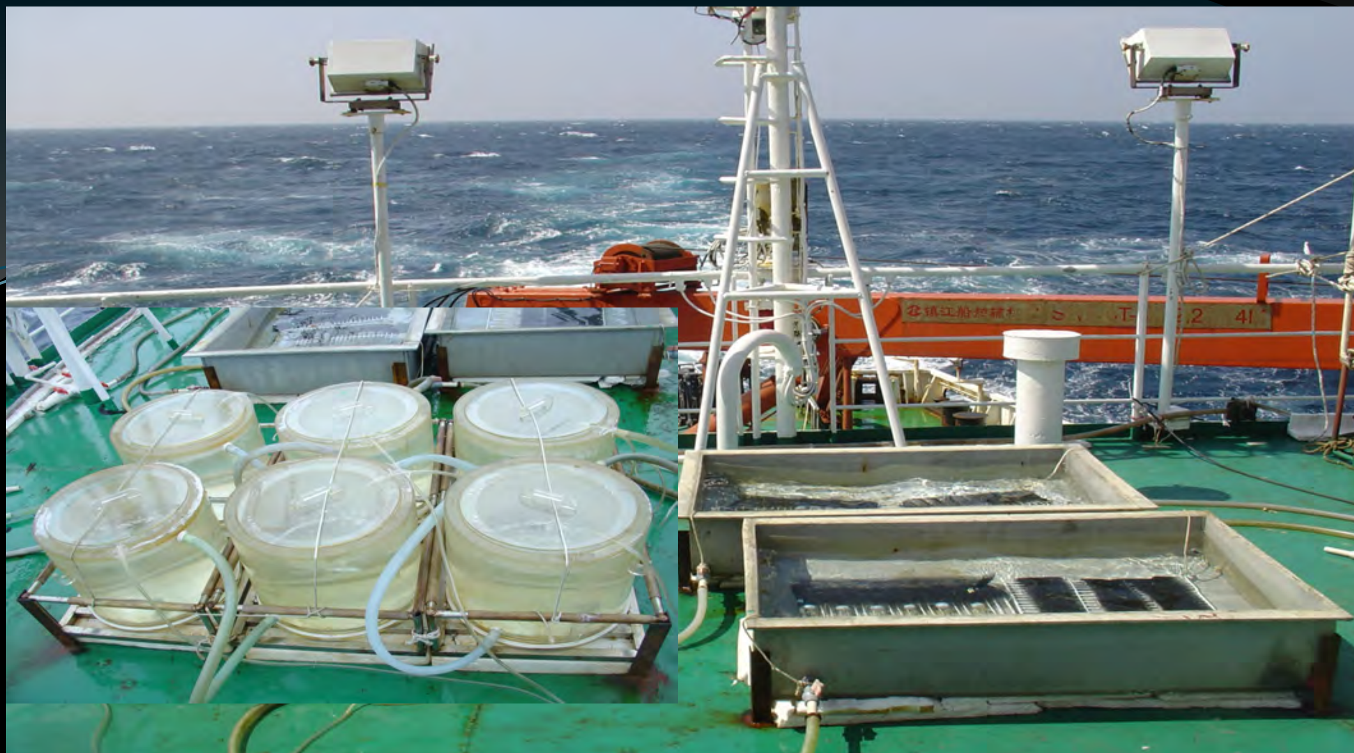
Growth/Photosynthesis/Respiration/Calcification/N_2 fixation pH

CO_2

$\Omega = [CO_3^{2-}]_{MEAS} / [CO_3^{2-}]_{CAL}$

Effects of ocean acidification?

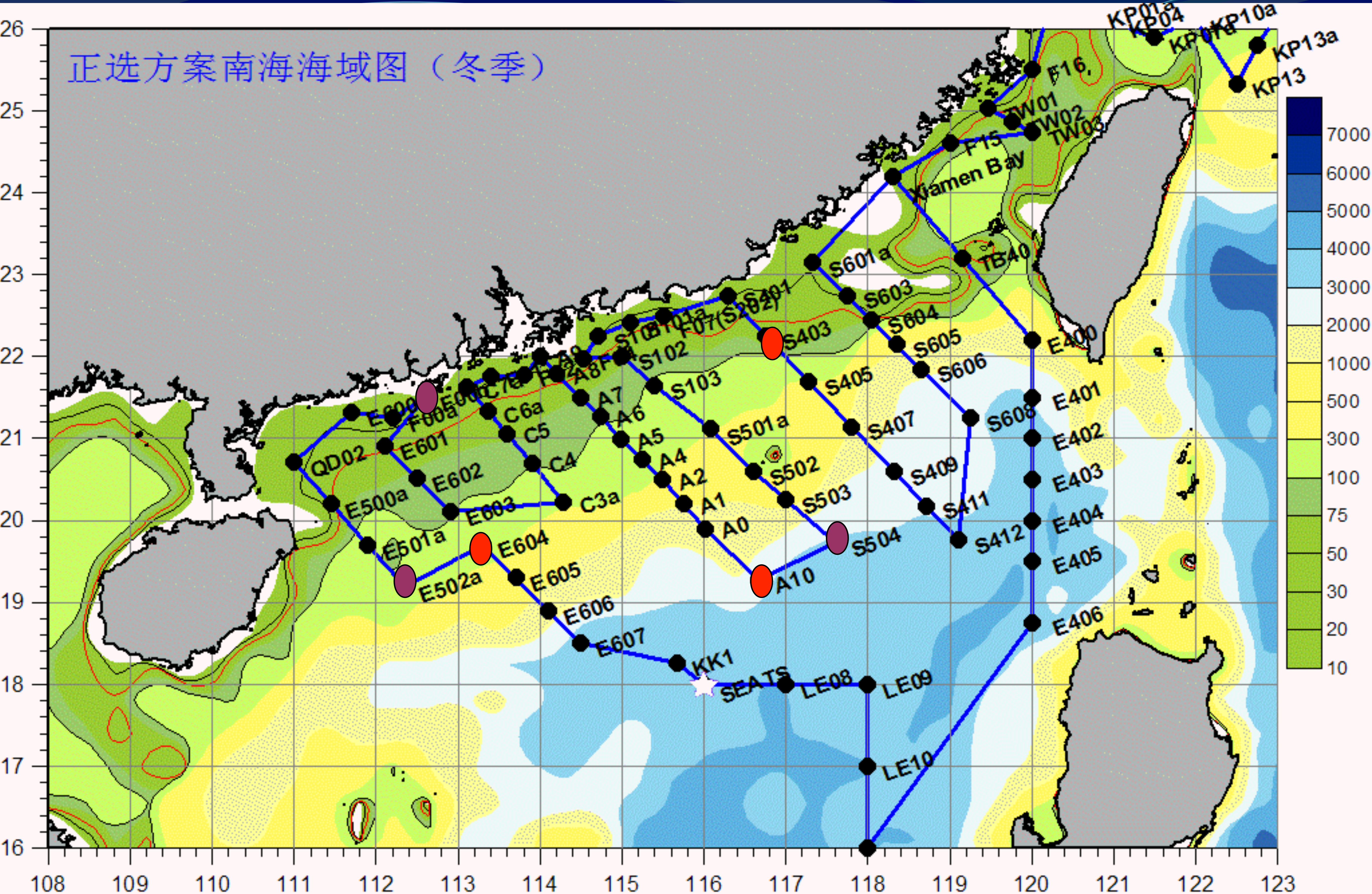
CO2 rise and acidic stress: double edged?



FOCE: Free Ocean CO2 Enrichment Exp.

Responses

1. Photosynthesis / Growth
2. Metabolic Pathways
3. Calcification （calcifying algae)
4. Combined impacts with other stressors



正选方案南海海域图（冬季）
KP01a
KP04
KP07a
KP10a
KP13a
KP13
F16
TW01
TW02
TW03
F15
Xiamen Bay
TB40
S601a
S603
S604
S605
S606
S608
E400
S401
F07(S202)
S403
S405
S407
S409
S411
S412
S101
S102
S103
S501a
S502
S503
S504
A10
A8
A7
A6
A5
A4
A2
A1
A0
C7a
C6a
C5
C4
C3a
E600
F00a
E601
E602
E603
E604
E605
E606
E607
QD02
E500a
E501a
E502a
KK1
SEATS
LE08
LE09
LE10
E401
E402
E403
E404
E405
E406
7000
6000
5000
4000
3000
2000
1000
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300
100
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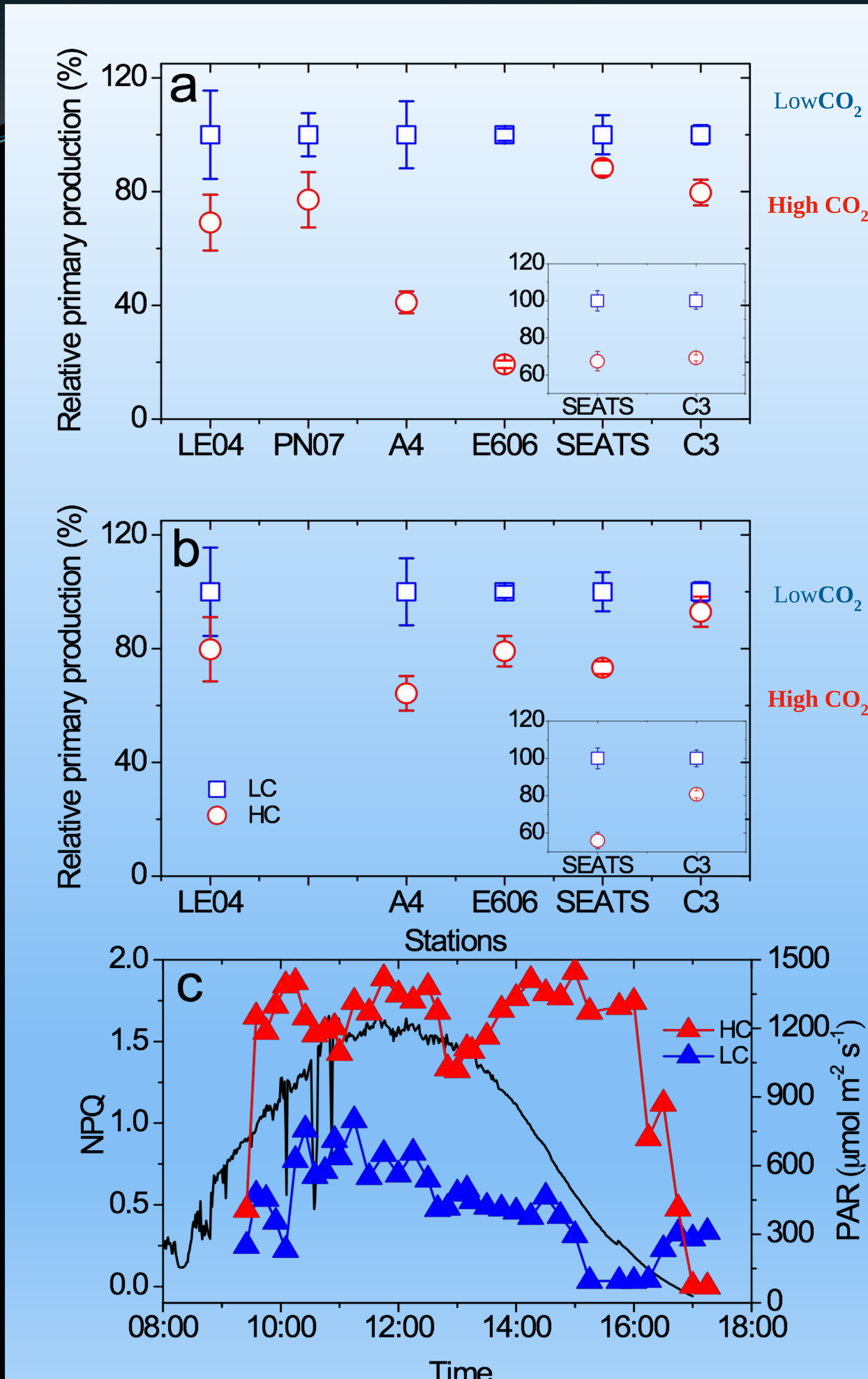
Supplementary Table 2. Locations of the stations, cruise information, sea surface temperature (SST, °C) and pH_T, $NO_3^- + NO_2$ (N, µmol L^{-1}) and PO_4^{3-} (P, µmol L^{-1}), solar PAR (mean, µmol photons m^{-2} s^{-1}) during ^{14}C-traced incubations, incubation time (h), surface seawater chlorophyll a concentration (Chl a, µg L^{-1}), chlorophyll a concentration (µg L^{-1}) of phytoplankton assemblages grown for 6-7 days under low CO_2 (LC,385 µatm) and high CO_2 (HC, 800 µatm for all stations except SEATS and C3, where 1000 µatm CO_2 was applied), and the primary productivity (PP, triplicate incubations, µg C $L^{-1}h^{-1}$) by the phytoplankton assemblages grown in the low CO_2 microcosms at the end (day 7) of the growth-out in the microcosms. BLQ stands for "below the limit of quantification". The concentrations of the nutrients were determined by the chemistry group of Xiamen Univ. during the cruises. Chlorophyll a concentration in the microcosms at station PN07 was not measured (nd).

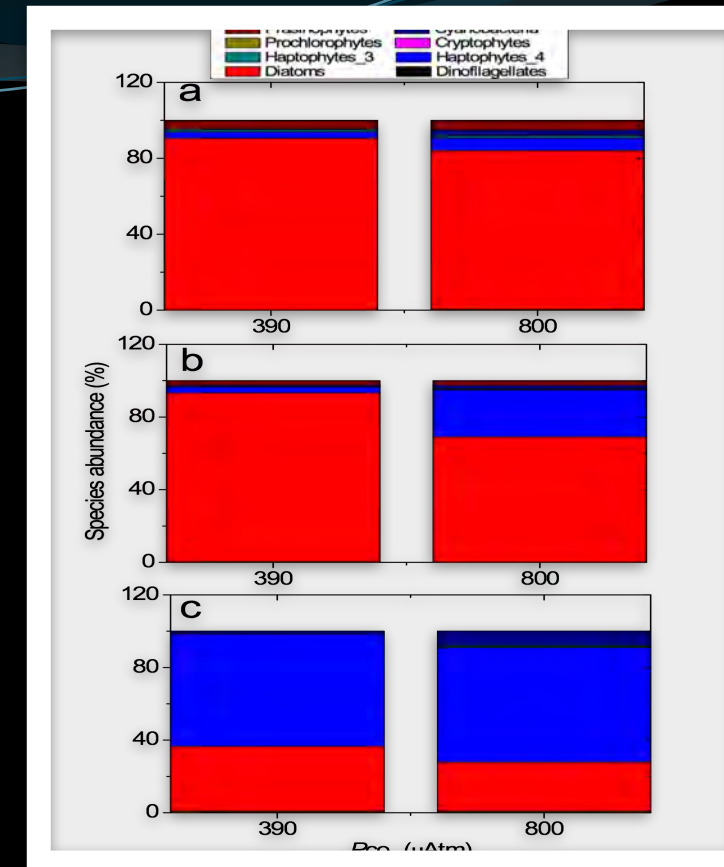
Station	Location	Season*	SST	pH_T	N	P	Solar PAR	Incubation time (h)	Chl *a*	Chl *a* (LC)	Chl *a* (HC)	PP
LE04	(18.0°N, 113.0°E)	Summer	29.5	8.03	BLQ	0.014	1681	6	0.05	0.15	0.13	0.10±0.08
PN07	(30.0°N, 124.5°E)	Summer	29.6	8.03	BLQ	0.019	1371	6	0.71	Nd	nd	0.18±0.12
A4	(20.8°N, 115.2°E)	Autumn	25.5	8.04	BLQ	0.156	794	6	0.44	1.08	0.69	2.73±0.32
E606	(18.9°N, 114.1°E)	Autumn	25.3	8.06	BLQ	BLQ	821	6	0.34	0.82	0.20	4.74±0.10
SEATS	(18.0°N, 116.0°E)	Spring	28.7	8.04	BLQ	0.037	1251	12 (24)	0.10	0.49	0.59	2.08±0.14 (19.80±1.09)**
C3	(20.6°N, 114.2°E)	Spring	28.5	8.03	BLQ	0.032	1027	12 (24)	0.21	0.42	0.36	1.83±0.06 (16.28±0.73)**

Gao et al. 2012 *Nature Climate Change (Supplementary data)*



Per volume of seawater

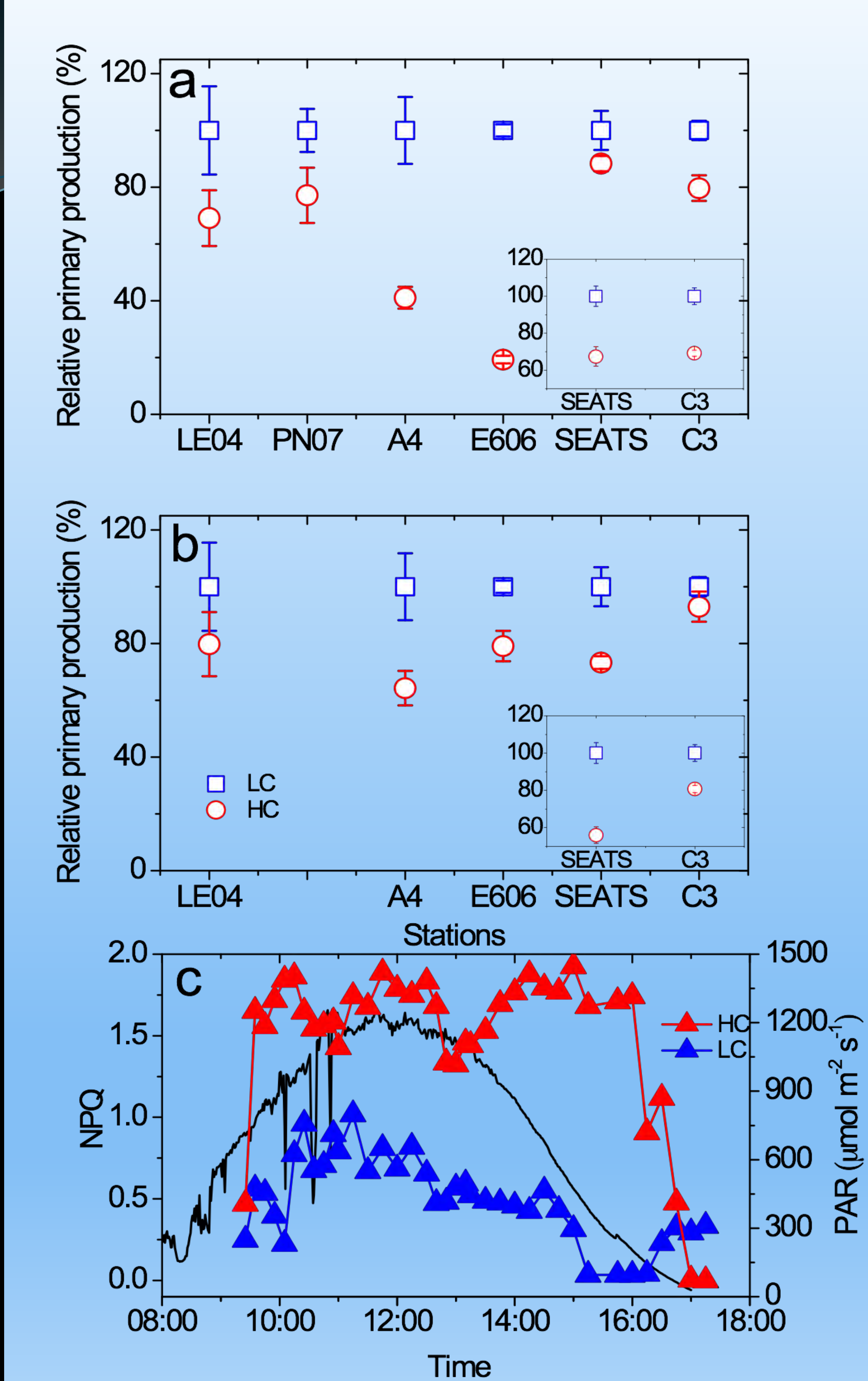
Per Chl. a



Diatom abundance

NPQ
High light-stress

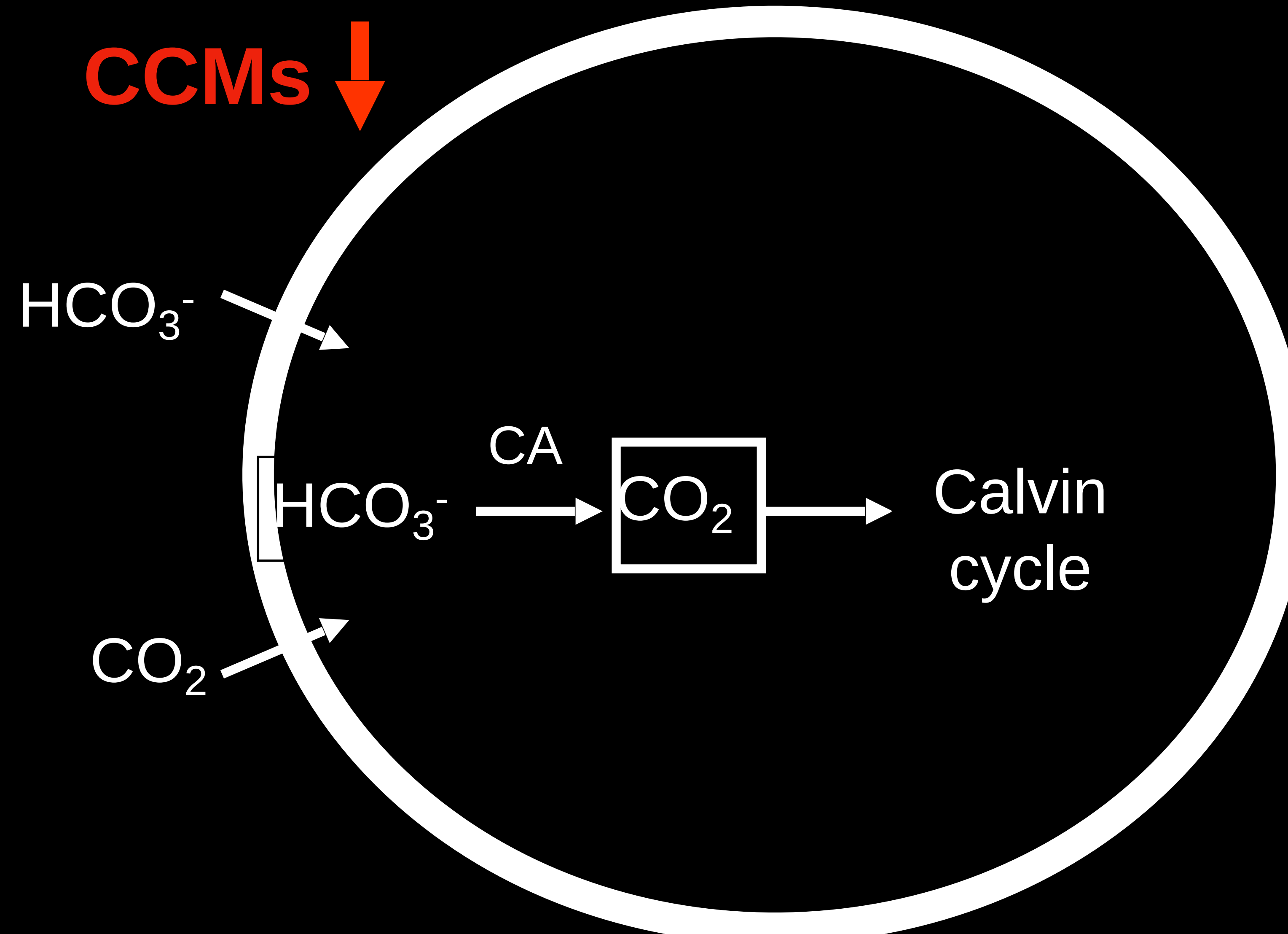
Gao et al. 2012 ***Nature Climate Change***



Gao et al. 2012 *Nature Climate Change*

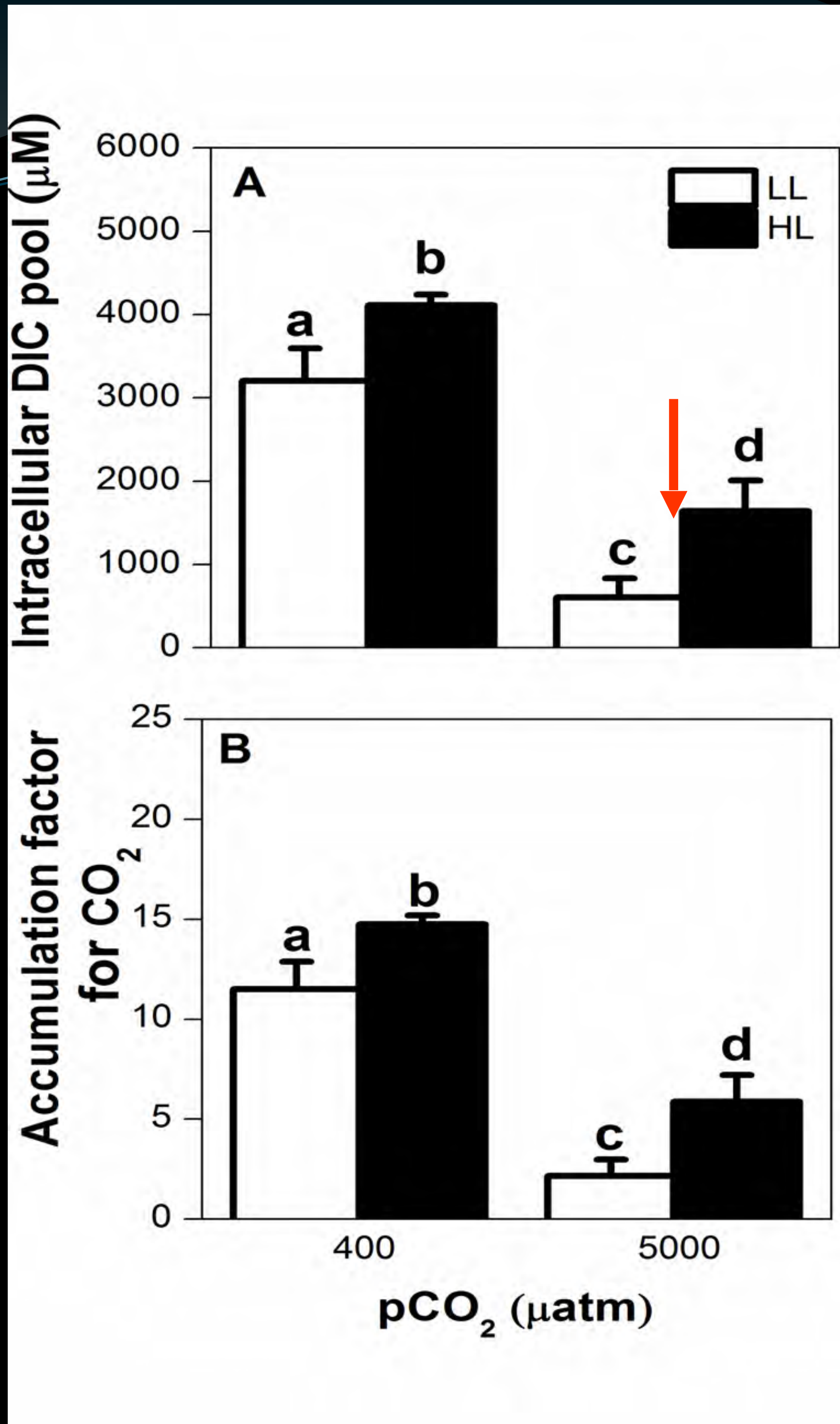
intracellular dissolved inorganic carbon concentration up to 1000 times that of milieu

CCMs ↓



Acidic stress + photorespiration ↗

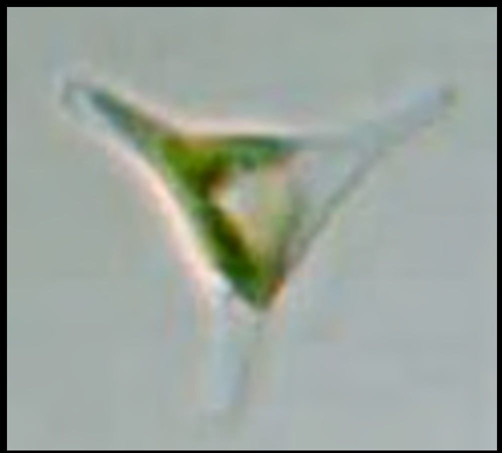
Primary production ↘



High-CO2 grown

Phaedactylum constatum

Lower (3-4 times) intracellular DIC



Liu et al. 2017 *Aquatic Microbial Ecology*

Carboxylation
Oxygenation

Rubisco →

Intracellular CO_2/O_2 ratio ↘

Oxygenation → Photore

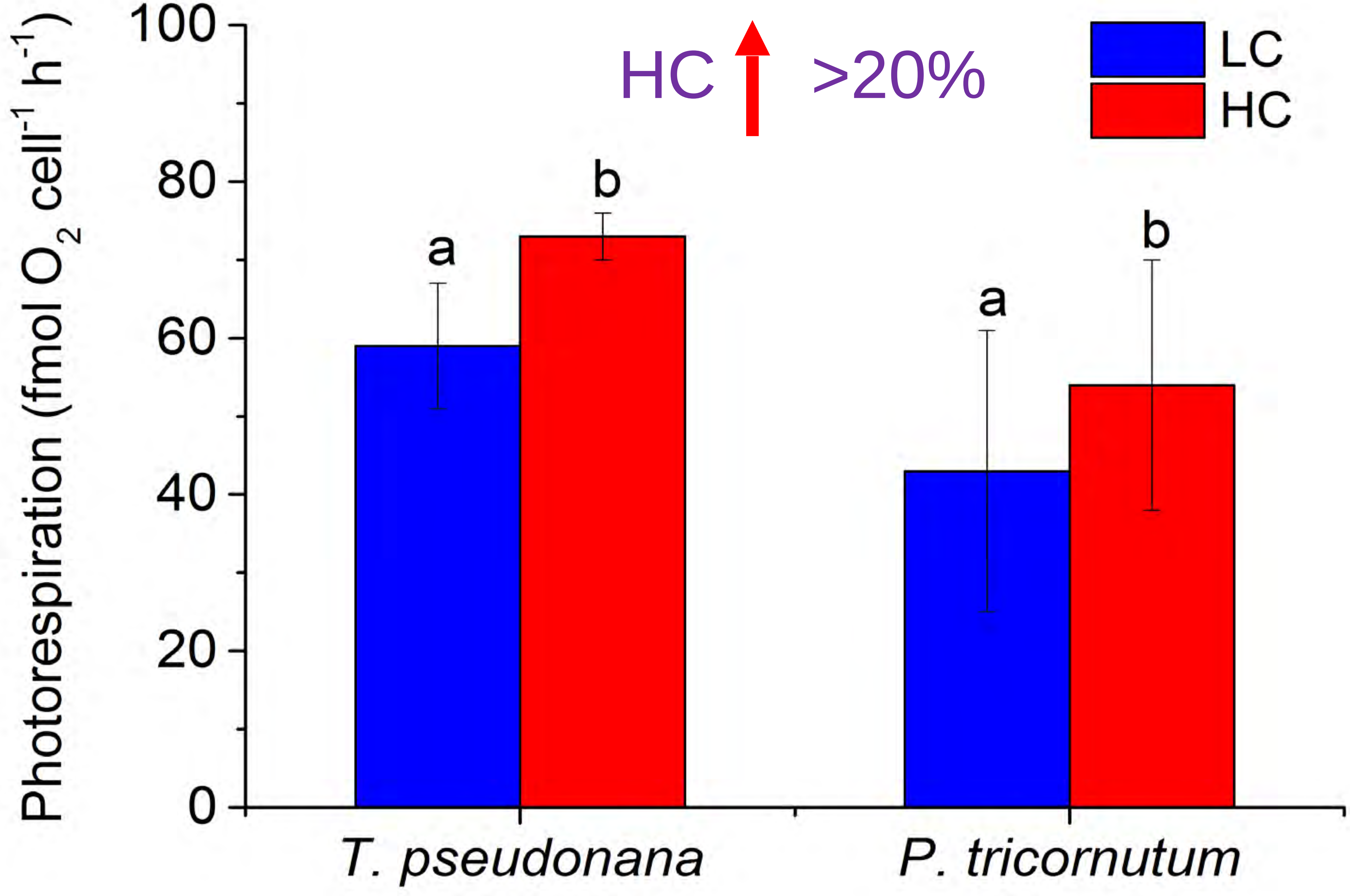
因此， 光呼吸的重要功能之一即为在光合CO2 缺少时保持卡 尔文循环的运转

Photorespiration alleviates

植物在强光下，“光反应”使叶绿体内积累大量的
ATP 和 NADPH + H +， 如果 CO2 供应不足或植物处于逆

costs extra energy, releas

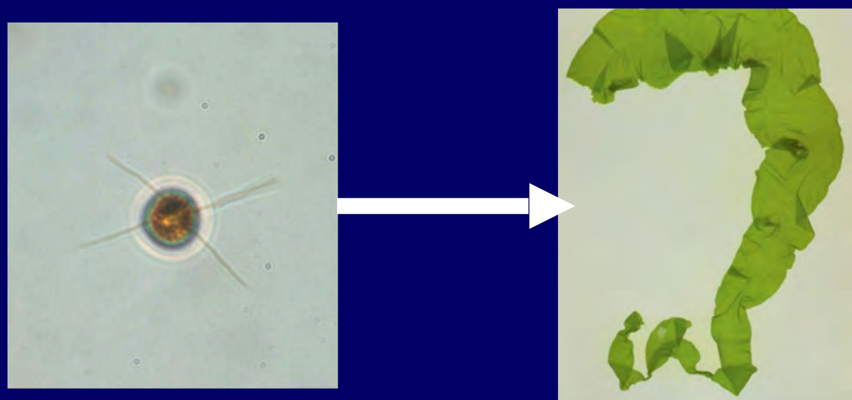
Photorespiration

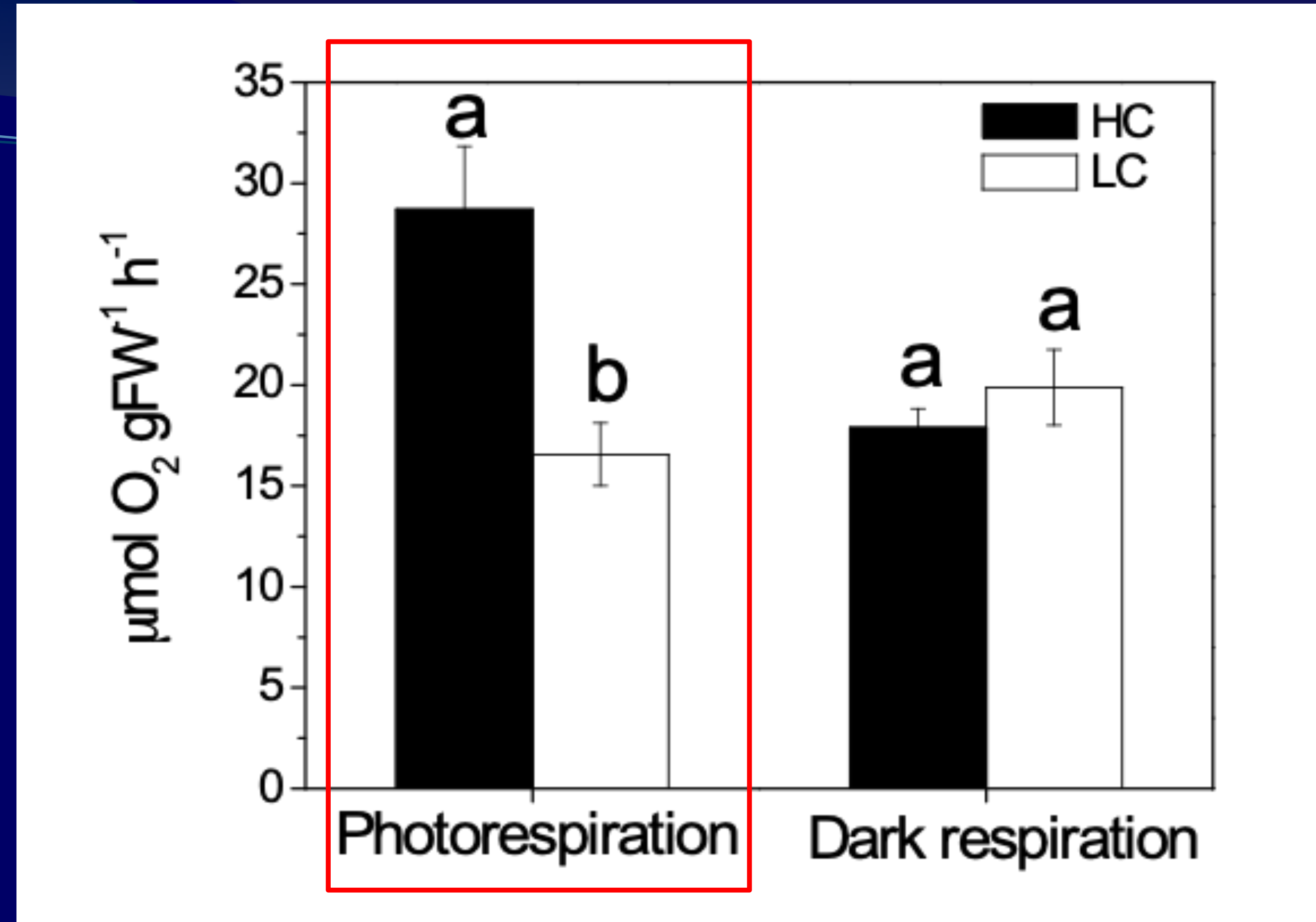


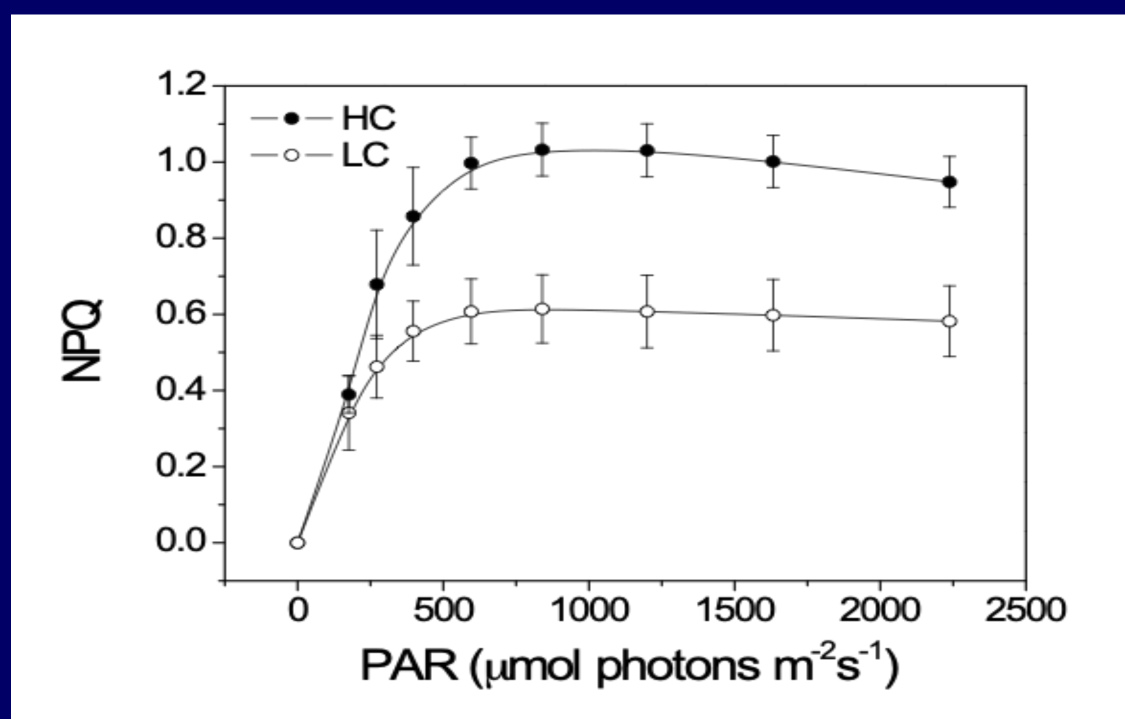
Gao et al. 2012 *Nature Climate Change*

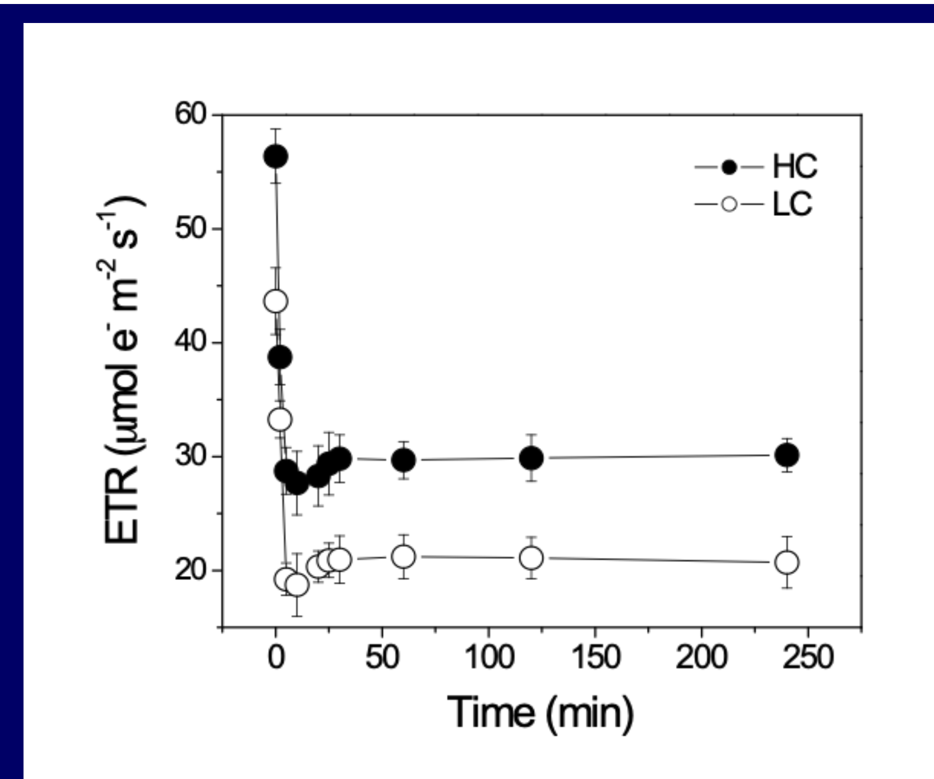
CO_2

HC 1000
LC 390 µatm





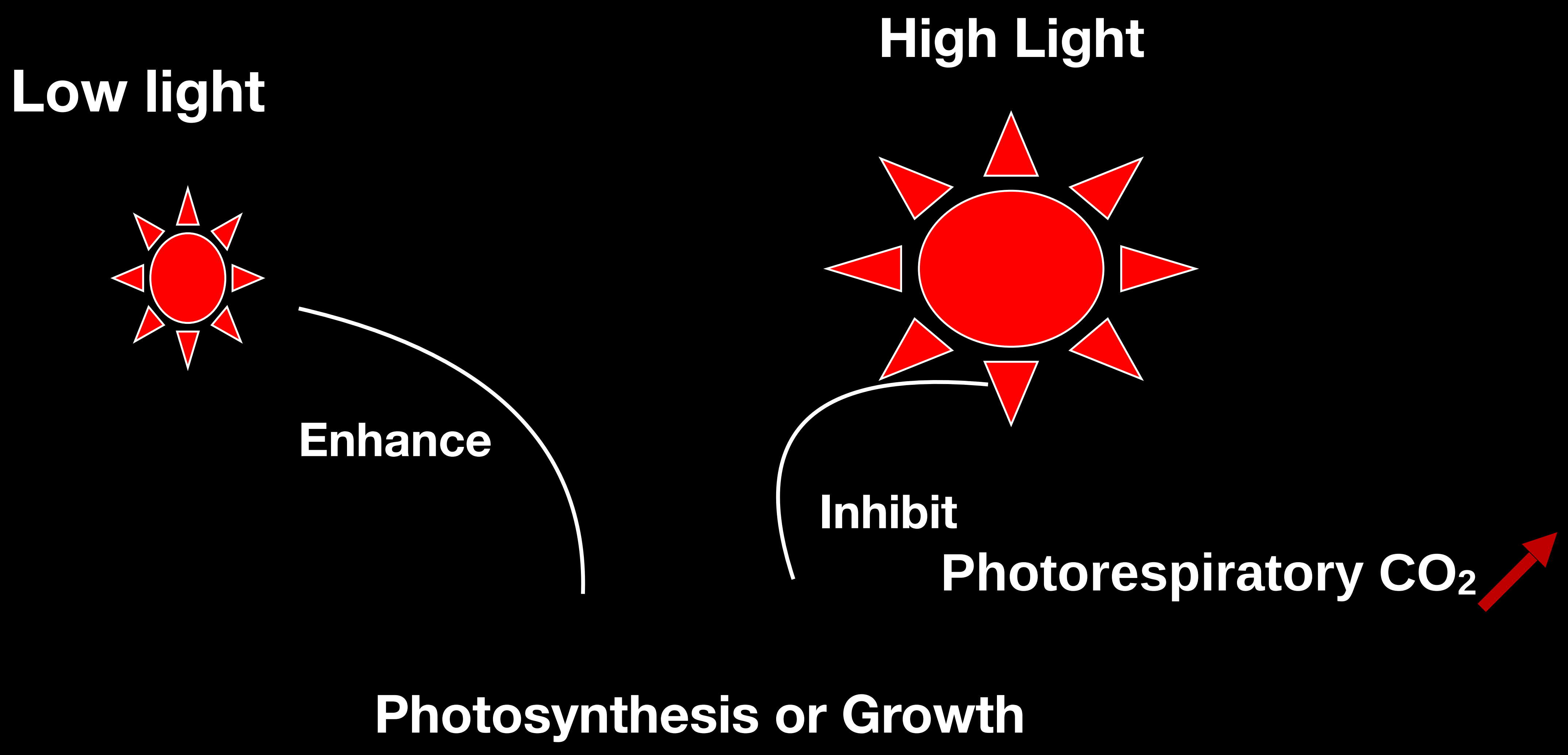




Xu and Gao 2012 Plant Physiol.

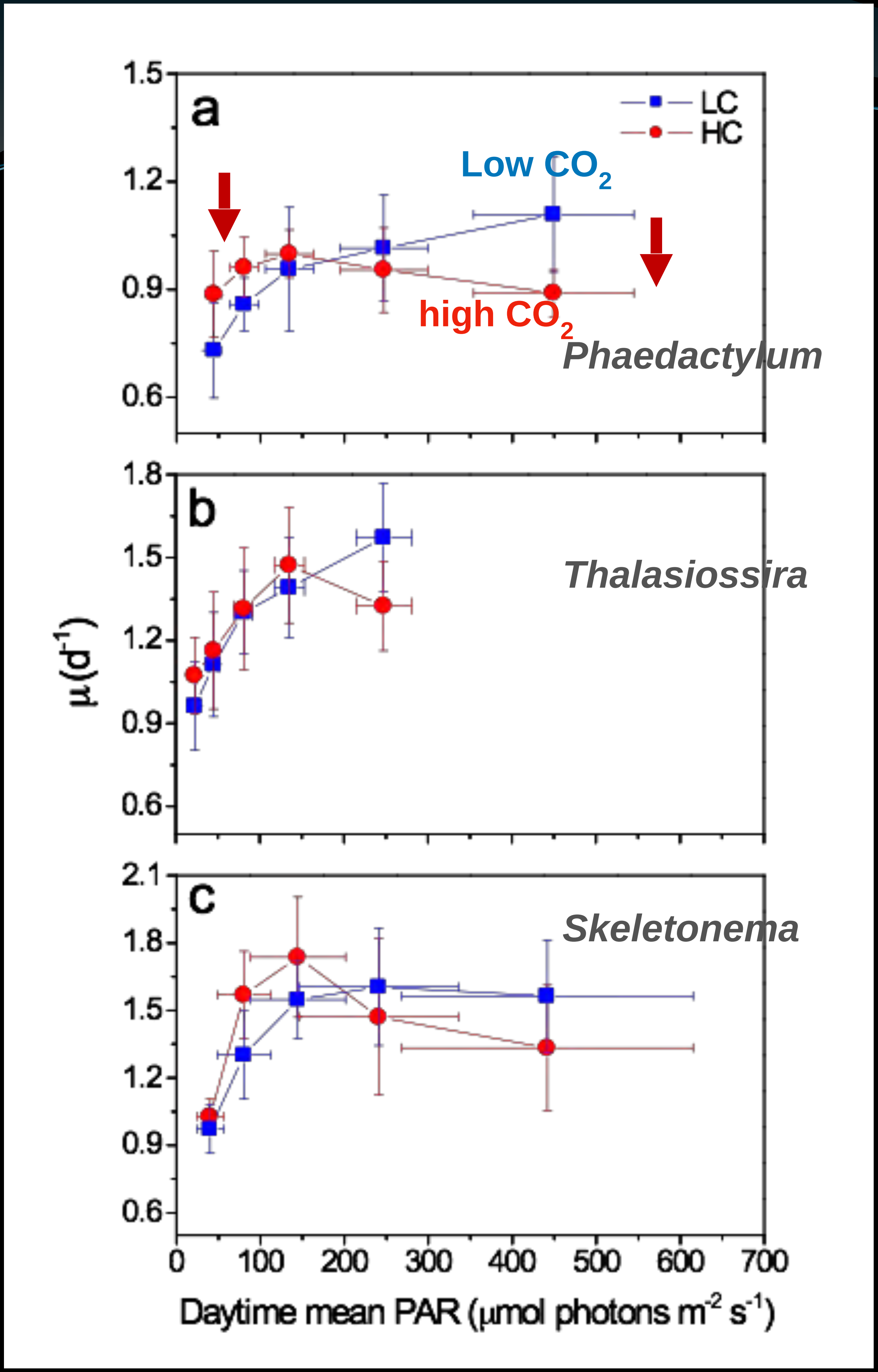
Energetic costs：CCMs↓，acidic stress/photo-stress↑

CO2-fertilization：CO_2、HCO_3^-↑



Gao KS 2017 *Bioenergetics*

Diatoms



Growth rate reversed at higher PAR levels, with the PAR thresholds (daytime mean PAR levels) at the reversion points being about 160, 125 and 178 μmol photons m^{-2} s^{-1} for *P. tricornutum*, *T. pseudonana* and *S. costatum*, respectively.

These light levels correspond to 22-36 % of incident surface solar PAR levels and are equivalent to PAR levels at 26-39 m depth in the South China Sea

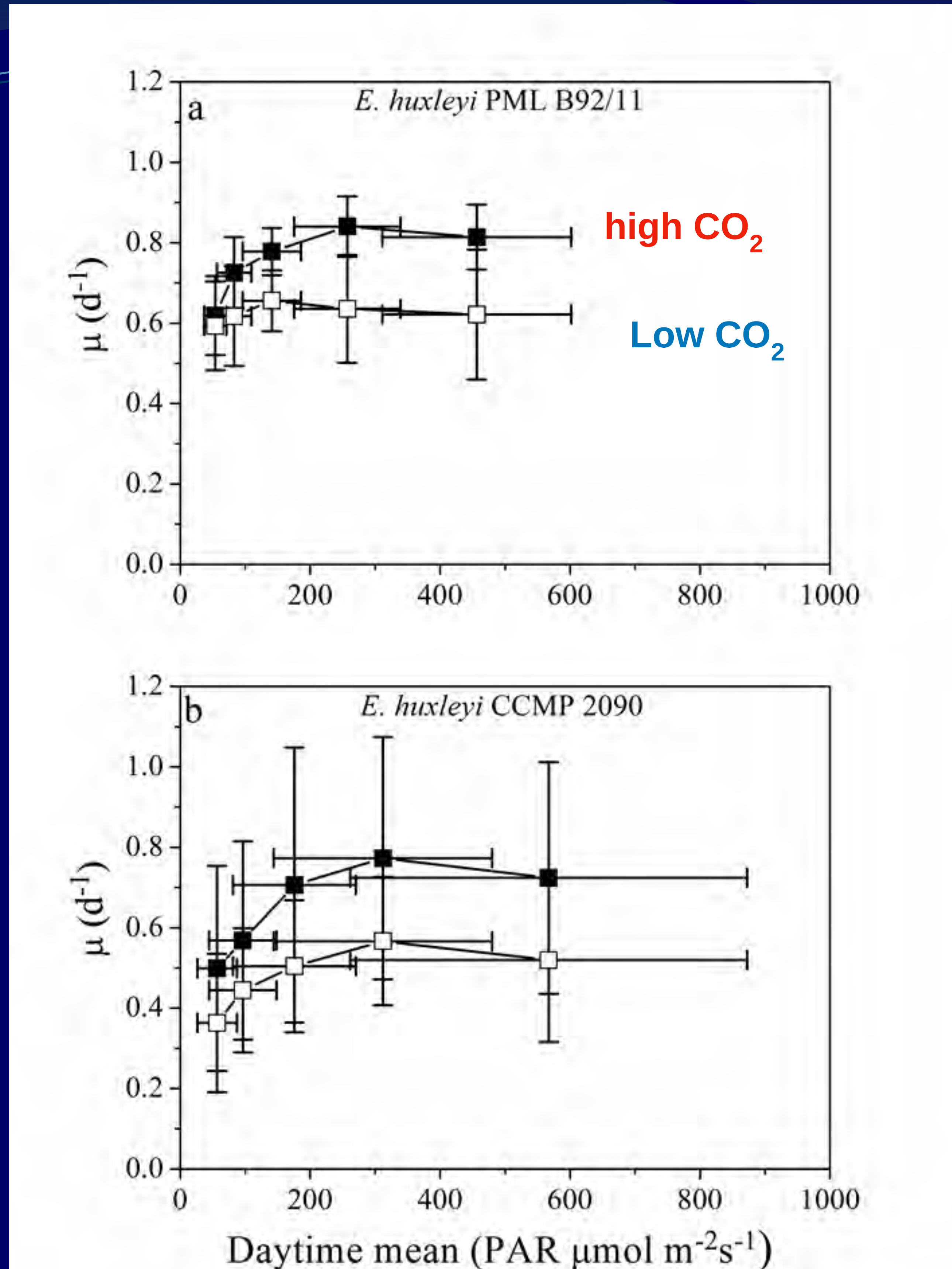
Gao et al. Nature Climate Change 2012

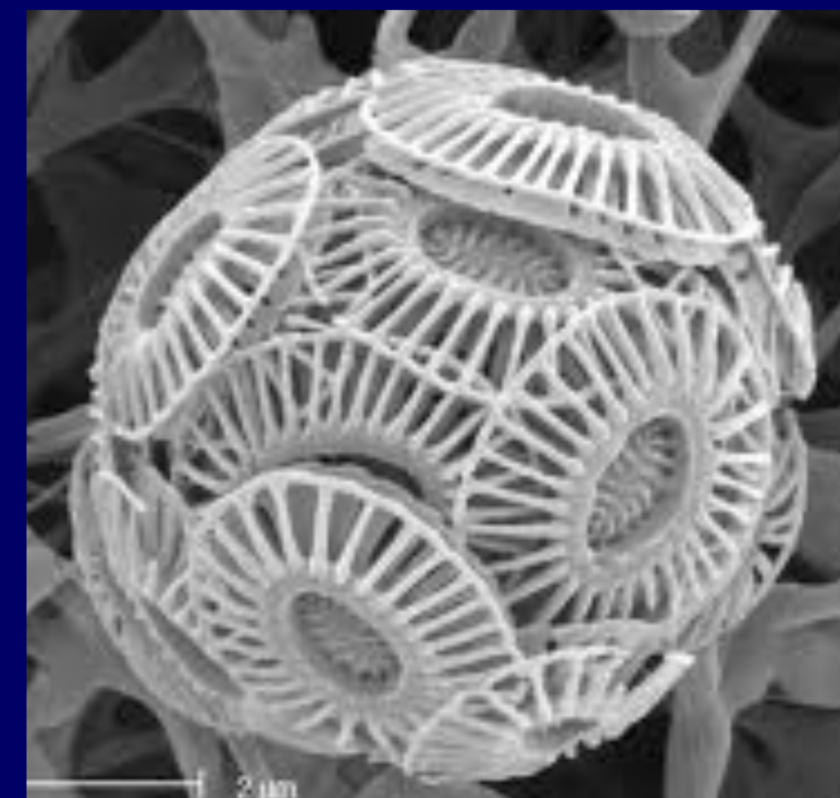
Ocean acidification (OA) down-regulates CCMs, reducing intracellular "CO2"

photorespiration ↑

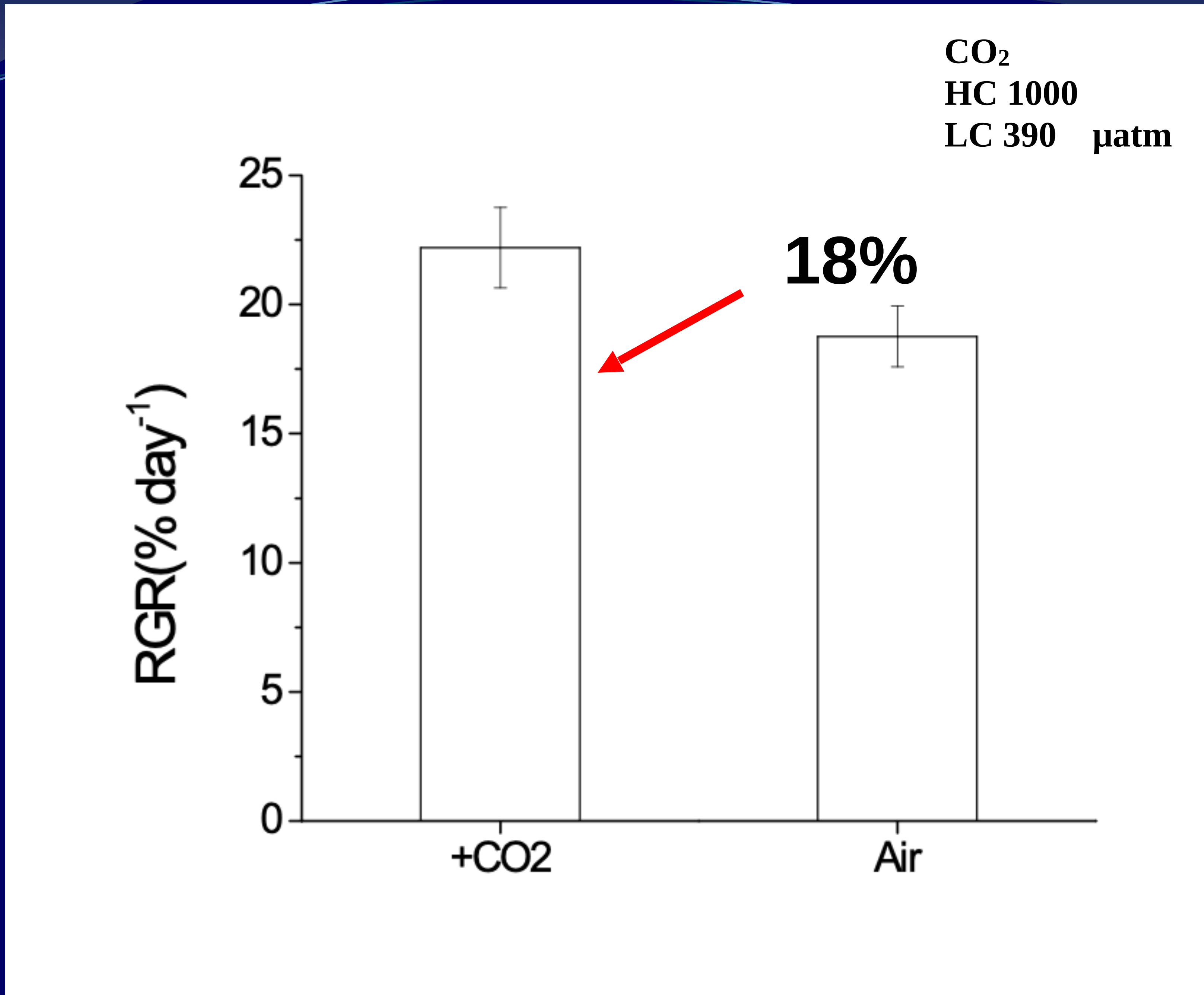
Primary productivity of the SCS oligotrophic surface seawaters ↓

Diatom growth response to OA , depending on sunlight exposures, faster under low but slower under high sunlight levels.





Jin et al. 2017 MEPS

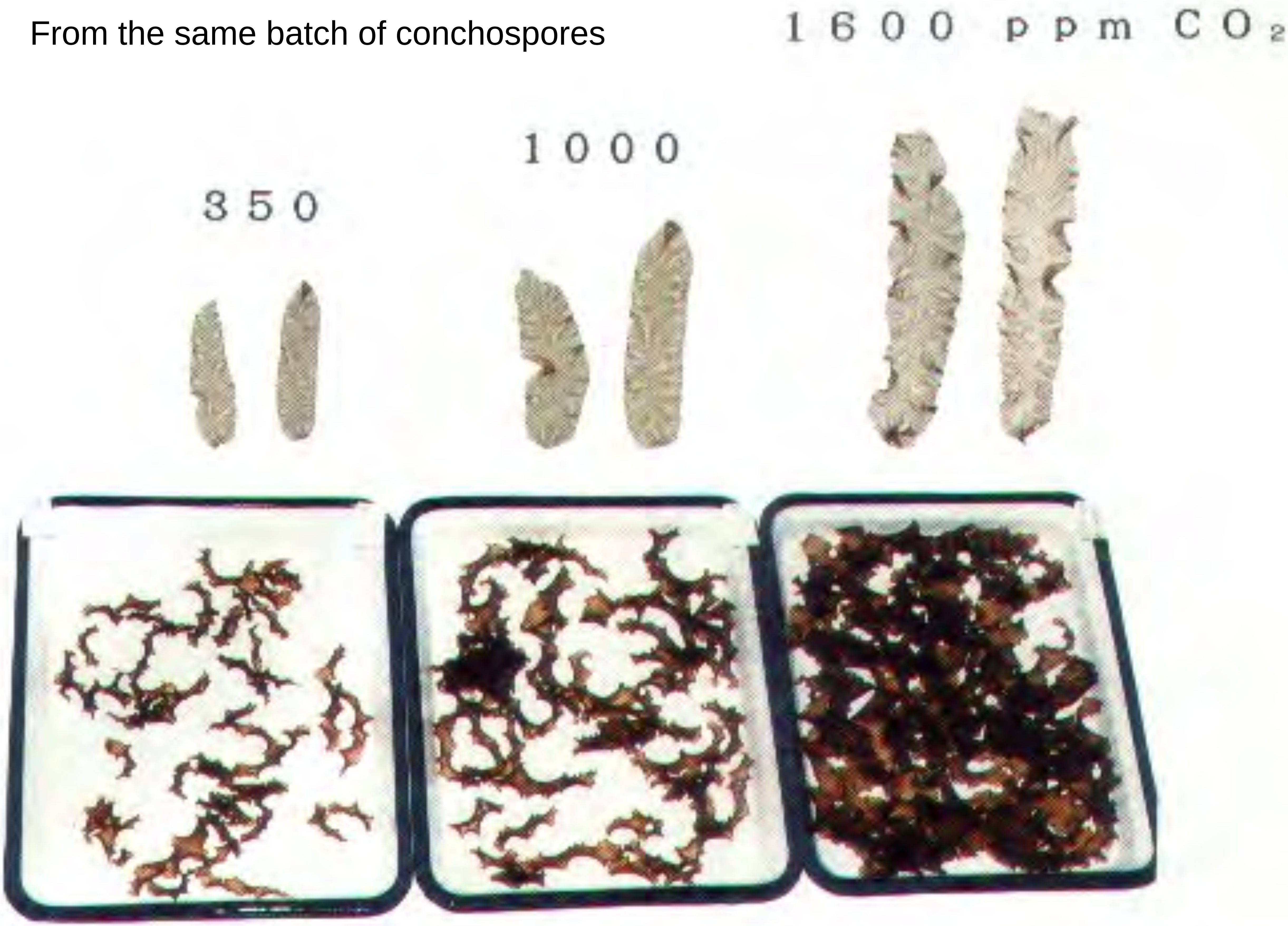


CO_2 → **Growth**

The RGR of the young sporophytes *Ulva prolifera* grov under low and high CO_2 condition.

Xu and Gao 2012 Plant Physiol.

From the same batch of conchospores



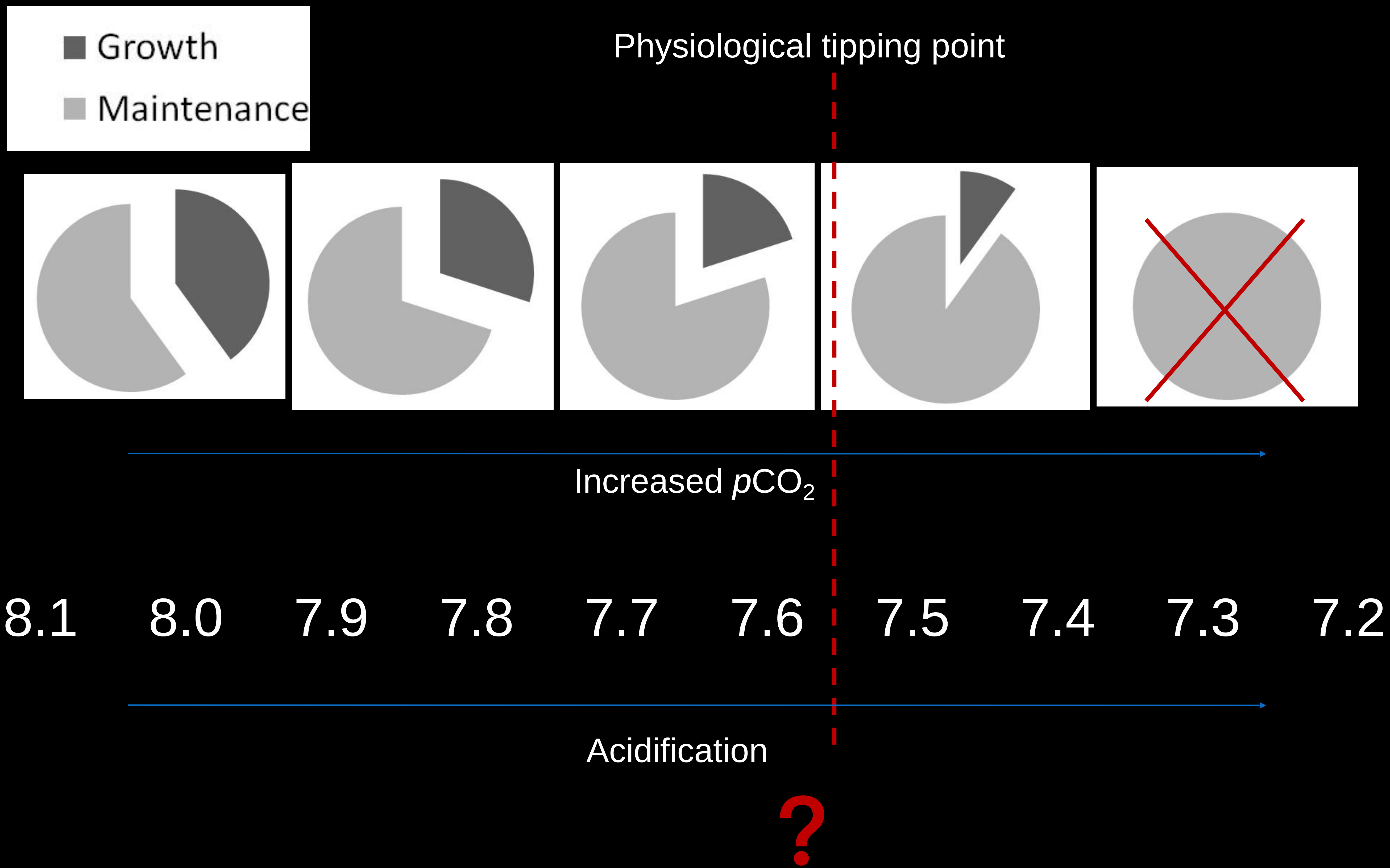
50 individuals each treatment

Gao et al. 1991

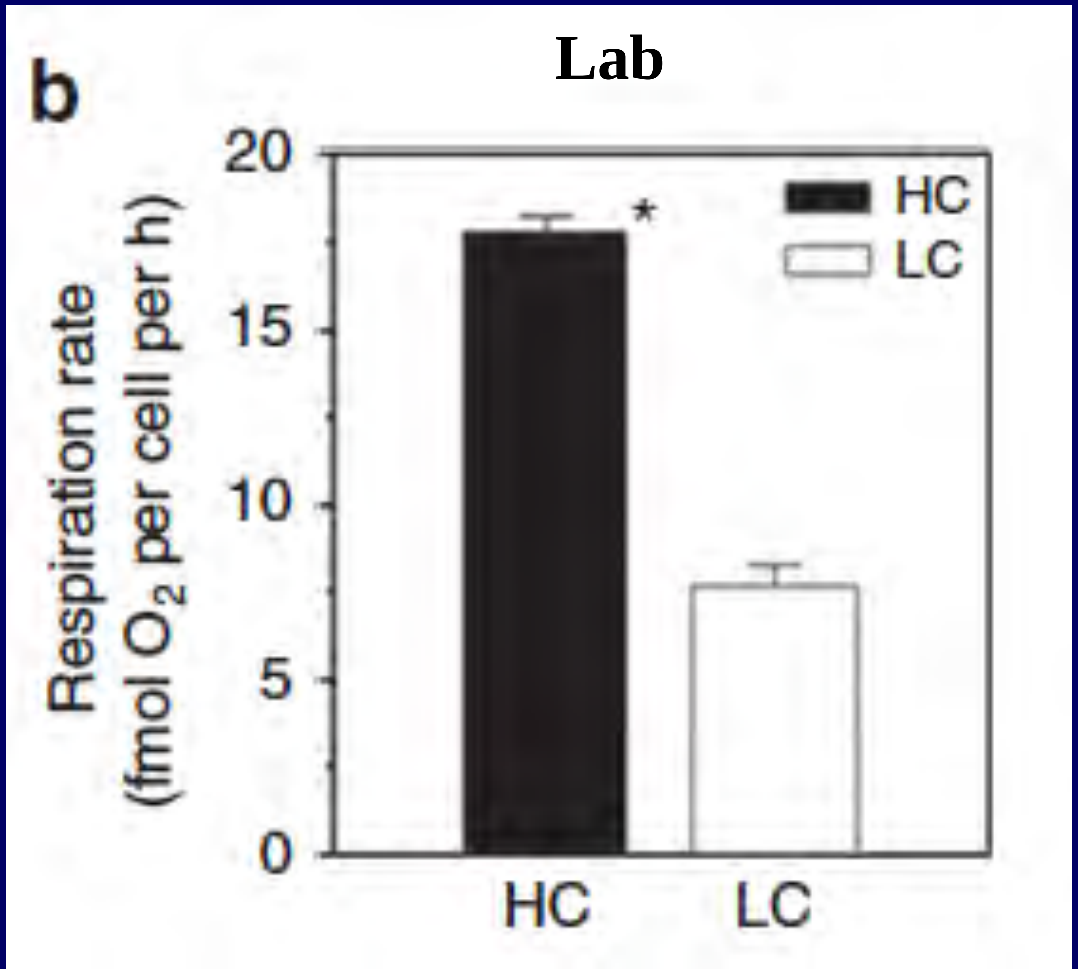
Responses

1. Photosynthesis / Growth
2. **Metabolic Pathways**
3. Calcification （calcifying algae)
4. Combined impacts with other stressors

Changes in Energetics

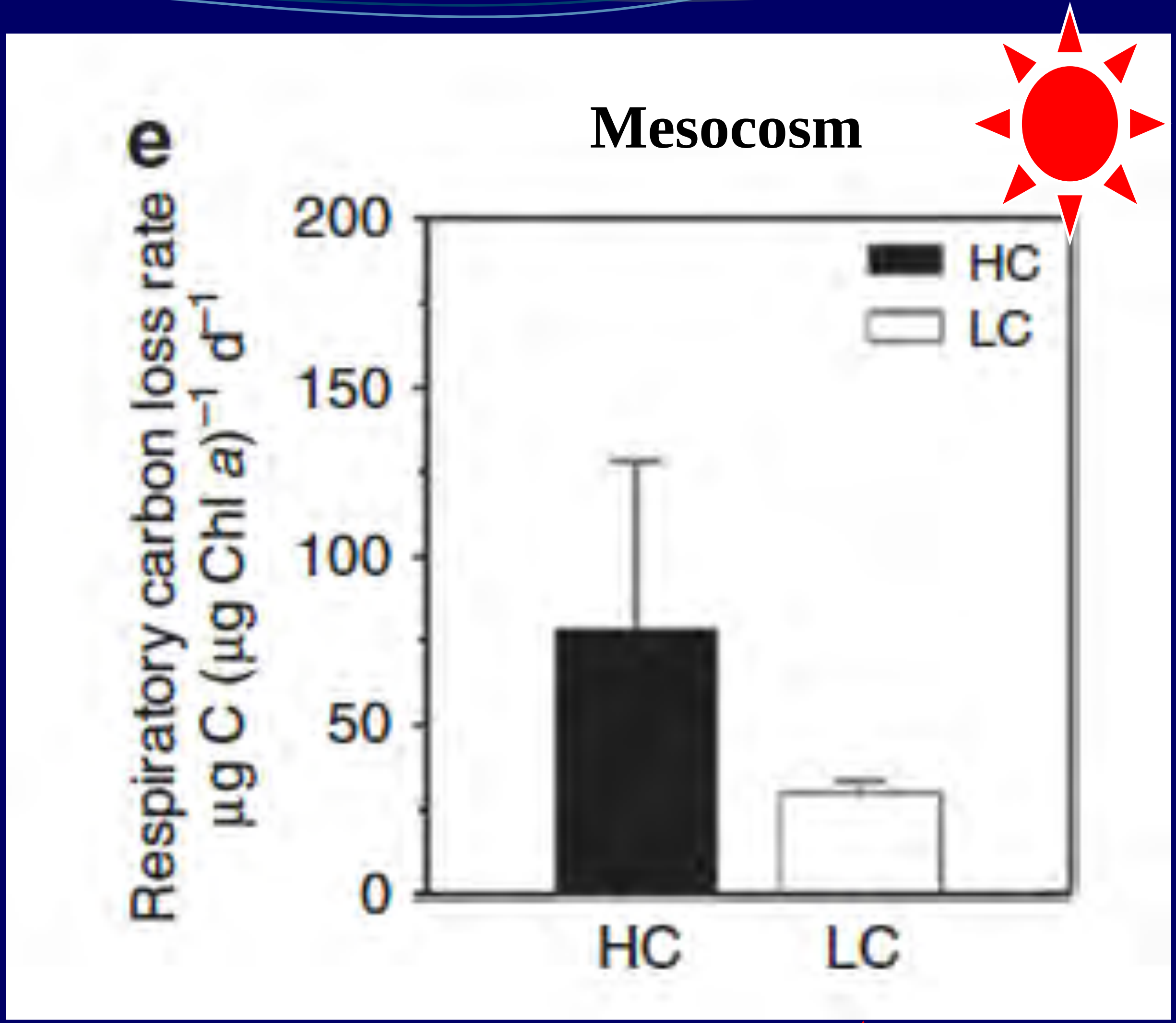


Respiration rate in phytoplankton under OA and control



130% ↑

Emiliania huxleyi



160% ↑

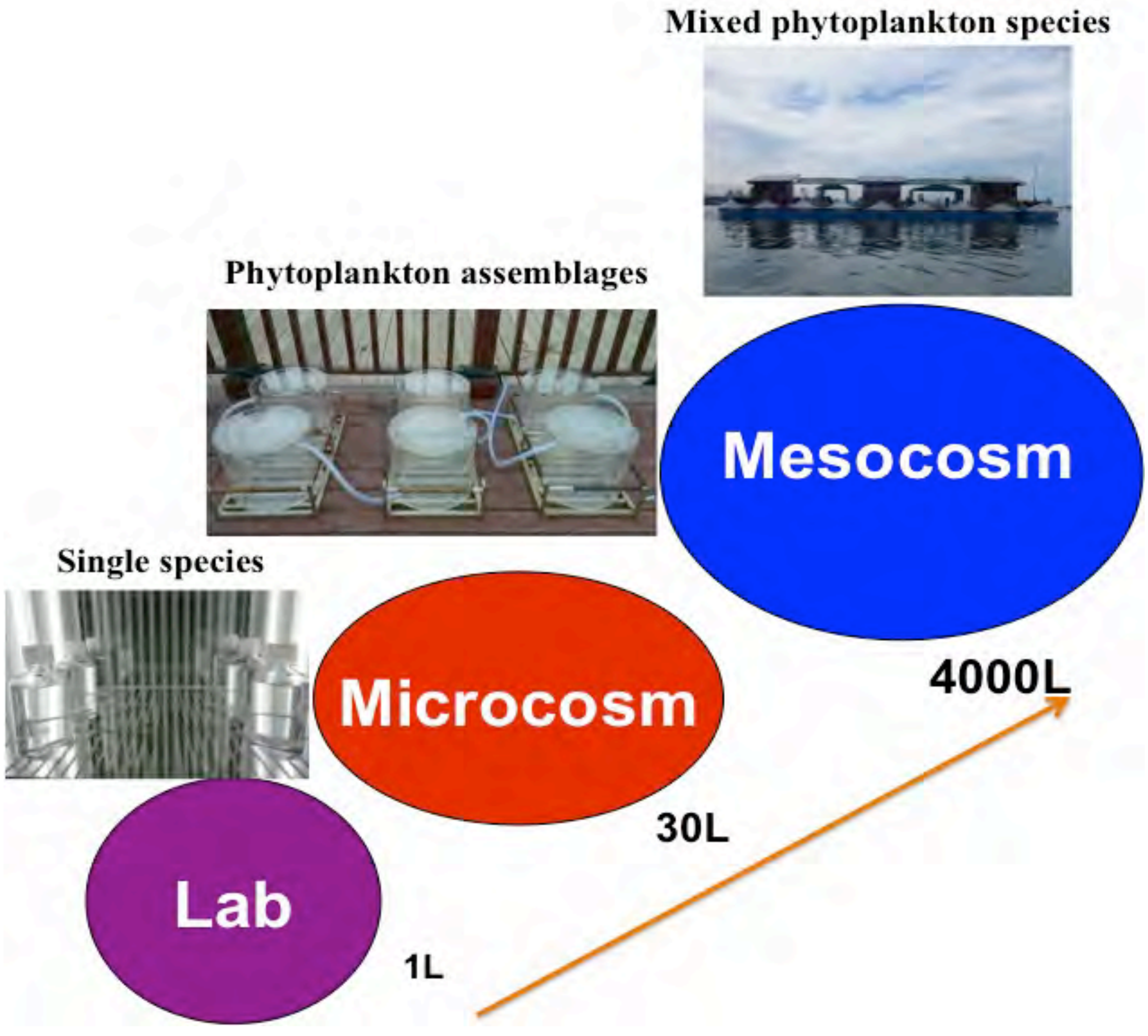
Phytoplankton assemblages

Jin et al. 2015 *Nature Communication*

Hypothesis

To cope with the acidic stress induced by elevated CO_2, microalgae need extra energy and may alter their metabolic pathways

Physiological test in different systems



Protein analysis

Sample preparation

Protein separation

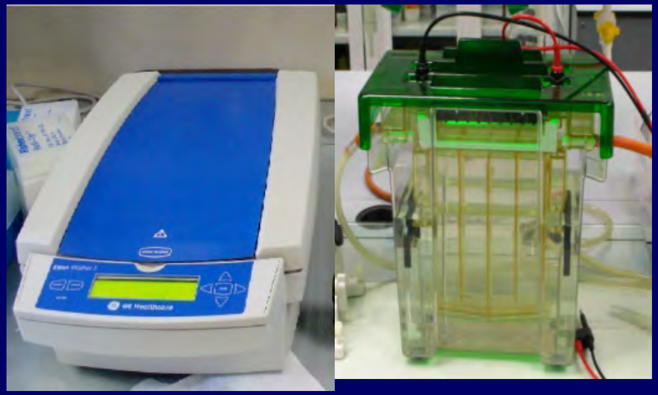


Image acquisition

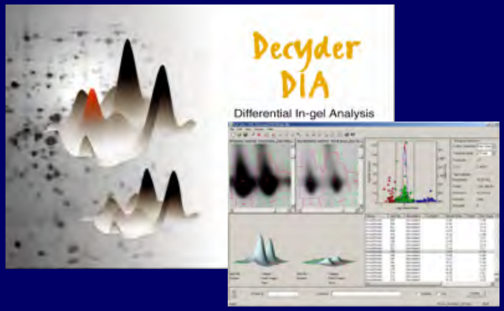


Image analysis

Spot cut

Protein lyse

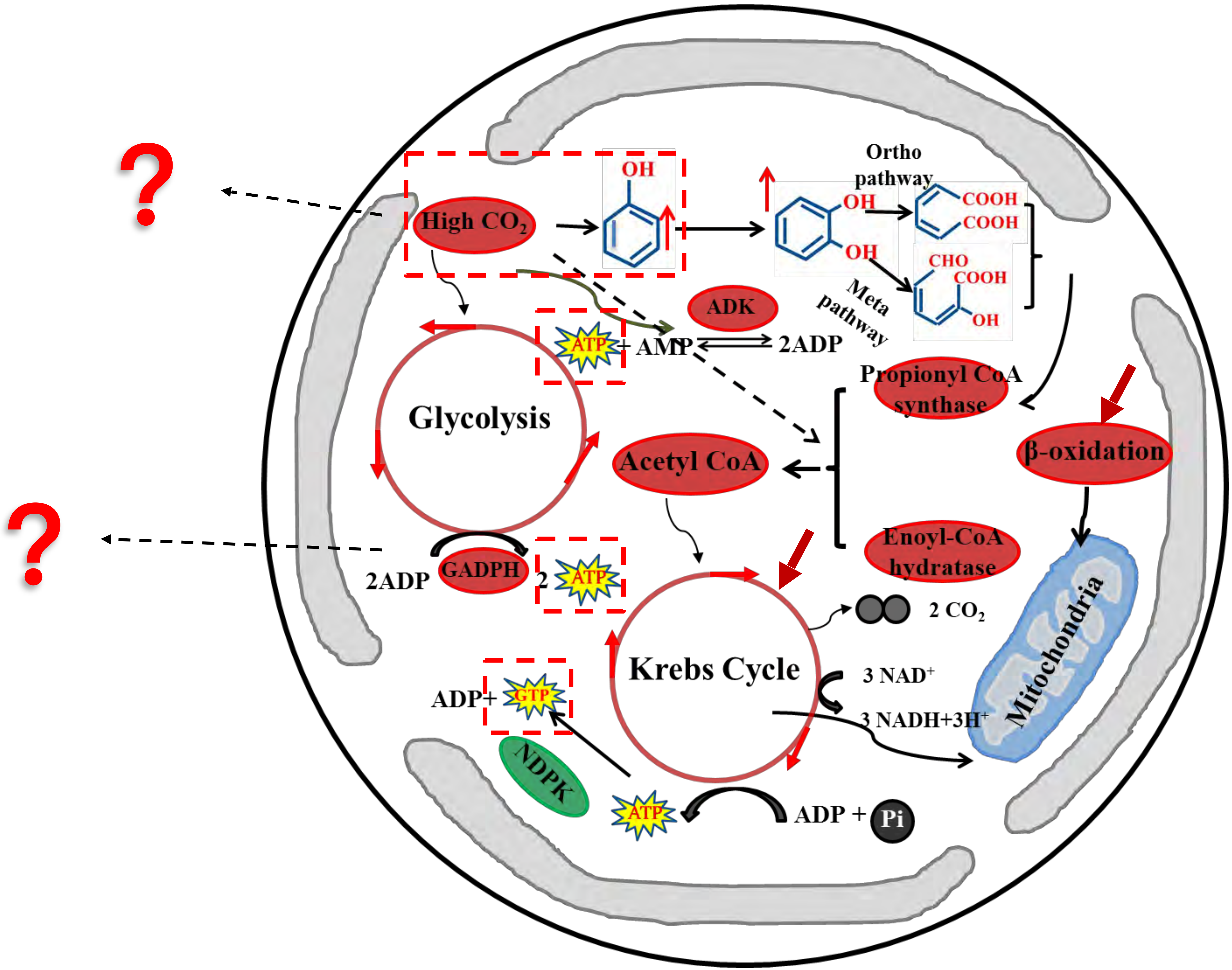
Protein spotting

TOF 5800
Proteomics Analyzer

Various proteins, that showed statistically significant alterations in abundance greater than 2-fold, in HC and LC treatments

Spot Id.	Protein identity	GI number	Protein score C.I. (%)	Total Ion C. I. %	Protein score (peptides)	MW/pI	Fold change		Function
							High CO_2	Low CO_2	
3	Propionyl CoA synthase	239994558	100	100	357(14)	69708.5/5.51	2.33	1.00	β-oxidation
4	Serine protein kinase	239995429	100	99.946	177(15)	74347.3/5.31	2.82	1.00	Protein kinase, signal transduction
9	Hypothetical protein AmacA_2	223994739	100	100	805(22)	51069.6/5.61	2.01	1.00	Unknown
11	Hypothetical protein MDMS009_211	254489880	100	100	440(11)	447891.1/4.87	1.00	4.34	Unknown
12	Methane/ phenol/ toluene hydroxylase	148260382	100	100	238(5)	39315.7/5.76	3.40	1.00	Phenol biodegradation
13	Acyl-CoA dehydrogenase family protein	83943662	100	100	438(18)	44108.4/5.55	1.00	2.66	β-oxidation
14	Chloroplast glyceraldehyde-3-phosphate dehydrogenase	77024139	100	100	336(7)	44096.1/5.2	2.92	1.00	Glycolysis
15	Conserved hypothetical protein (bacterium S5)	288797257	100	99.996	166(7)	21306.1/4.87	2.50	1.00	Unknown
17	Enoyl-CoA hydratase	83955054	99.996	98.89	115(8)	28178.9/5.51	3.82	1.00	β-oxidation
19	Adenylate kinase	239993306	100	100	600(16)	23693/4.99	2.12	1.00	ATP synthesis
21	TRAP-T family protein transporter periplasmic binding protein	83943788	100	100	811(17)	39967.7/4.56	3.04	1.00	Substrate-binding protein (SBP)-dependent secondary transporters
24	Nucleoside diphosphate kinase	114765301	100	100	352(6)	15293.7/4.93	1.00	2.10	Catalyze the transfer of a phosphate from a NTP to NDP

Altered metabolic pathways under OA

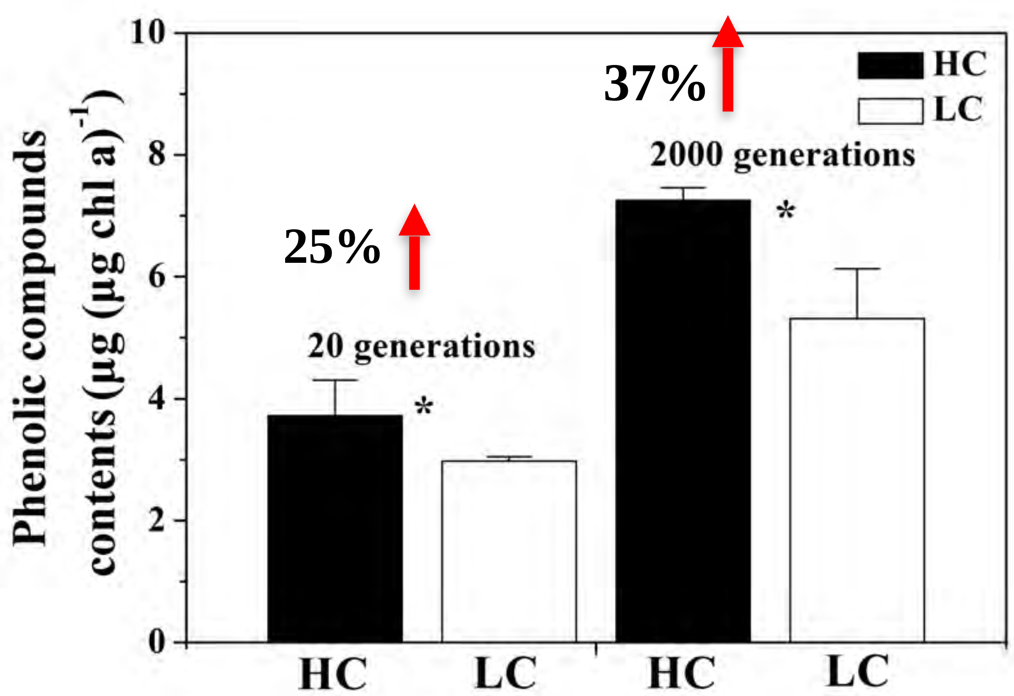


Jin et al. 2015 *Nature Communications*

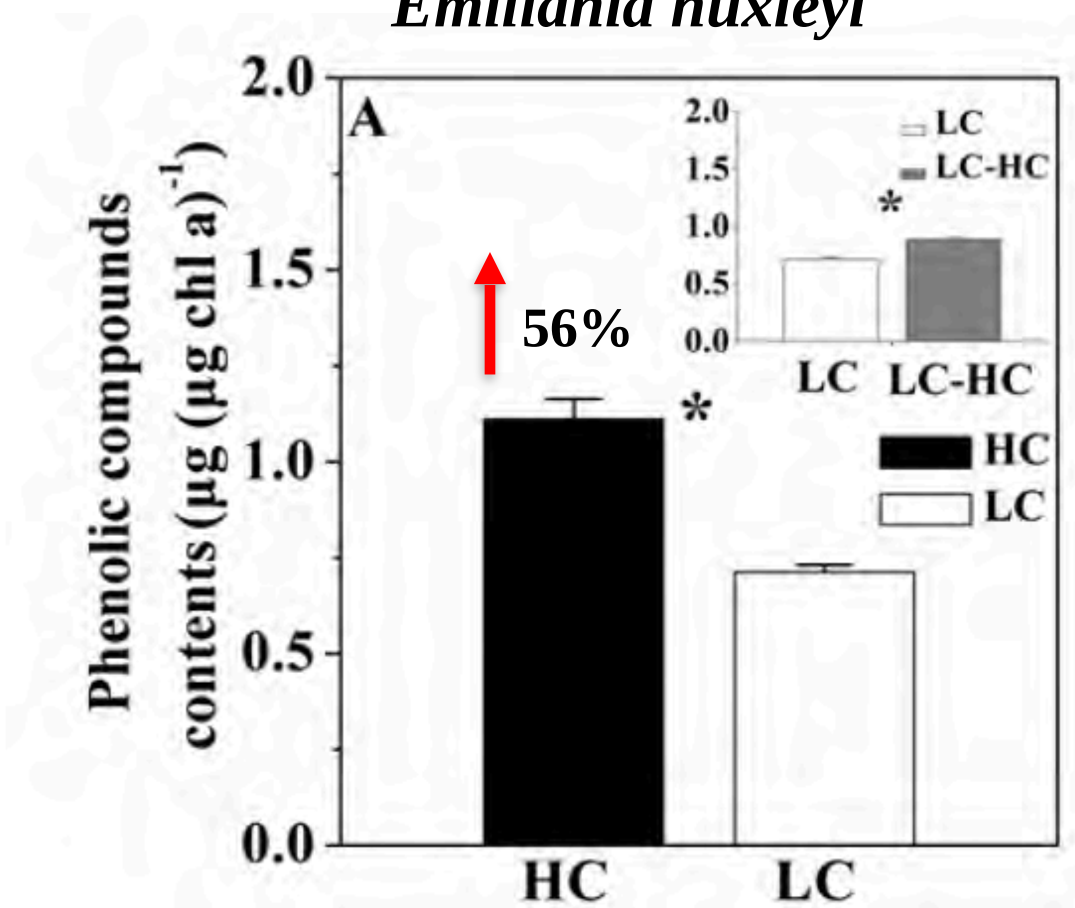
Contents of phenolic compounds in phytoplankton

Lab test

Phaeodactylum tricornutum



Emiliania huxleyi

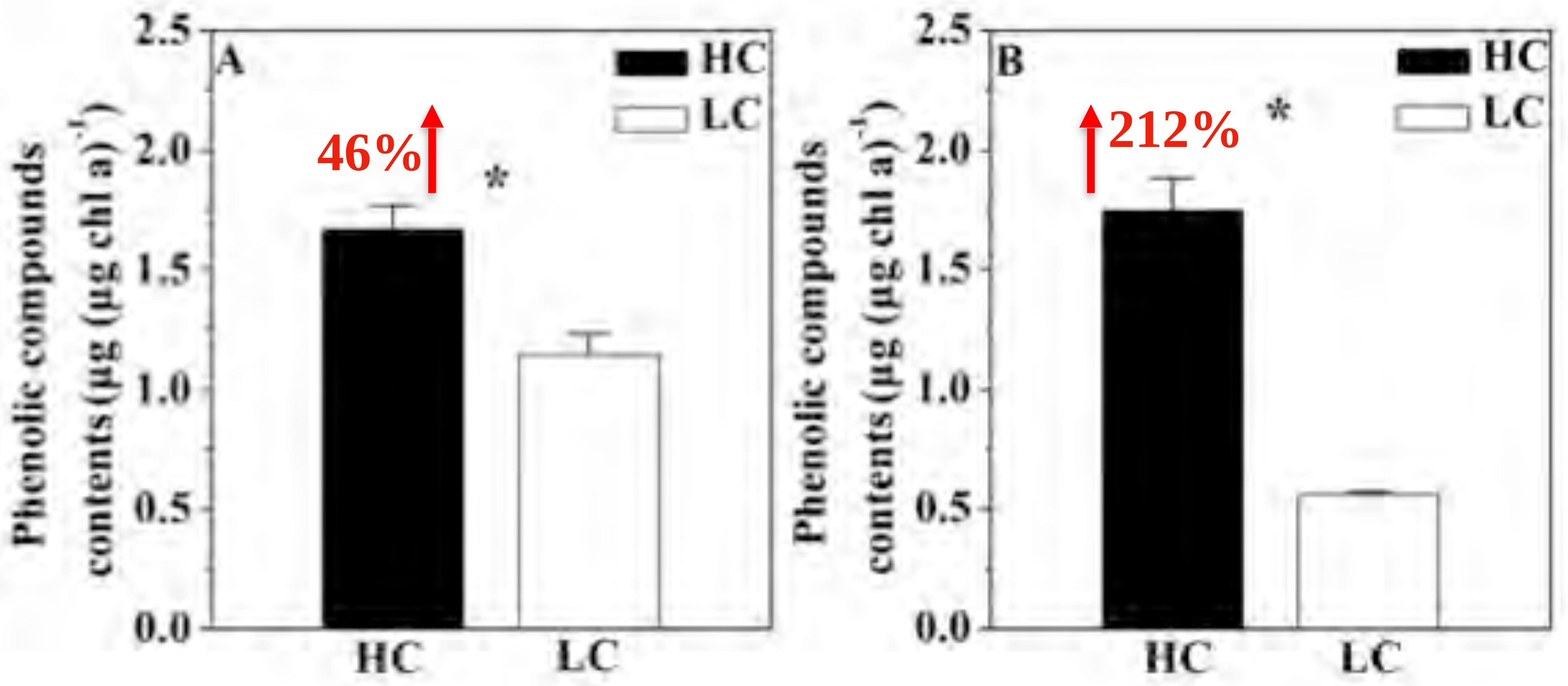


Former Ph D stdudent: Yahe Li

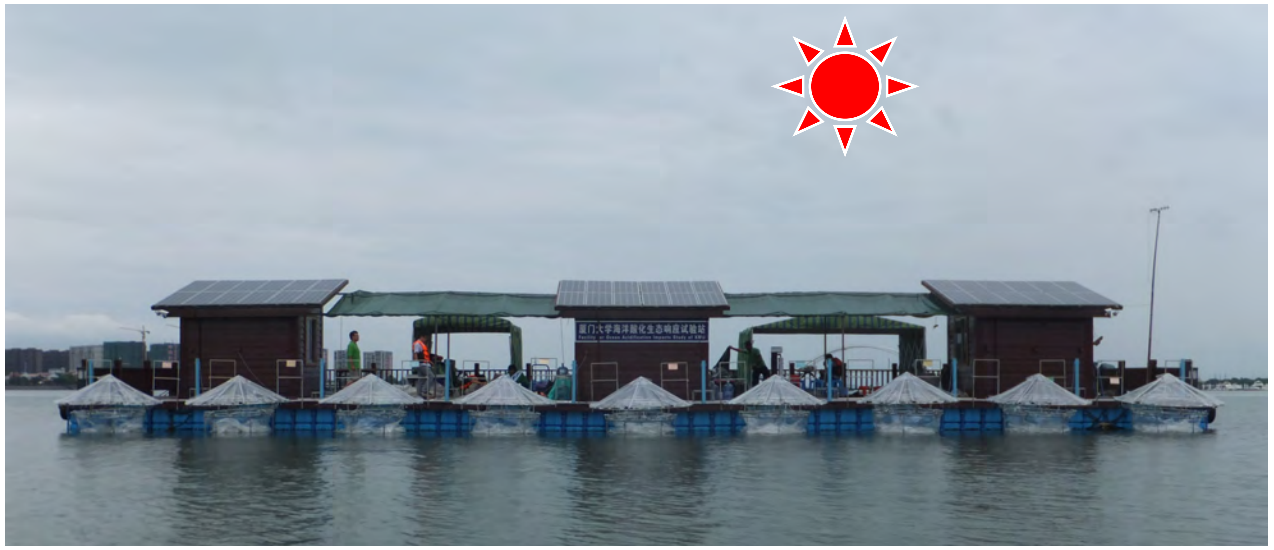
Former Ph D student: Peng Jin

Jin et al. 2015 *Nature Communications*

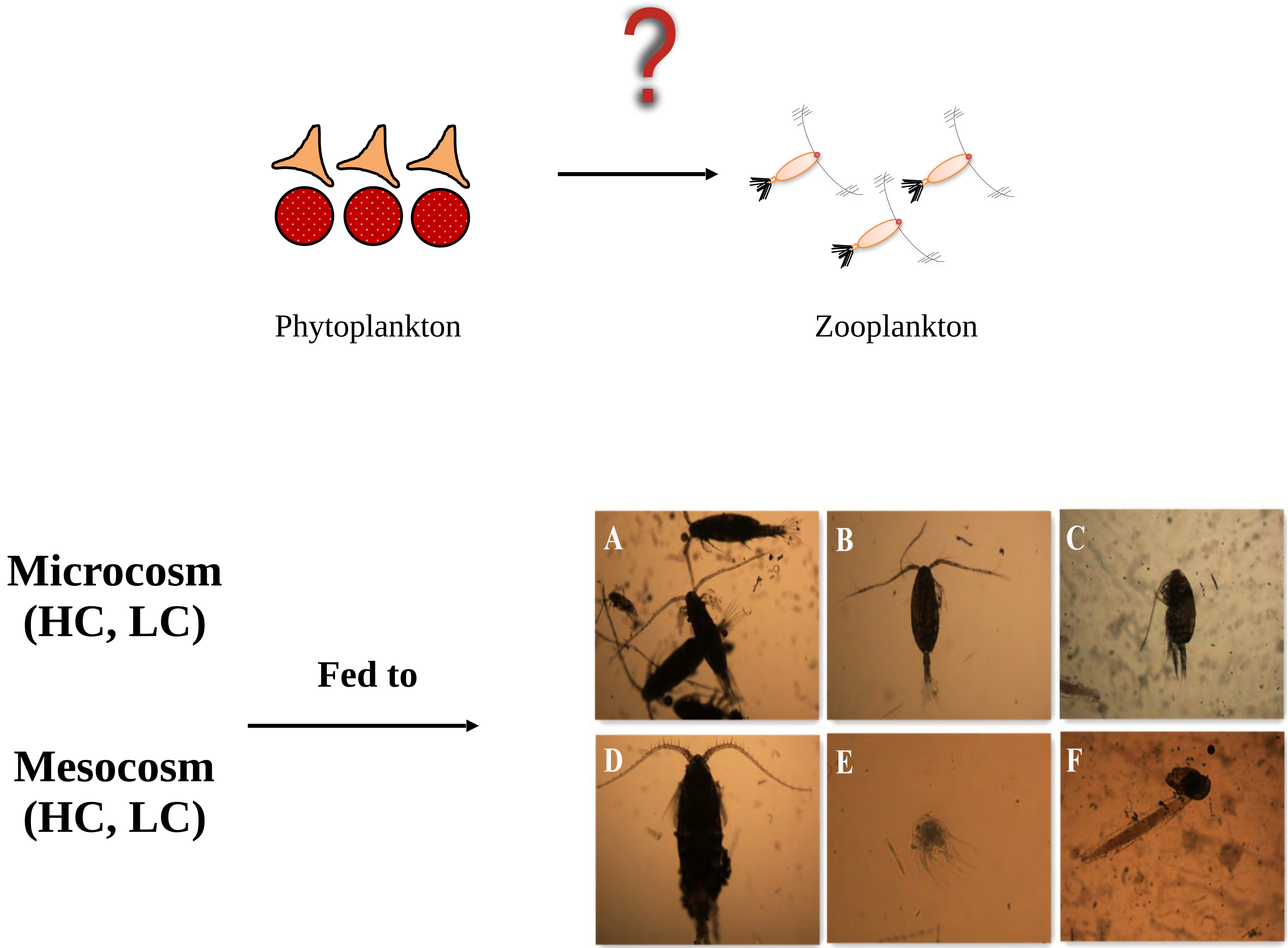
Microcosm (30L) Mesocosm (4000L)



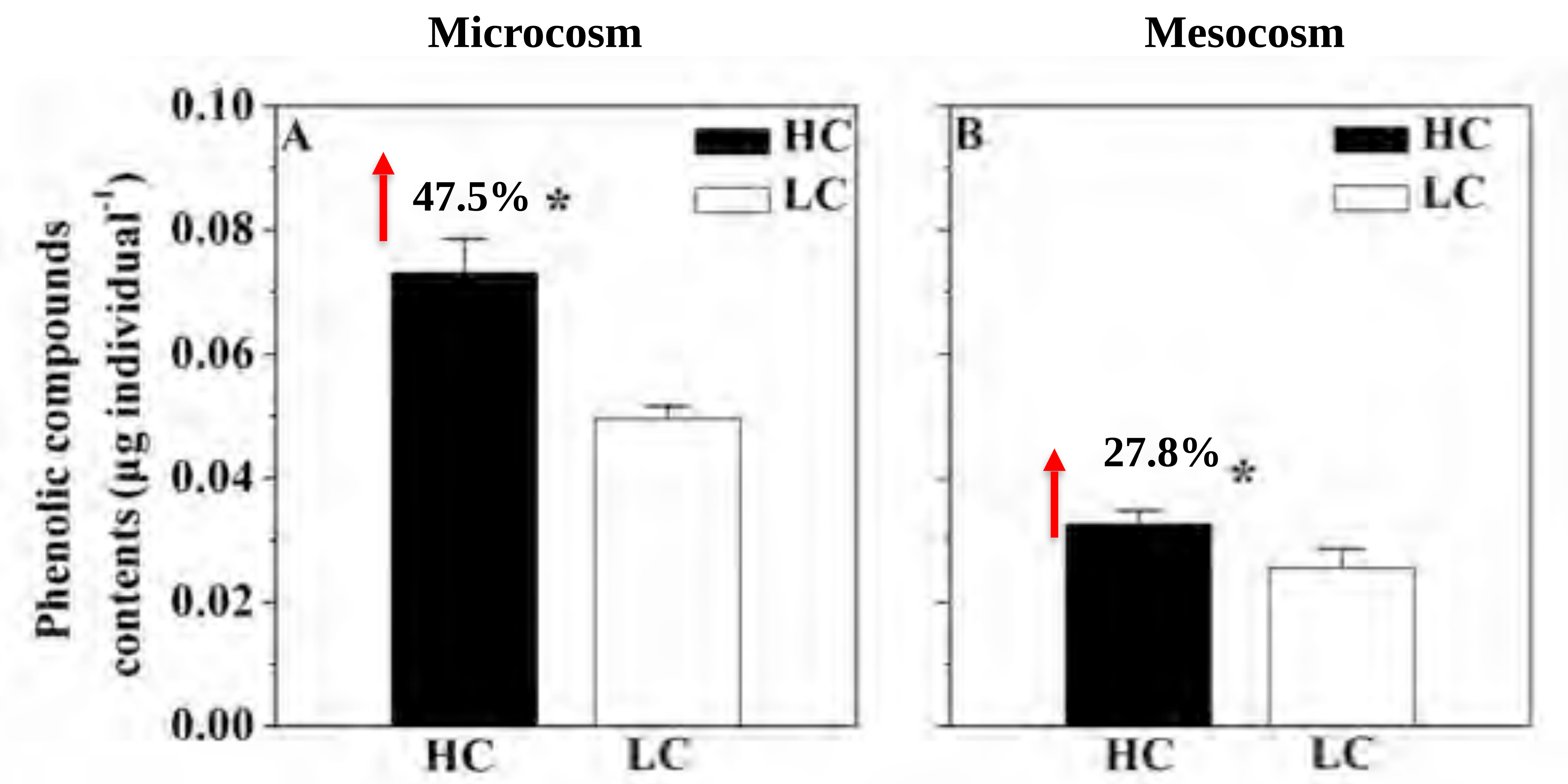
- **Kreb cycle and β-oxydation pathways are upregulated under OA, leading to higher contents of toxic phenolics**



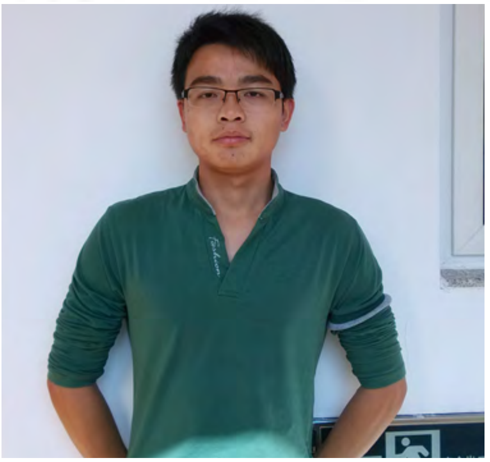
Jin et al. 2015 ***Nature Communications***


?
Phytoplankton
Zooplankton
Microcosm
(HC, LC)
Fed to
Mesocosm
(HC, LC)
A
B
C
D
E
F

Contents of phenolic compounds in zooplankton that were fed on phytoplankton (HC, LC) from microcosm and mesocosm systems



Former Ph D student: Peng Jin



Ph D student: Tifeng Wang

Jin et al. 2015 *Nature Communications*

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ARTICLE

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Ocean acidification increases the accumulation of toxic phenolic compounds across trophic levels

Peng Jin[1,†], Tifeng Wang[1], Nana Liu[1], Sam Dupont[2], John Beardall[3], Philip W. Boyd[4], Ulf Riebesell[5] & Kunshan Gao[1]

Ecological implications:

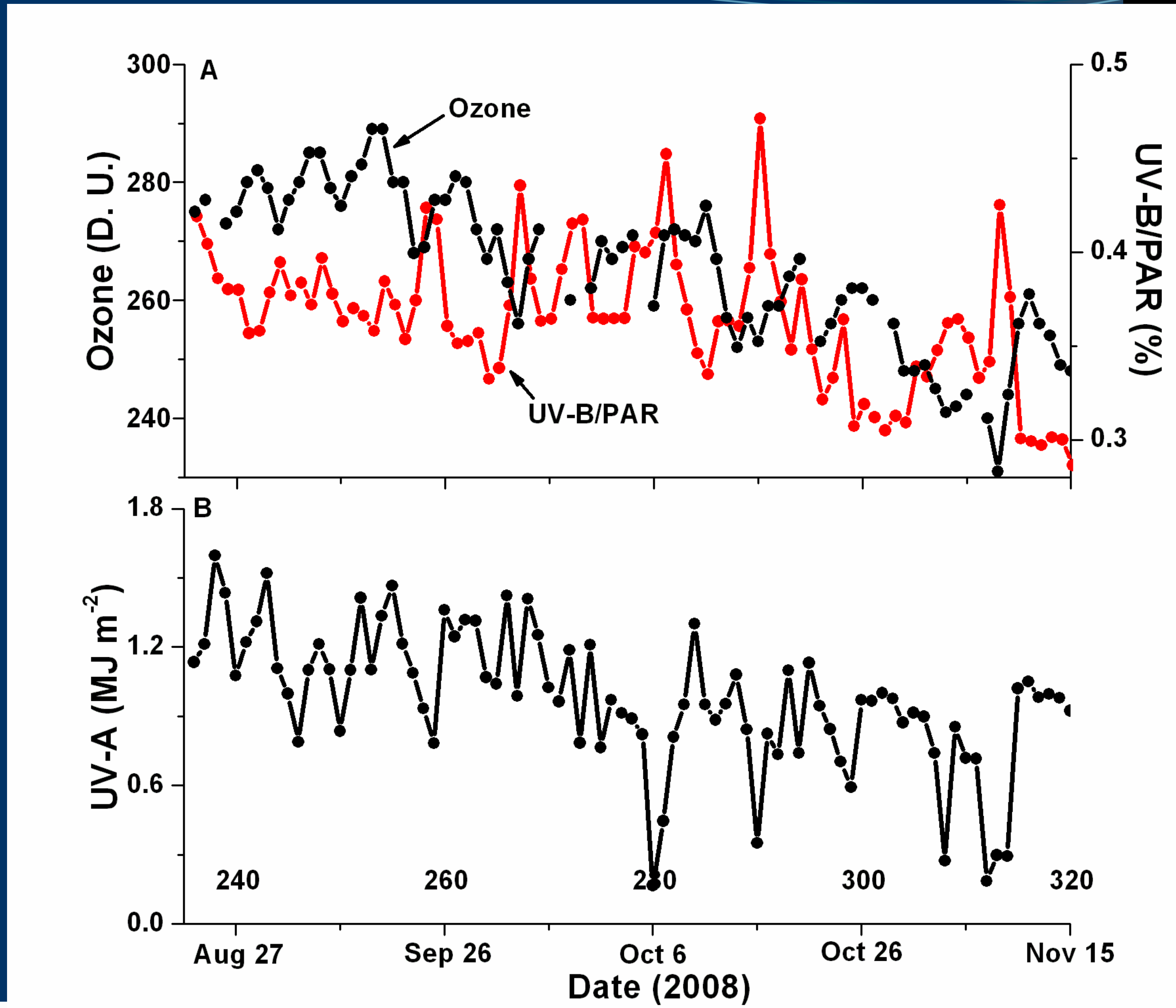
Increased accumulation of phenolic compounds in phytoplankton and zooplankton, implying a food chain impact.

Responses

1. Photosynthesis / Growth
2. Metabolic Pathways
3. Combined impacts with other stressors (UV & Virus)

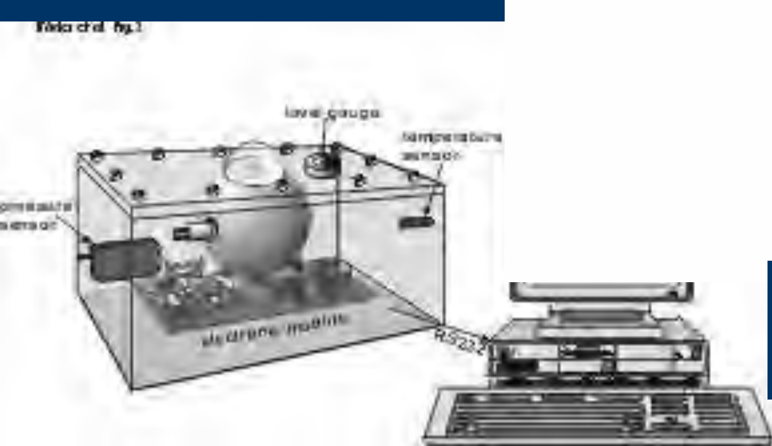
Xiamen

Monitored every second



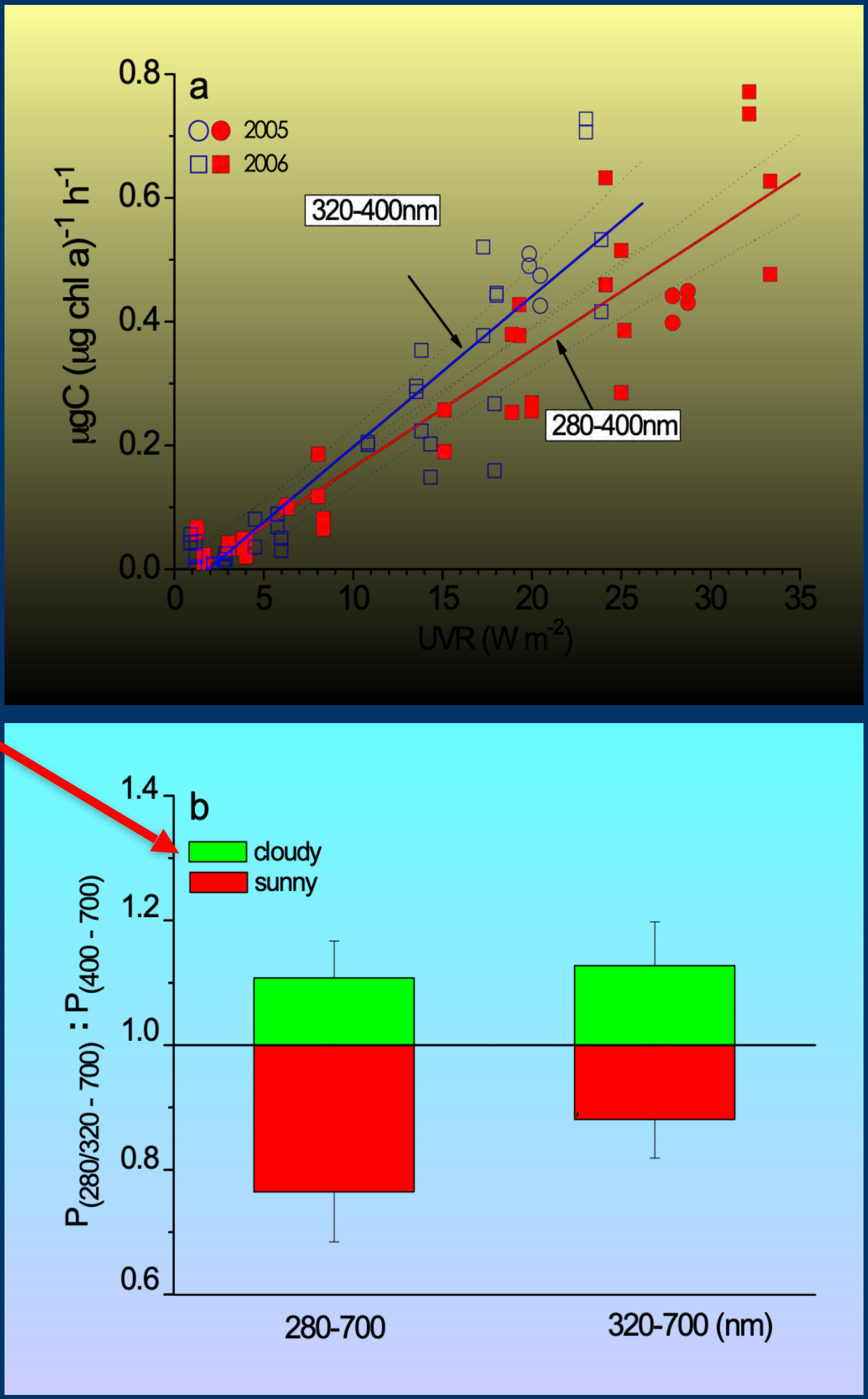
UVB/PAR

UVA



UV penetration deep down to 50-70 m

1. Evidence that UV-A alone drives photosynthesis
2. UV-A enhances photosynthetic carbon fixation on cloudy days



UV-A

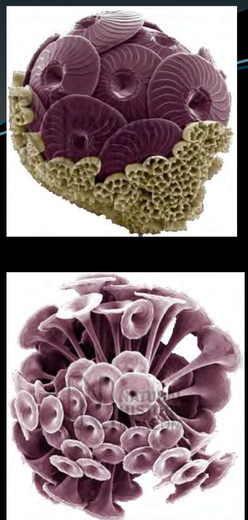
"+"

"__"

UV-B "__"

Gao et al. 2007 Plant Physiology

颗石藻 (coccolithophore) Corralling algae



Photosynthesis
C-sink

Calcifying algae

Calcification
C-source

Modulating air-sea
CO_2 flux

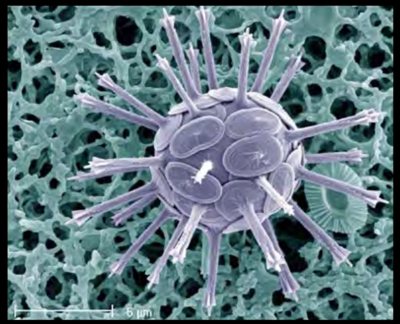
Hypothesis

Calcified layer or “shell" of calcifying algae may play protective roles against UV

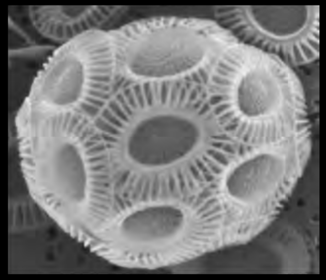
Decreased carbonate ions associated with OA may decrease calcification

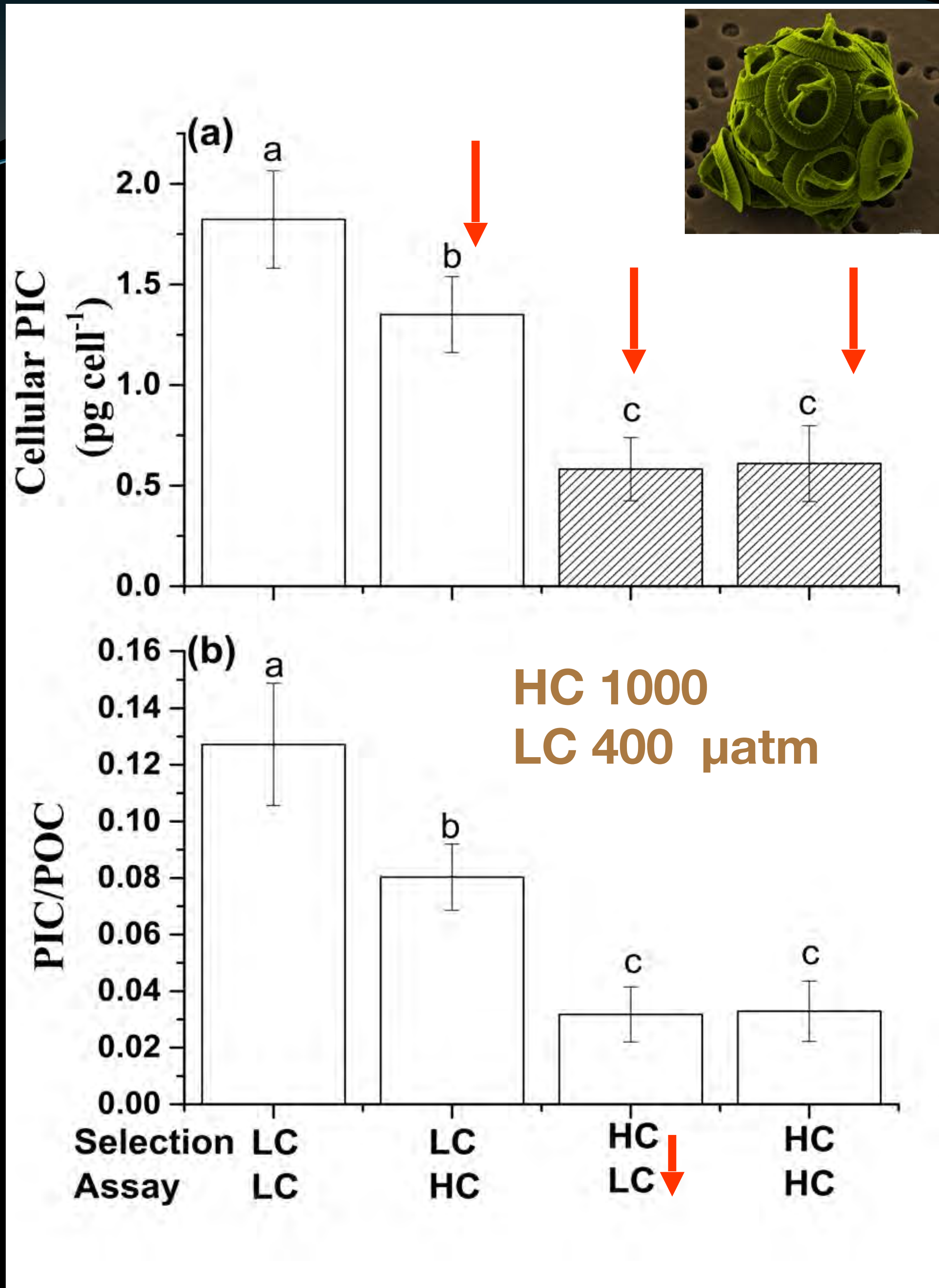
Synergestic impacts of OA + UV are expected

Coccolithophores (calcifying marine phytoplankton)

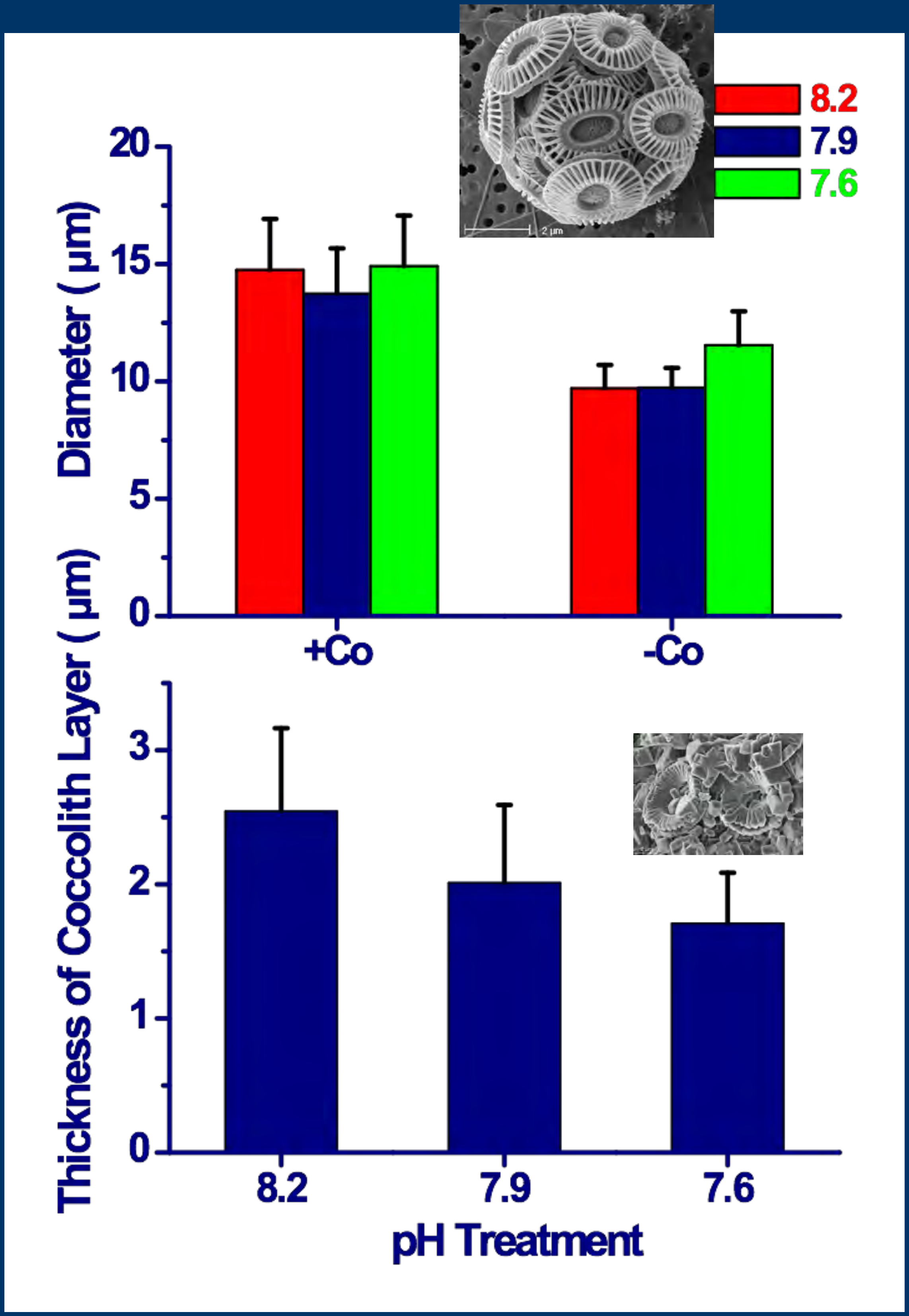


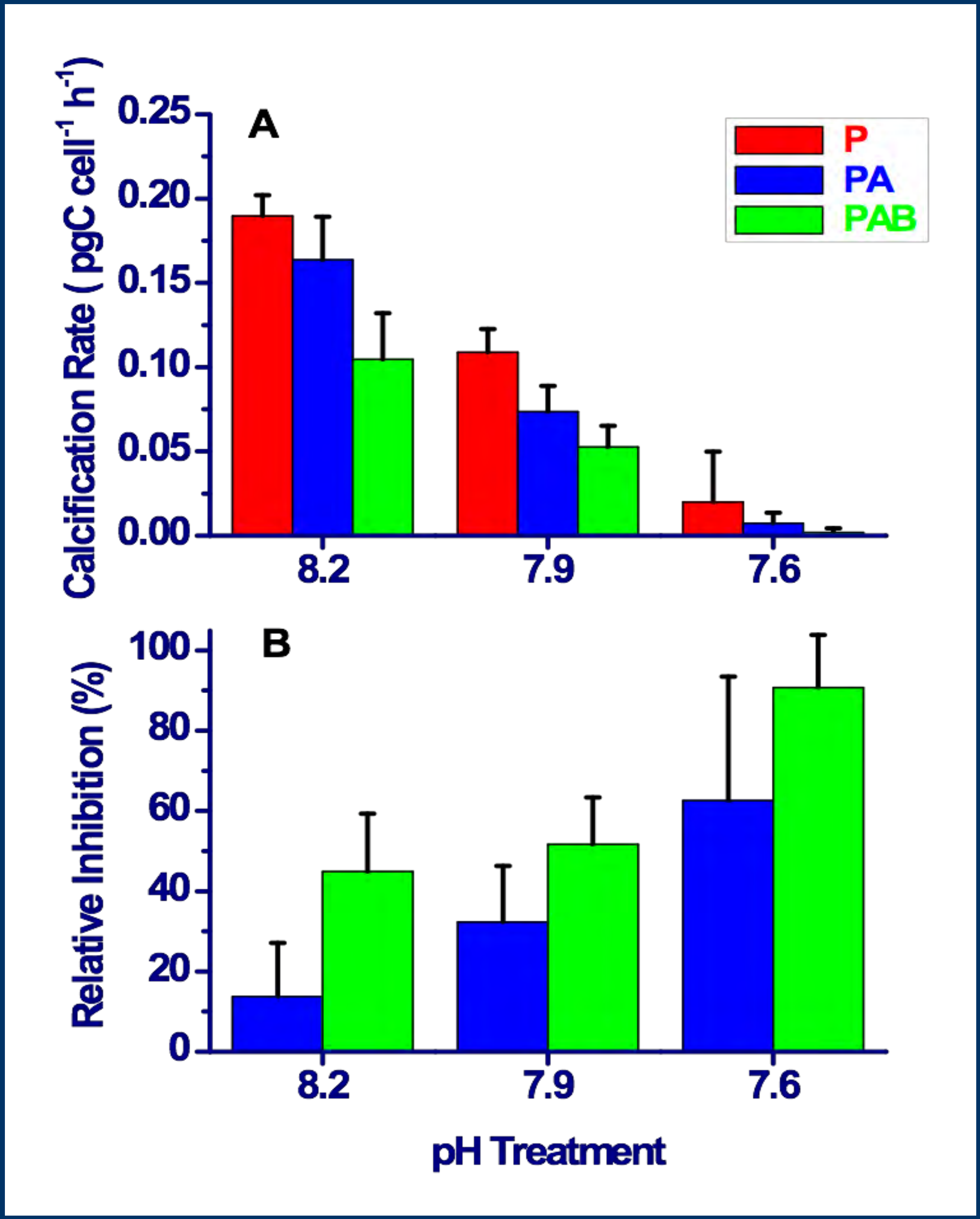




After growth under OA condition for 1000 generations, declined calcification could not be recovered even after transferred to ambient low CO2 conditions and grown for 20 generations, reflecting an evolutionary response

Tong et al. 2018 *Global Change Biology*

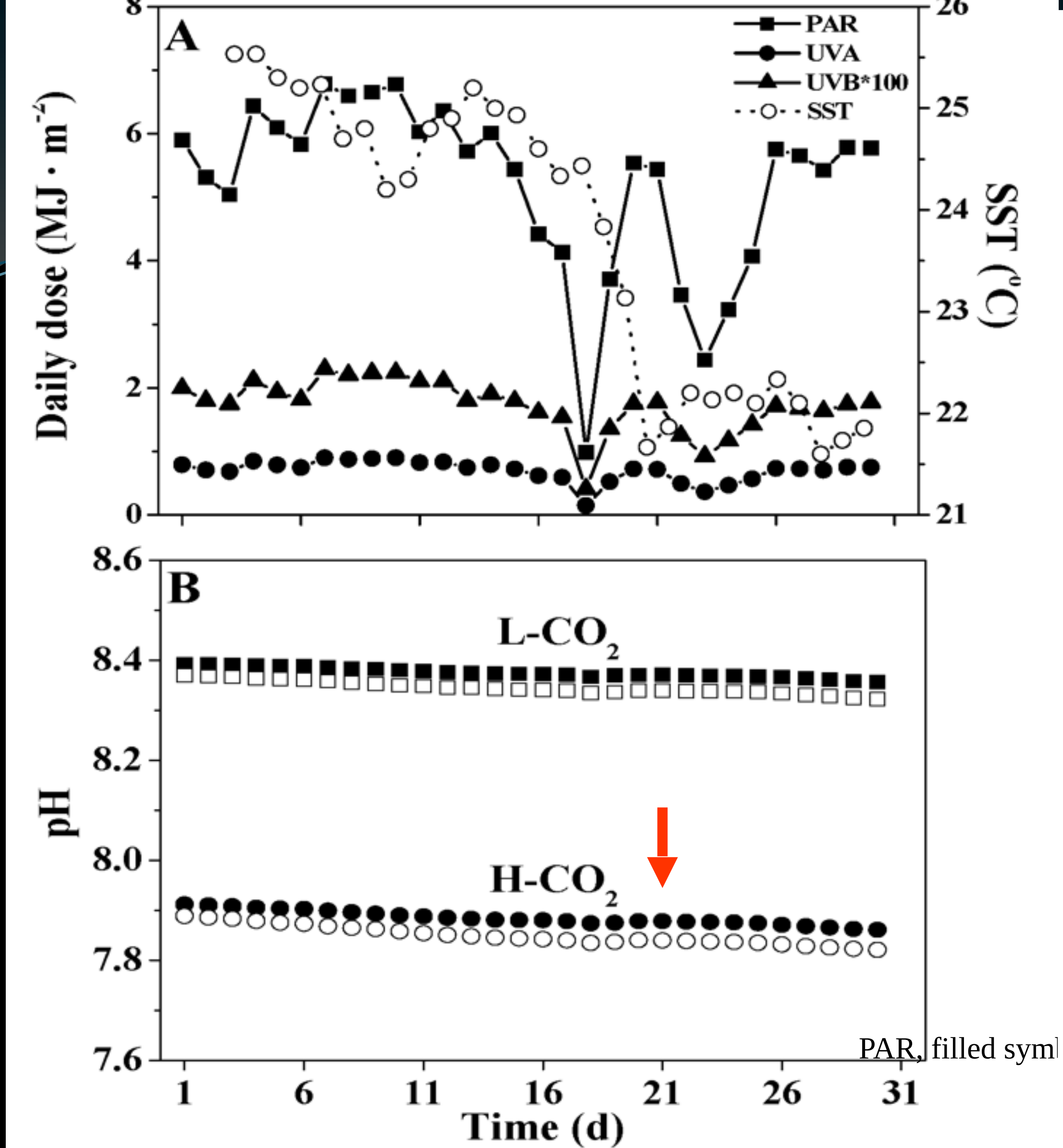


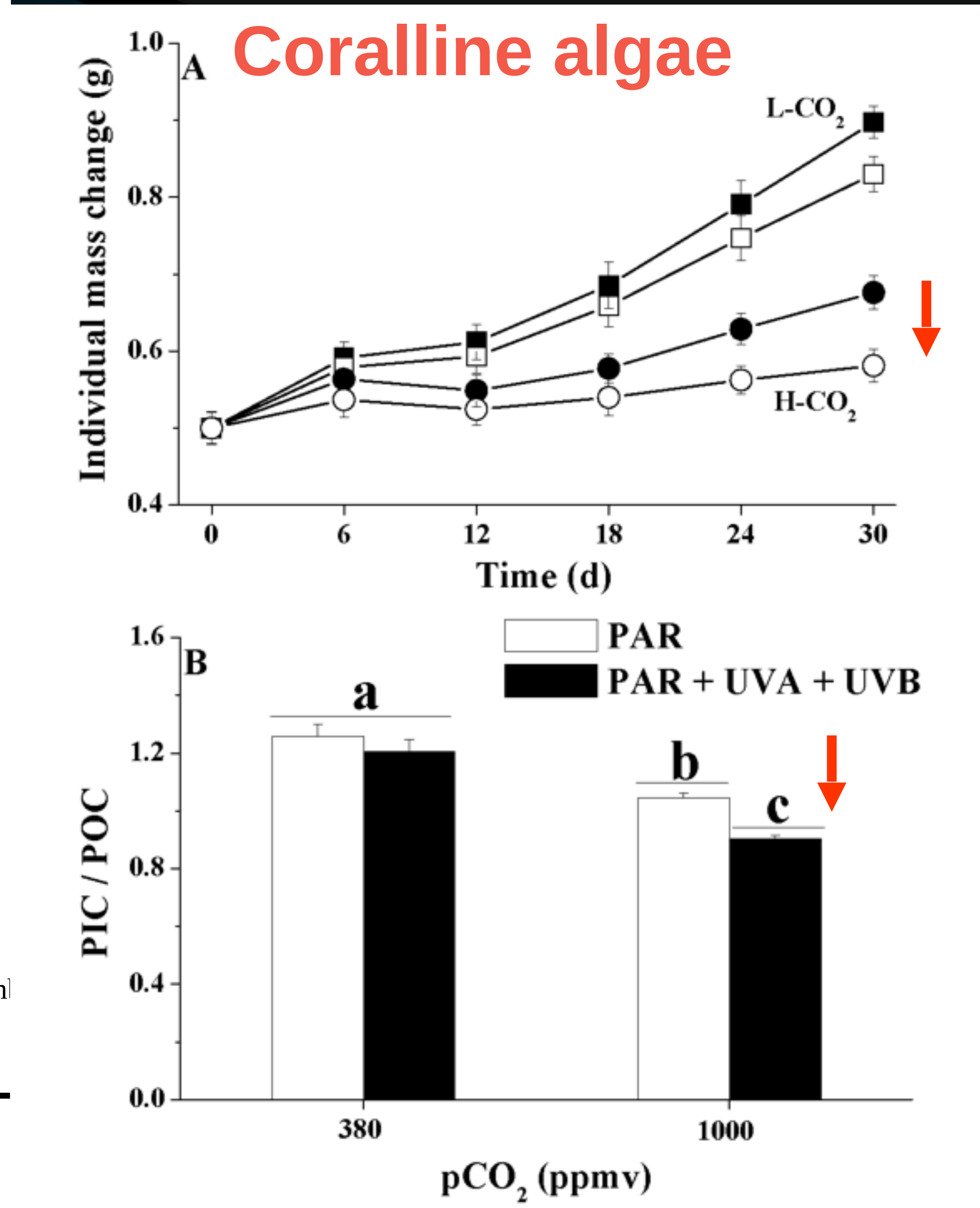


P: PAR
PA: PAR+UVA
PAB: PAR+UVA+B

Gao et al. 2009 *Limnol. Ocean.*

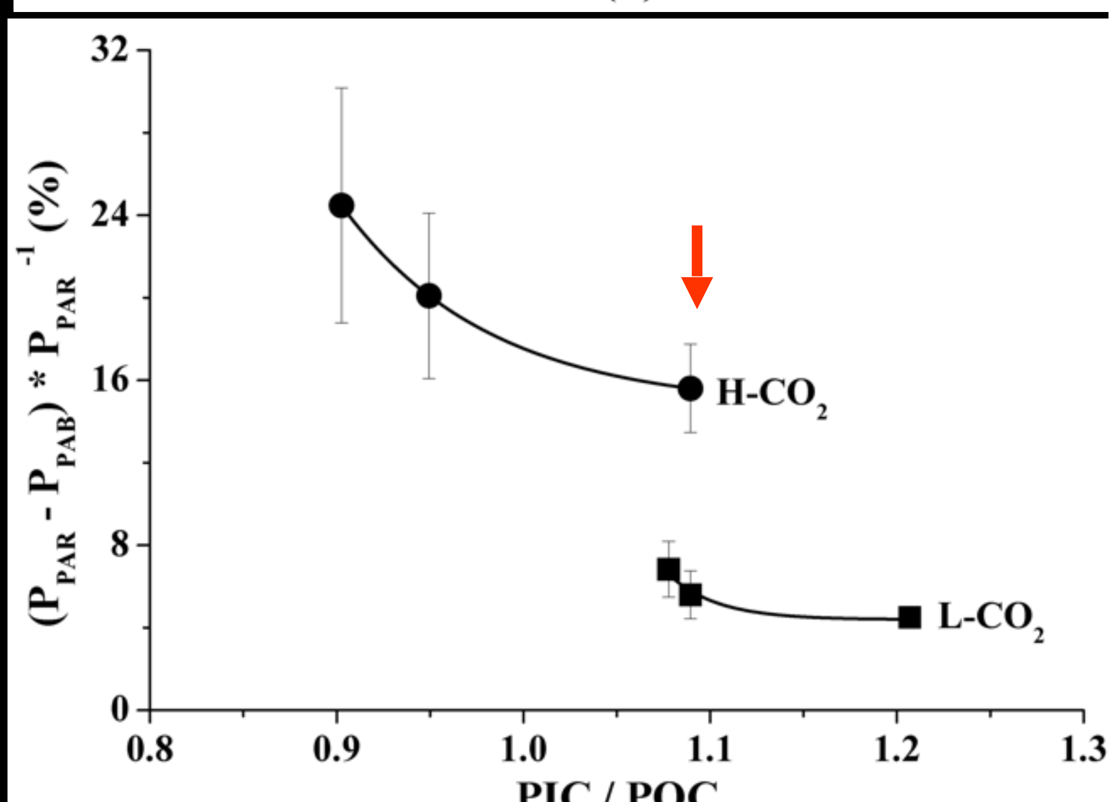
Corraline algae





Less growth

Lower calcification

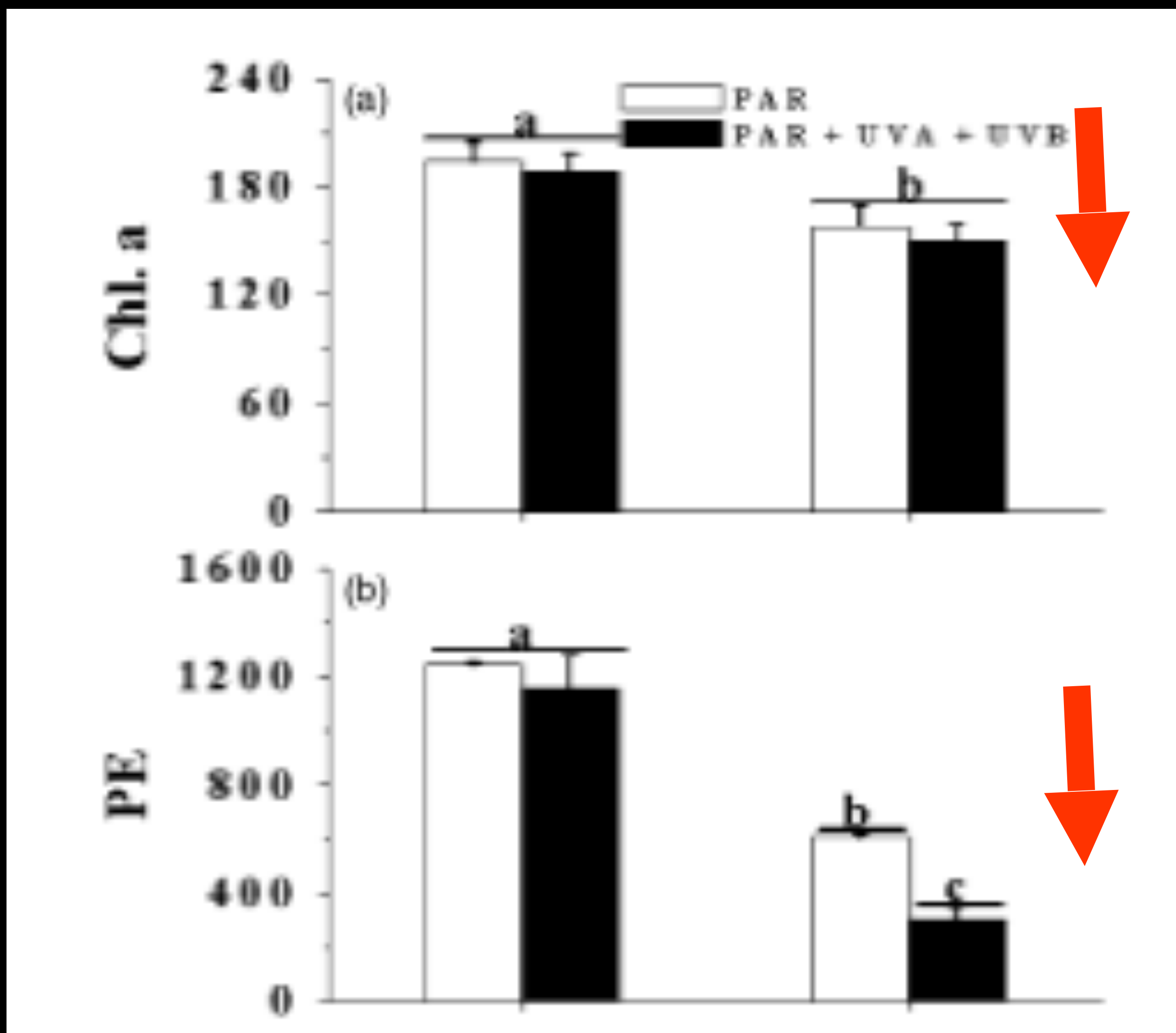


UV-induced photosynthetic inhbition

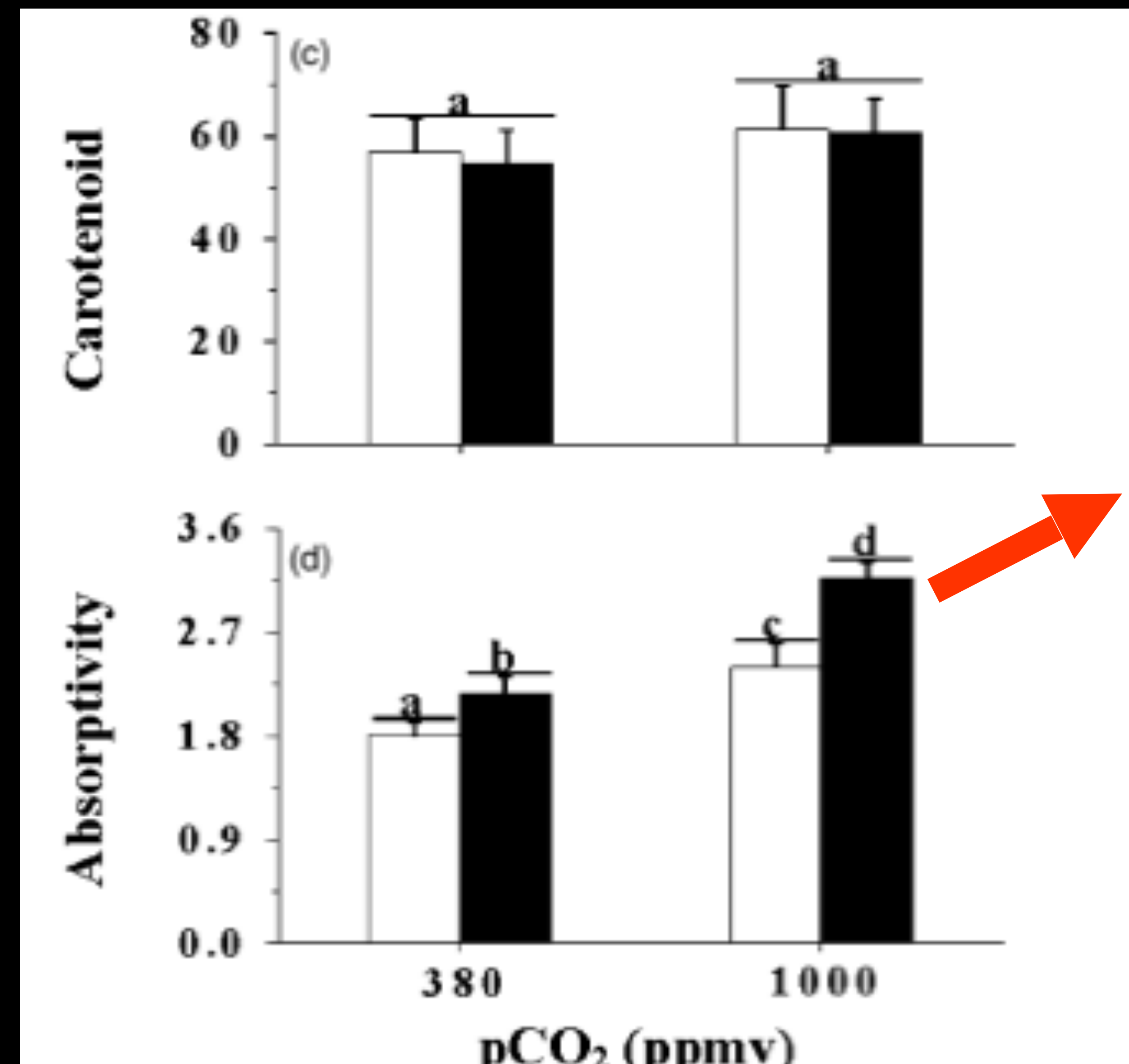
Gao and Zheng 2010 *Global Change Biol*.

UV-absorbing compounds

Photosynthetic pigments

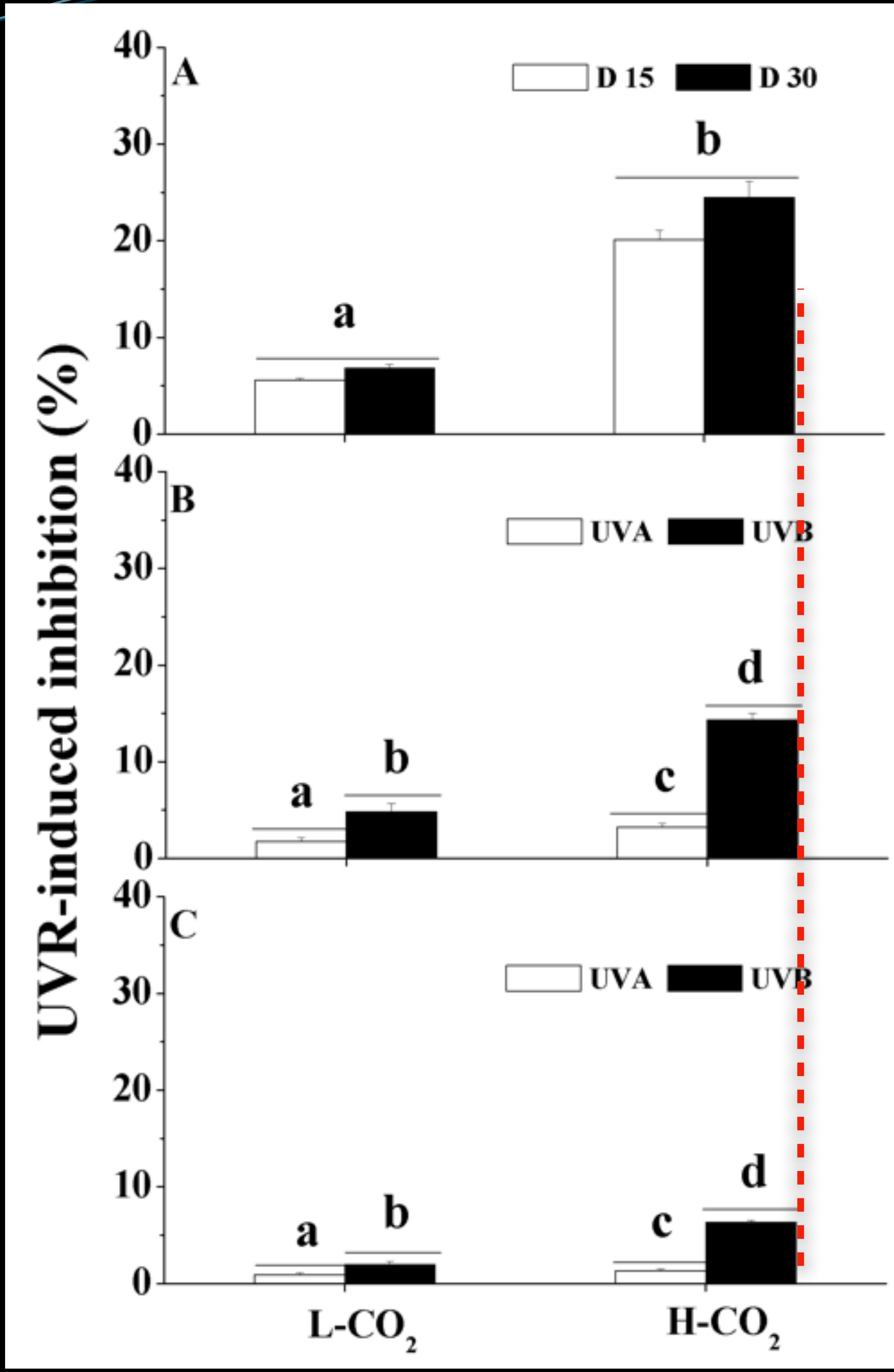


380 1000 ppmv



Gao and Zheng 2010 *Global Change Biol*.

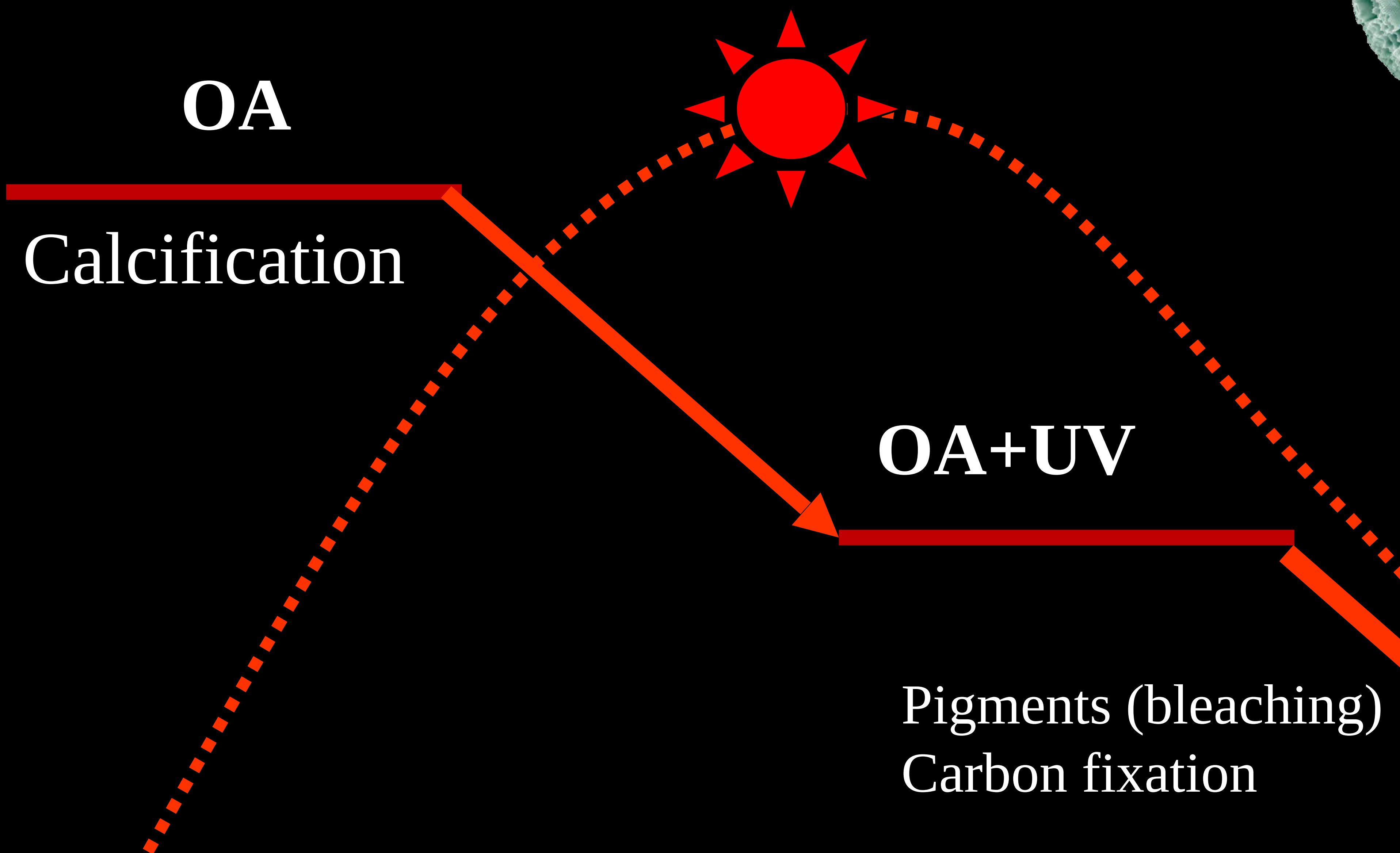
UVB（0.5-0.8% of PAR in terms of engergy) results in higher inhibition than UVA（14-16% of PAR） under influence of ocean acidification



Photosynthesis

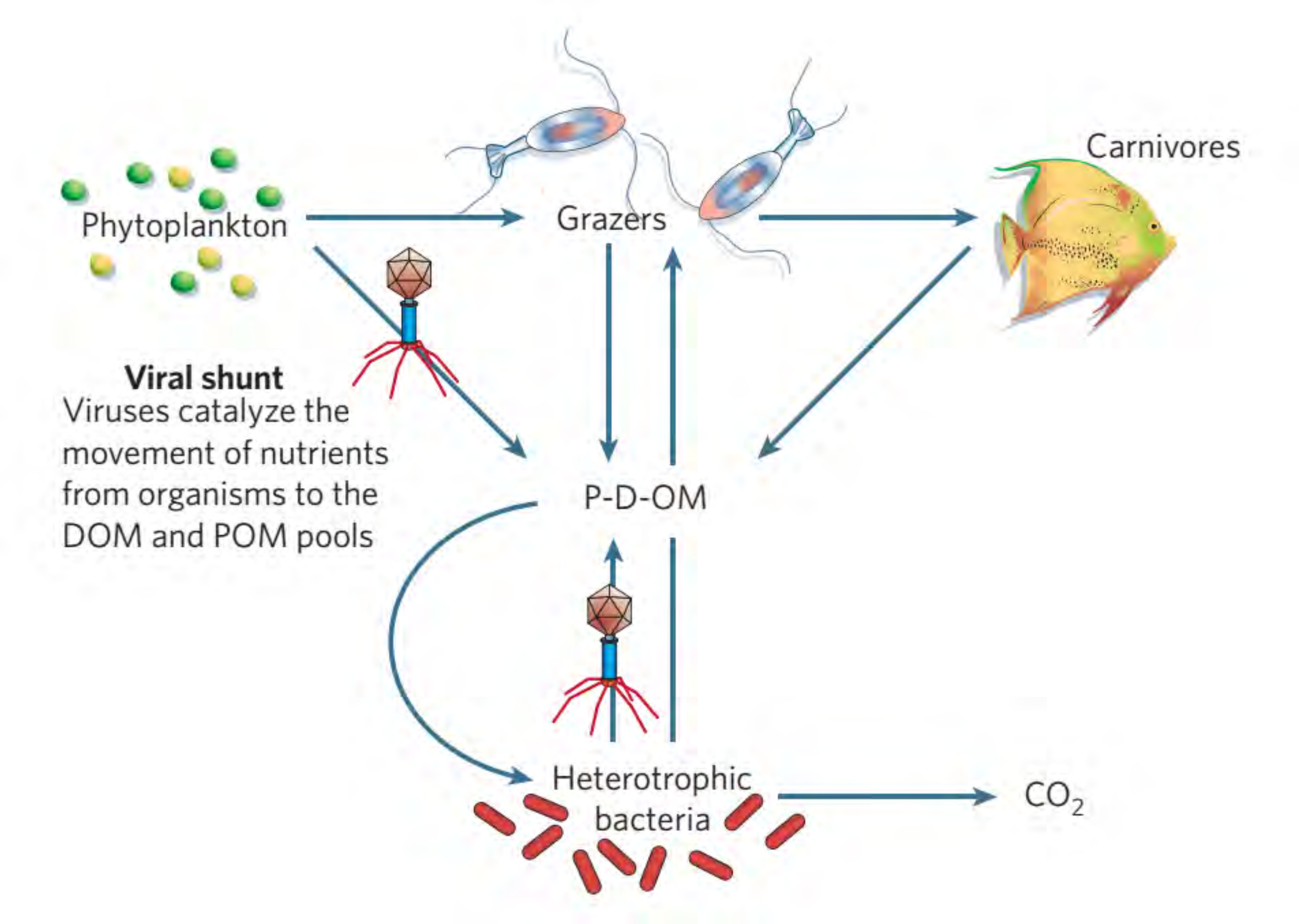
Calcification

Gao and Zheng 2010 *Global Change Biol*.

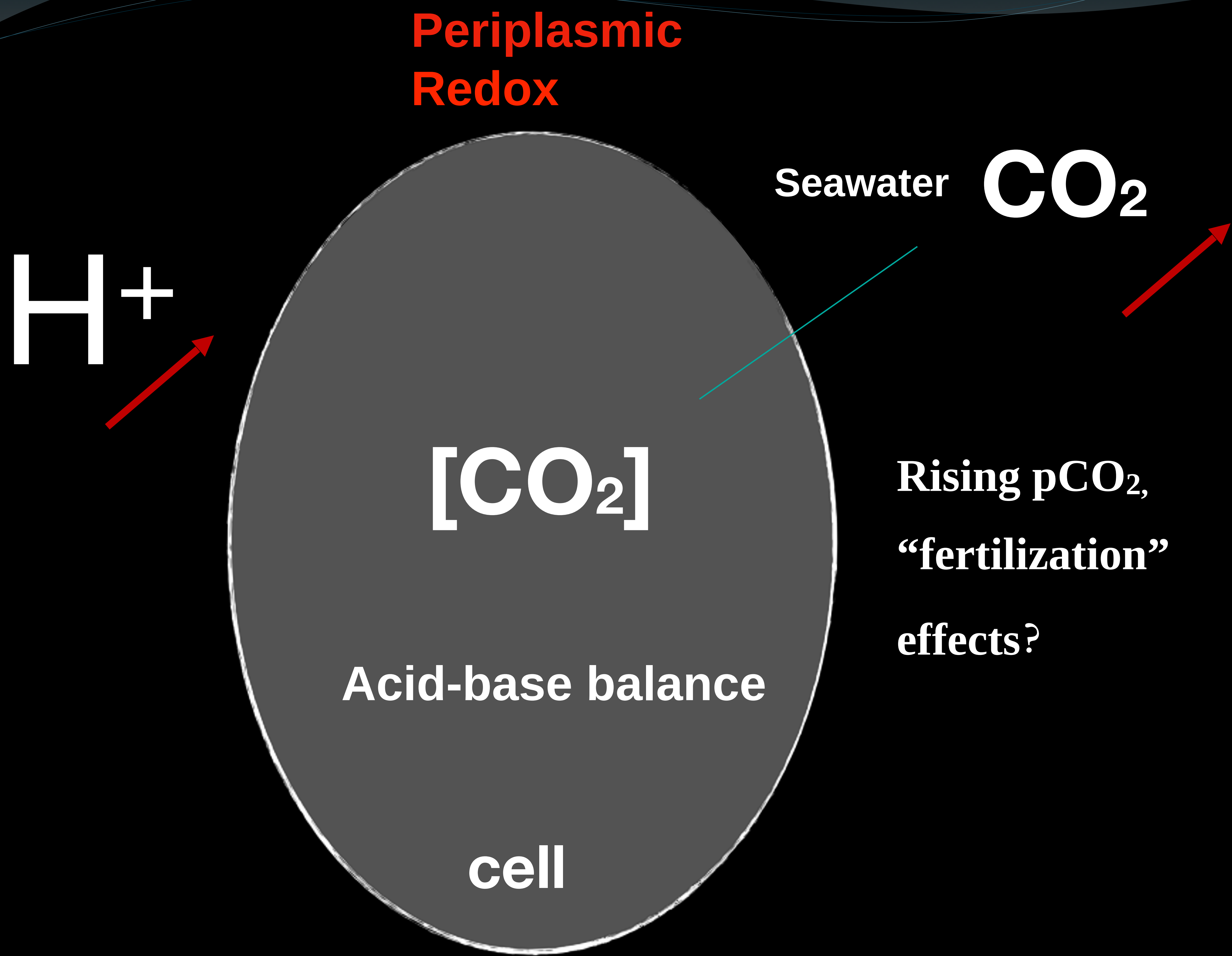


Ocean acidification decreases calcification of calcareous algae， enhancing solar exposures, leading to bleaching and further decrease in calcification

Virus as a bio-stressor



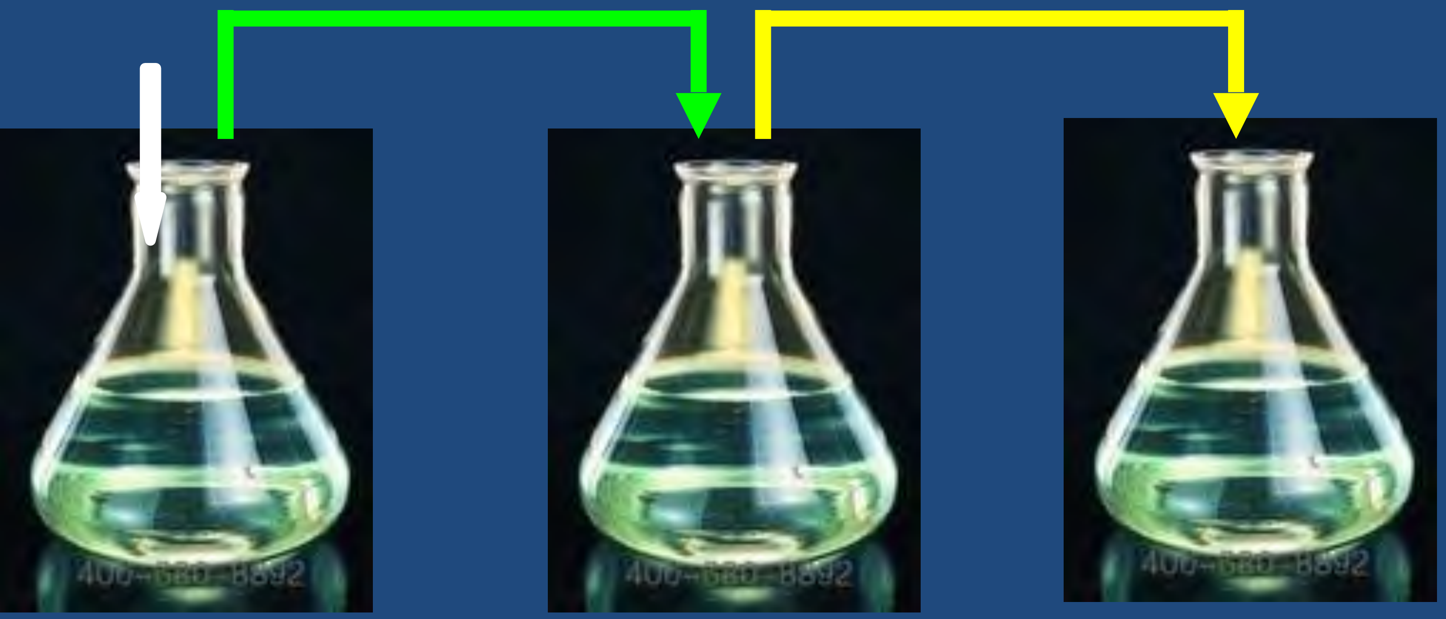
Viral abundance in natural seawater 10^4-10^8 particles mL^{-1}

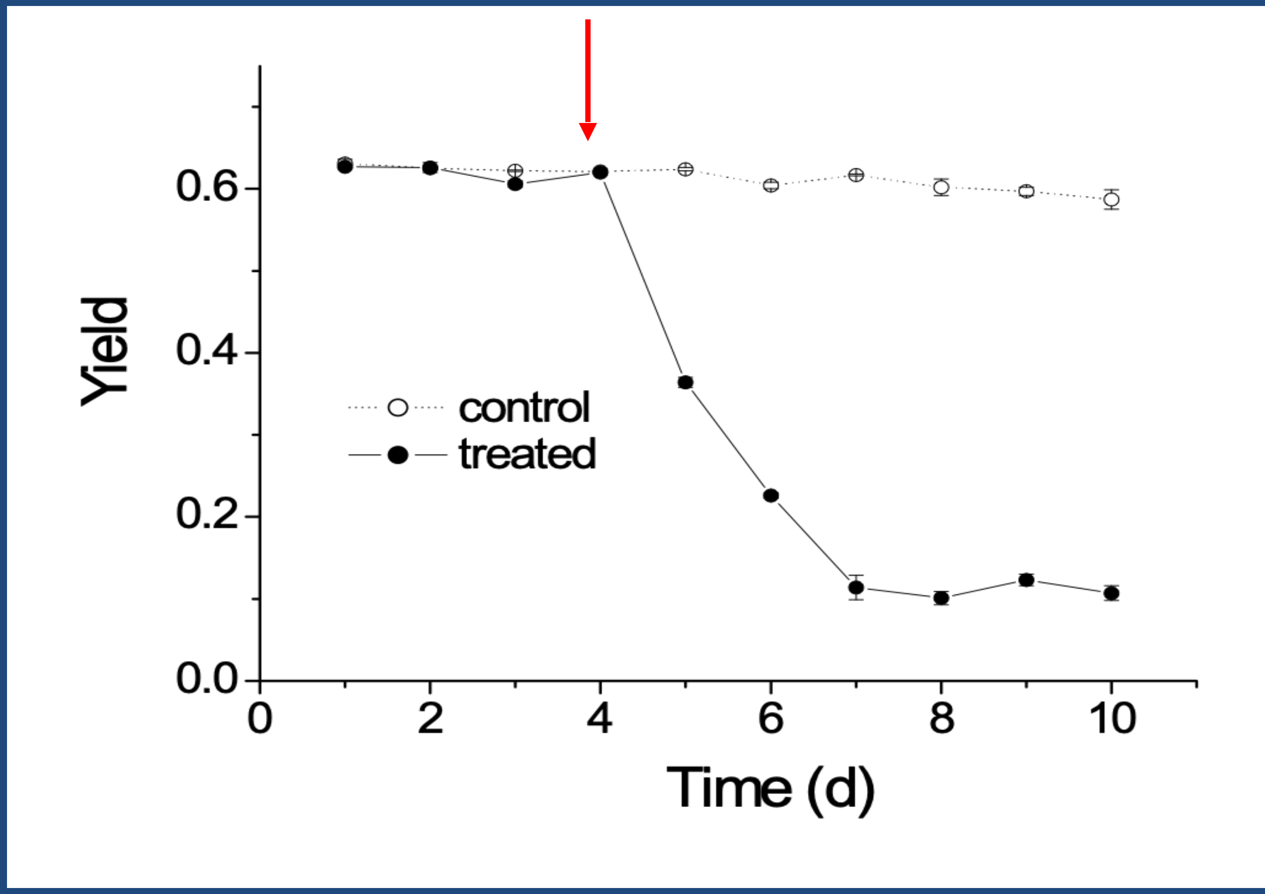

Periplasmic
Redox
Seawater CO_2
H^+
$[CO_2]$
Acid-base balance
cell
Rising pCO_2,
"fertilization"
effects?

General hypothesis

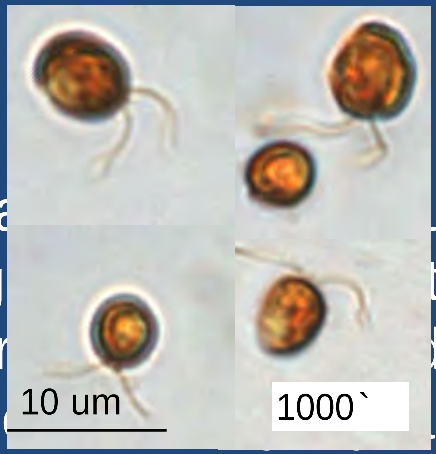
- Changes in carbonate chemistry, induced by OA, can influence Redox activity at cell surface
- Such changes may affect viral attack to the host

Isolation and cloning of PgV





Viruses of *Phaeocystis globosa* (PgVs) were isolated, in November 2007, from the coastal waters of Shantou (23.3 °N, 116.6°E), when the algal bloom occurred. Seawater (10 L) was sampled at the end point of the algal bloom and filtered through 0.2 μm pore-size cellulose acetate filters .The filtrate was then concentrated, by an ultrafiltration disc to 100 mL.The concentrated virus-size fraction was used for inocula, and the clonal isolate of PgV was obtained by a modified serial infection procedure .



Modified serial infection procedure: The virus-size fraction concentrate wa… ures of *P. globosa* at 1% (vol/vol) and incubated for 10 days, during which time algal g… tored via *in vivo* chlorophyll fluorescence. Samples from cultures in which lysis occurr… through 0.2 μm pore-size cellulose acetate filters and a crude PgV lysate was obtain… te was added at 10% (vol/vol) to exponentially growing *P. globosa* cultures and incubated for 7 days, during which time algal growth was monitored again as above. The clonal lysate was obtained after the above procedure was repeated six times.

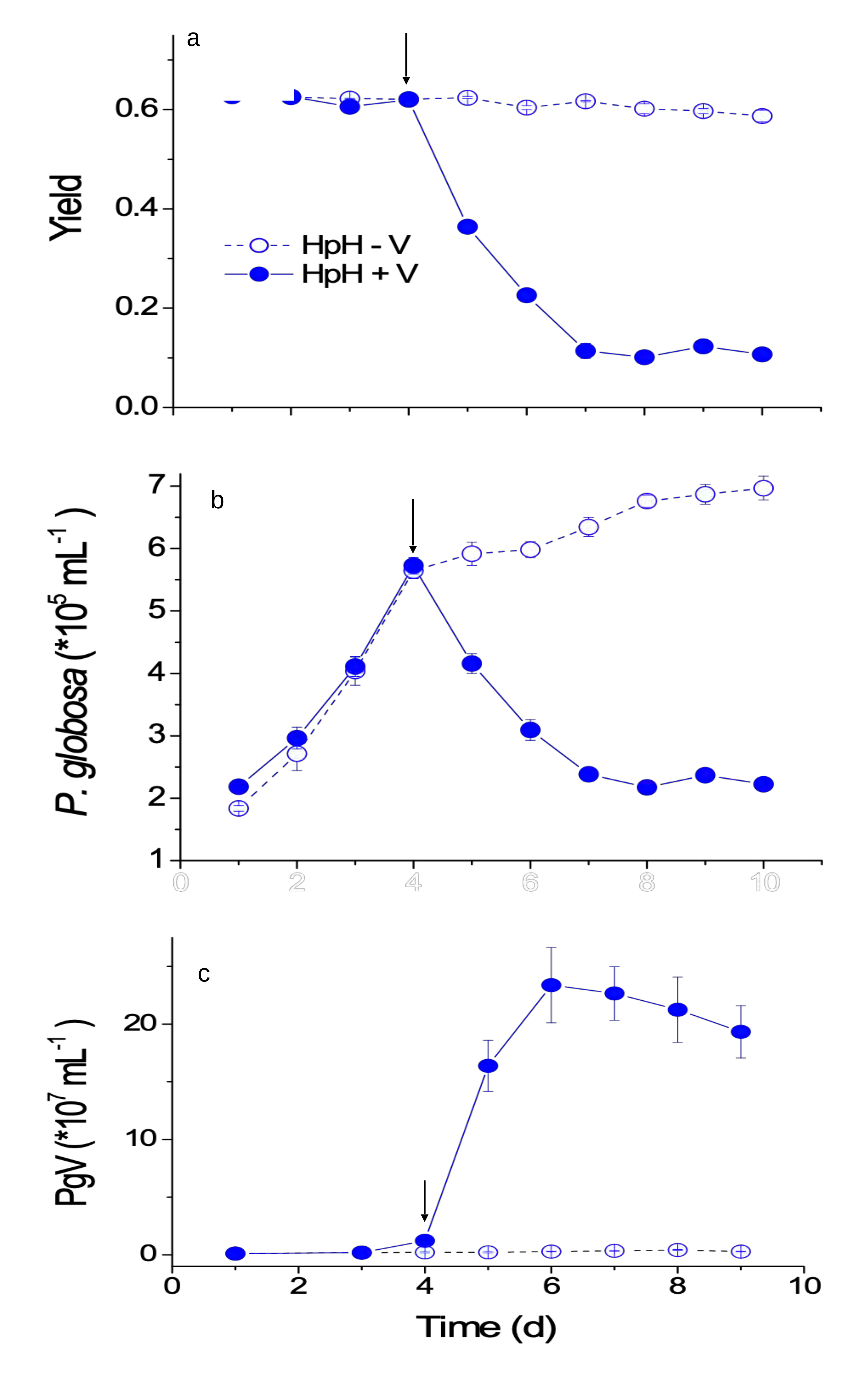


Fig. S2 Effective photochemical quantum yield (a) and cell density (b) of *P. globosa* and abundance of PgV (c) during viral infection of ambient-air-grown cultures. Open symbols represent uninfected cultures, while the solid symbols represent cultures to which PgVs were added at the time indicated by the arrows. The data represent the mean ± SD (n=3, triplicate cultures).

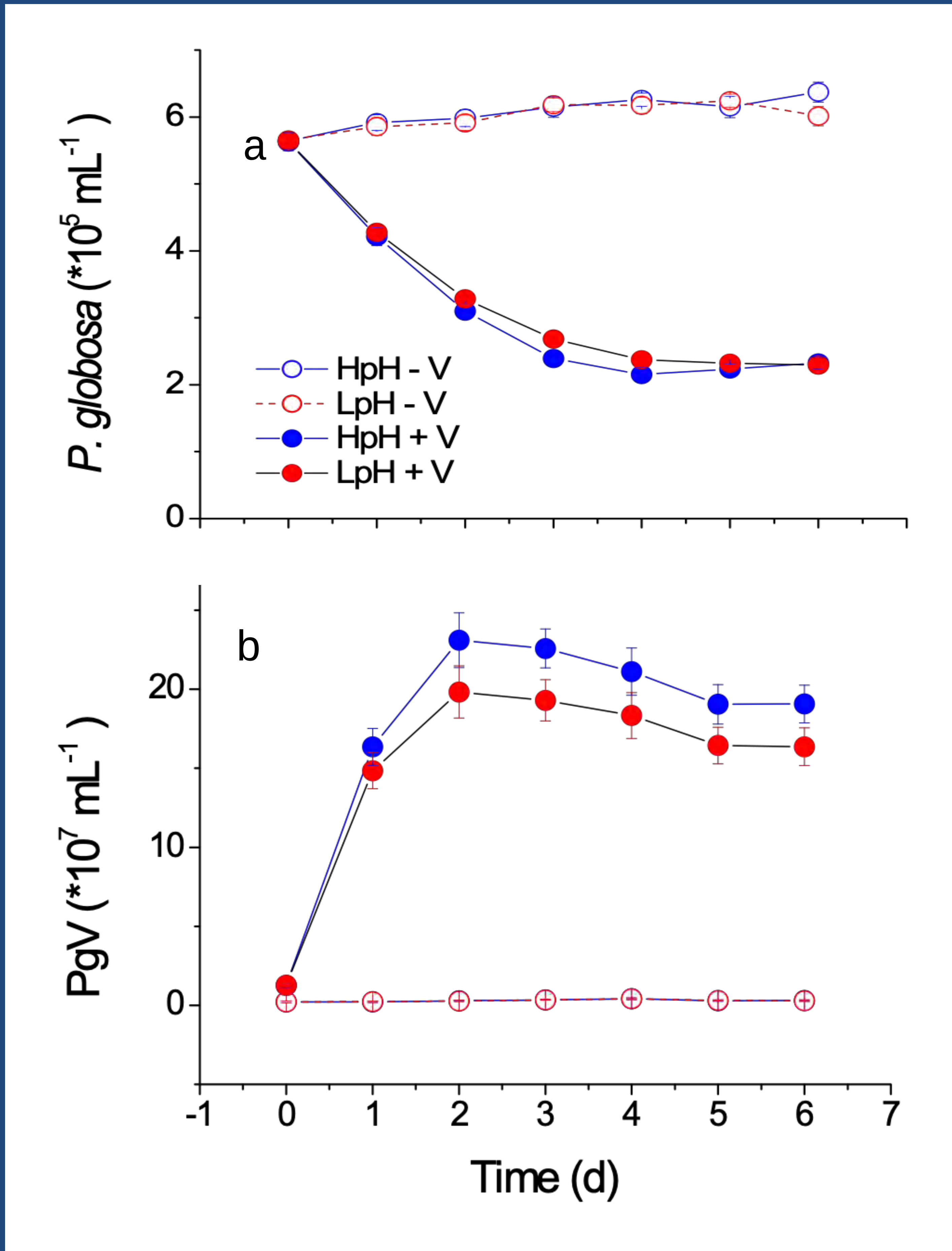
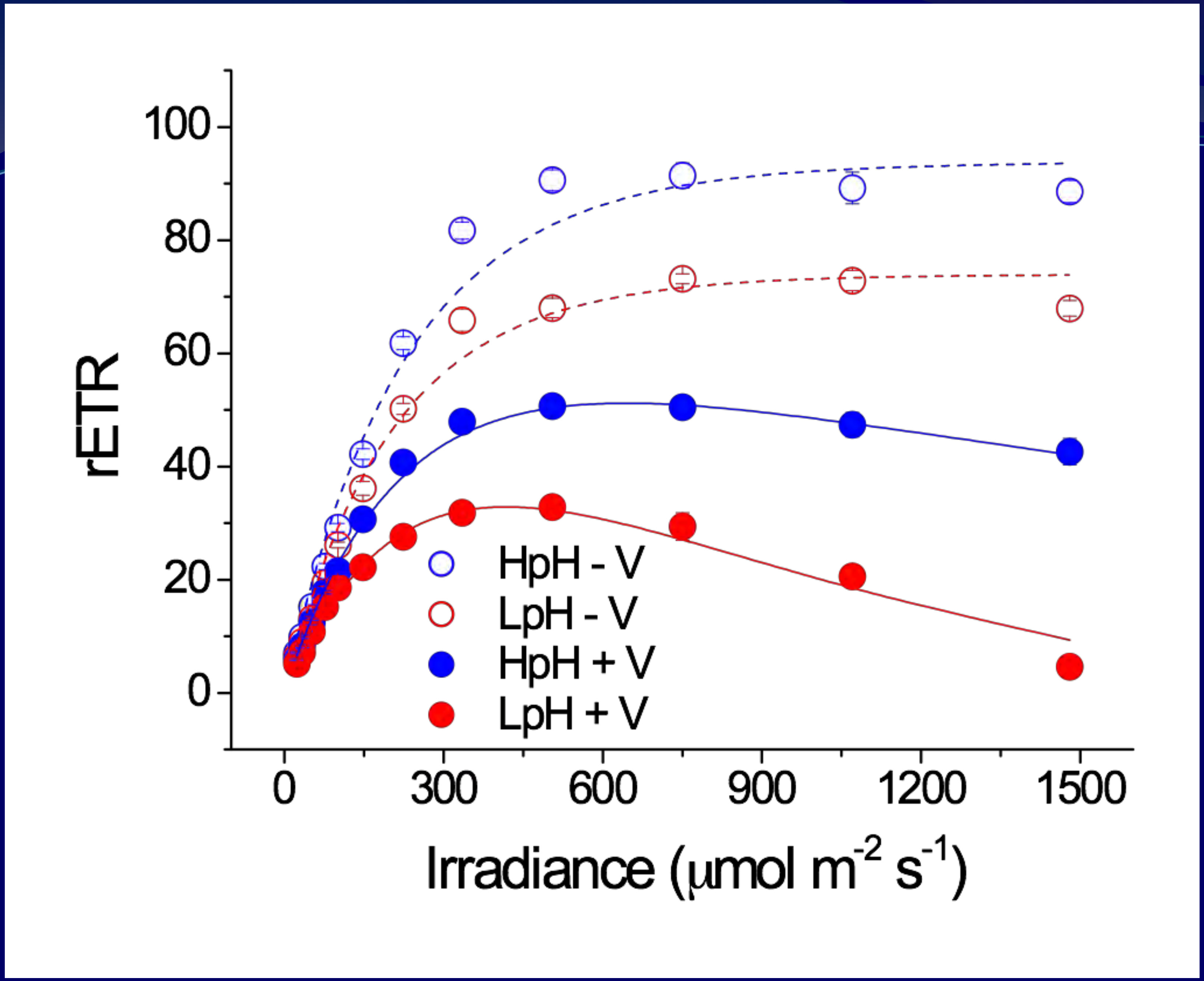
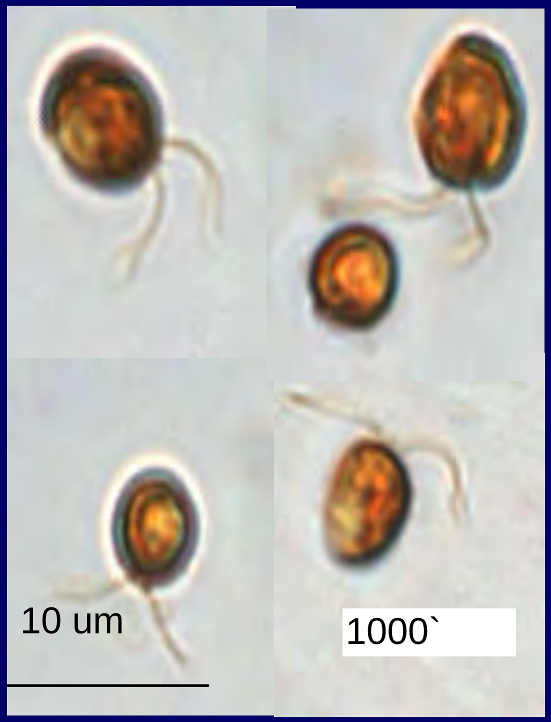


Fig. 3 Changes in cell (a) and virus (b) concentration of *P. globosa* during the burst size determination under different CO_2 (pH) treatments. HpH (= ambient CO_2) represents pH_{nbs}8.07; LpH (=high CO_2), pH_{nbs}7.70; V, virus. The data represent mean ± SD (n = 3, triplicate cultures).





Chen et al. 2015 *Global Change Biology*

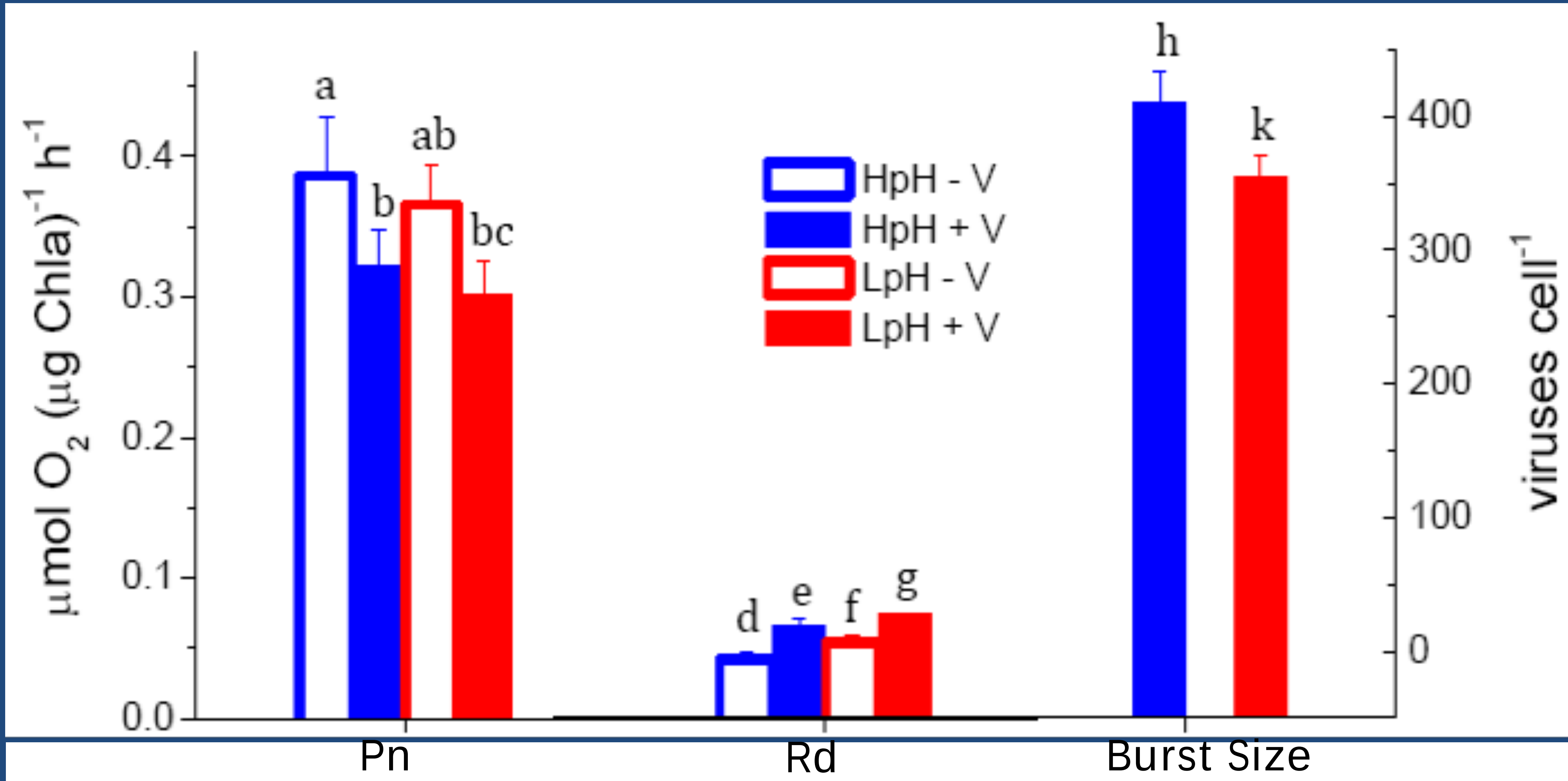


Fig. 2 Effects of ocean acidification on the interaction of *P. globosa* with its virus. HpH represents pH_{nbs}8.07; LpH, pH_{nbs}7.70; V, virus; P_n, net photosynthesis; R_d, dark respiration. Different superscript letters represent significant differences ($p<0.05$) among the treatments. The data represent means ± SD (n = 3).

Viral infection reduced the Pn by 16.6% in the high-pH and by 16.7% in the low-pH grown *P. globosa*. Both Low pH treatment and viral infection significantly increased the alga's mitochondrial respiration by 28.6% and 56.7%, respectively. In the ambient CO_2 grown cells, the stimulation of respiration following infection was 57.4%, but in the high CO_2 grown cells, the stimulation after infection was 38.8%.

Chen et al. 2015 *Global Change Biology*

Summary

- Ocean acidification (OA) (pCO2 rise) enhances diatoms growth under low and inhibits it under high levels of solar radiation

- OA increases phenolics contents in micro algae, stimulating Kreb cycle and ß-oxidation

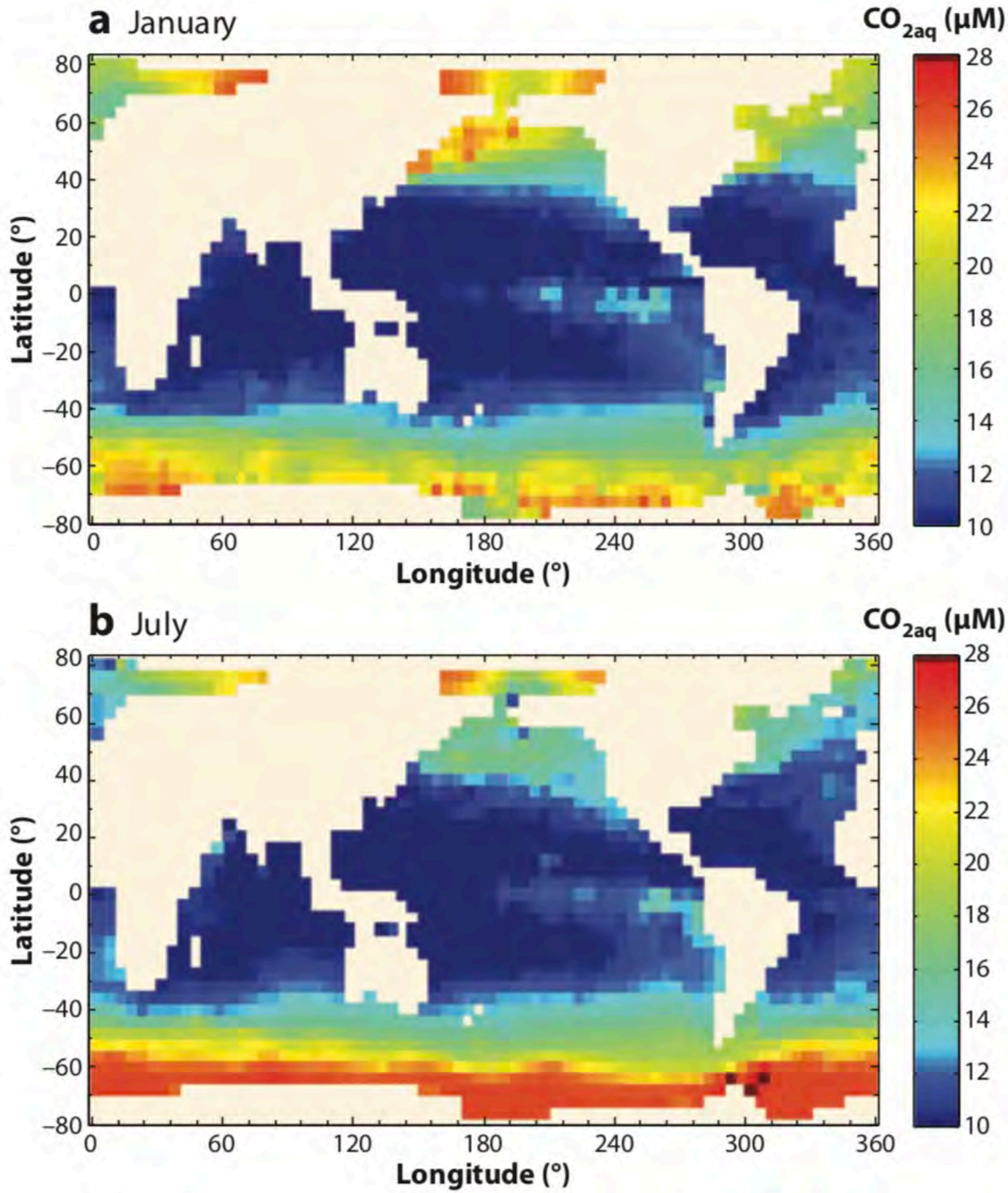
- OA and UV synergistically reduce calcification of coralline algae and coccolithophores

- Seawater acidification exacerbates virus attack to the red tide alga

Funded by

NSF-C
MOST

2018 FOCE mesocosm Experiment（involved 12 Labs from 2 Universities）



Surface Seawater pCO_2

July, much of the area of high CO_{2aq} in the

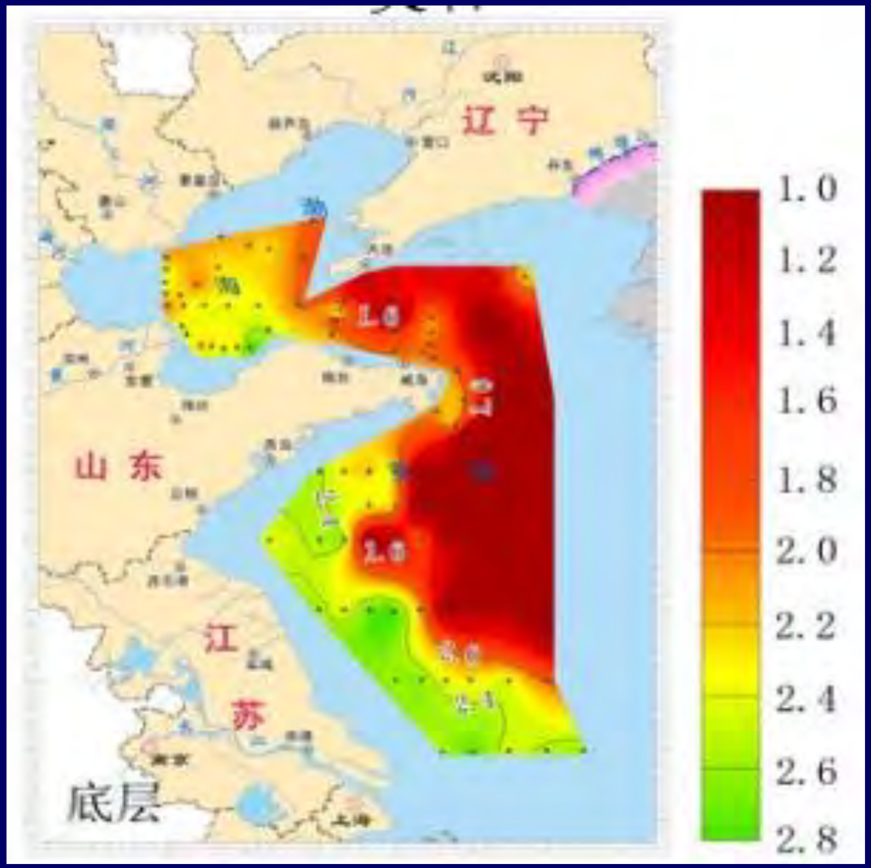
Southern Ocean south of 60 S is under ice

Reinfelder 2011
Ann Rev Mar Sci

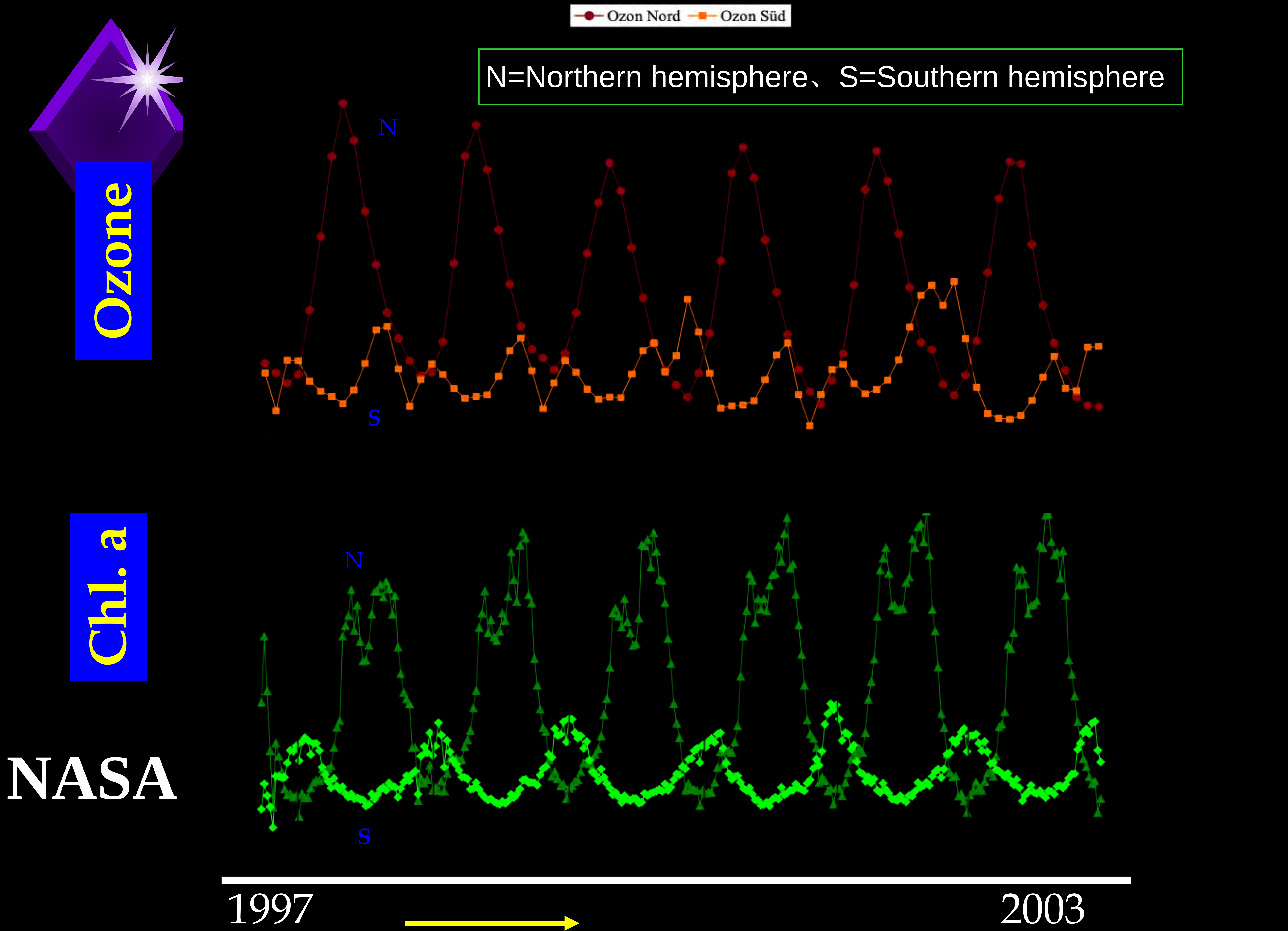
Documented low pH in the Chinese coastal waters

Regions	Low pH
The Bohai Sea (***Chinese Science Bulletin 2012)***	7.64 H^+ rise by 220%
The Yellow Sea（***Biogeosciences 2014***）	7.80
The East China Sea (***Biogeosciences 2013)***	7.80
The Northern South China Sea（*JGR* 2011)	7.9

Ocean acidification is occurring in the Chinese waters.



"中国海洋环境公报 2012"
China marine Environ Report



Ozon Nord
Ozon Süd
N=Northern hemisphere、S=Southern hemisphere
Ozone
N
S
Chl. a
N
S
NASA
1997
2003

Enhanced UV-B (280-315 nm, <1% solar PAR) due to ozone depletion is harmful to most organisms

Normal levels of solar UV-B is also harmful, damage bio-molecules/DNA

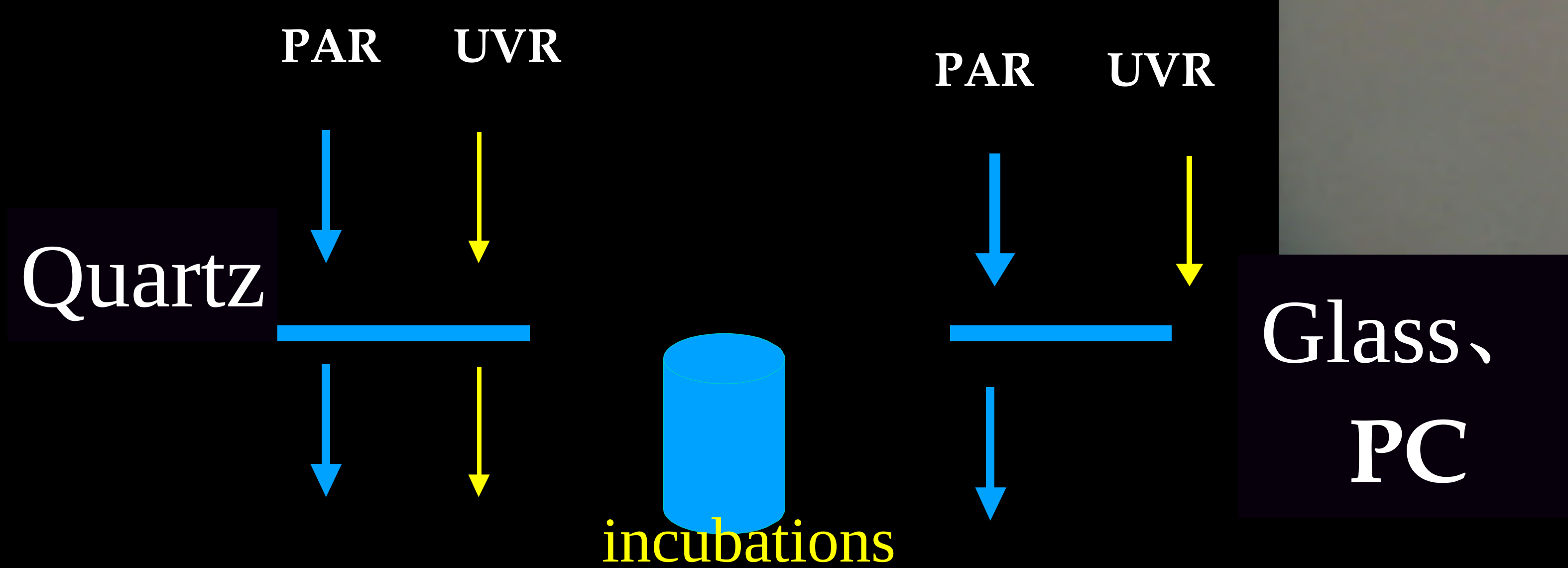
Solar UV-A (315-400 nm, about 14-16% solar PAR) could be harmful or stimulative in terms of repairing UV-B-induced damages and enhancing photosynthesis, depending on its exposure levels

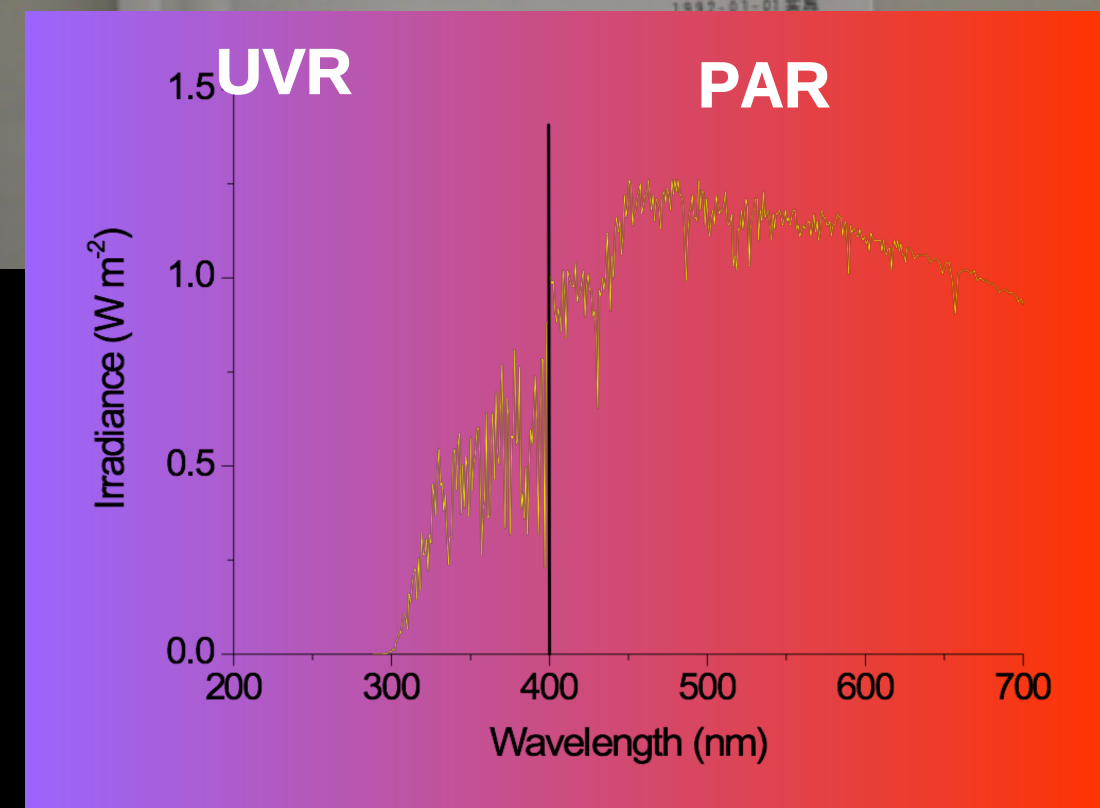
JGOFS Protocols

Chapter 19. Primary Production by ^{14}C

1.0 Scope and field of application

5.8 *Incubation Bottles:* Polycarbonate 0.25 l bottles are used for the productivity incubations. New bottles are soaked for 72 hours in a 5% solution of Micro detergent. Bot-





Marine Primary productivity investigation protocols have neglected UV radiation