Effects of Ocean Acidification on Primary Producers

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(Xiamen University)
Ocean Acidification

Ocean acidification alters carbonate chemistry

\[ \Omega = \frac{[\text{CO}_3^{2-}]_{\text{MEAS}}}{[\text{CO}_3^{2-}]_{\text{CAL}}} \]

Gattuso et al. 2015 Science
Marine Photosynthesis drives oceanic biological CO2 pump that takes up (per hr) over 100 million tons of fossil fuel CO₂. Marine Photosynthesis accounts for about 50%. 

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)

- Stimulating
- Neutral
- Inhibitive


Growth/Photosynthesis/Respiration/Calcification/N₂ fixation
CO2 rise and acidic stress: double edged?

Effects of ocean acidification?

\[ \Omega = \frac{[\text{CO}_3^{2-}]_{\text{MEAS}}}{[\text{CO}_3^{2-}]_{\text{CAL}}} \]
FOCE: Free Ocean CO2 Enrichment Exp.
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）
Supplementary Table 2. Locations of the stations, cruise information, sea surface temperature (SST, °C) and pH7, NO3−+NO2− (N, μmol L−1) and PO43− (P, μmol L−1), solar PAR (mean, μmol photons m−2 s−1) during 14C-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 CO2 (LC,385 μatm) and high CO2 (HC, 800 μatm for all stations except SEATS and C3, where 1000 μatm CO2 was applied), and the primary productivity (PP, triplicate incubations, μg C L−1 h−1) by the phytoplankton assemblages grown in the low CO2 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).

<table>
<thead>
<tr>
<th>Station</th>
<th>Location</th>
<th>Season*</th>
<th>SST</th>
<th>pH7</th>
<th>N</th>
<th>P</th>
<th>Solar PAR</th>
<th>Incubation time (h)</th>
<th>Chl a</th>
<th>Chl a (LC)</th>
<th>Chl a (HC)</th>
<th>PP</th>
</tr>
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<tbody>
<tr>
<td>LE04</td>
<td>(18.0°N, 113.0°E)</td>
<td>Summer</td>
<td>29.5</td>
<td>8.03</td>
<td>BLQ</td>
<td>0.014</td>
<td>1681</td>
<td>6</td>
<td>0.05</td>
<td>0.15</td>
<td>0.13</td>
<td>0.10±0.08</td>
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<tr>
<td>PN07</td>
<td>(30.0°N, 124.5°E)</td>
<td>Summer</td>
<td>29.6</td>
<td>8.03</td>
<td>BLQ</td>
<td>0.019</td>
<td>1371</td>
<td>6</td>
<td>0.71</td>
<td>Nd</td>
<td>nd</td>
<td>0.18±0.12</td>
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<tr>
<td>A4</td>
<td>(20.8°N, 115.2°E)</td>
<td>Autumn</td>
<td>25.5</td>
<td>8.04</td>
<td>BLQ</td>
<td>0.156</td>
<td>794</td>
<td>6</td>
<td>0.44</td>
<td>1.08</td>
<td>0.69</td>
<td>2.73±0.32</td>
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<tr>
<td>E606</td>
<td>(18.9°N, 114.1°E)</td>
<td>Autumn</td>
<td>25.3</td>
<td>8.06</td>
<td>BLQ</td>
<td>BLQ</td>
<td>821</td>
<td>6</td>
<td>0.34</td>
<td>0.82</td>
<td>0.20</td>
<td>4.74±0.10</td>
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<td>SEATS</td>
<td>(18.0°N, 116.0°E)</td>
<td>Spring</td>
<td>28.7</td>
<td>8.04</td>
<td>BLQ</td>
<td>0.037</td>
<td>1251</td>
<td>12 (24)</td>
<td>0.10</td>
<td>0.49</td>
<td>0.59</td>
<td>2.08±0.14  (19.80±1.09)**</td>
</tr>
<tr>
<td>C3</td>
<td>(20.6°N, 114.2°E)</td>
<td>Spring</td>
<td>28.5</td>
<td>8.03</td>
<td>BLQ</td>
<td>0.032</td>
<td>1027</td>
<td>12 (24)</td>
<td>0.21</td>
<td>0.42</td>
<td>0.36</td>
<td>1.83±0.06  (16.28±0.73)**</td>
</tr>
</tbody>
</table>
Gao et al. 2012 Nature Climate Change

Per volume of seawater

Per Chl. a

Diatom abundance

NPQ

High light-stress

Gao et al. 2012 Nature Climate Change
Acidic stress + photorespiration

Primary production

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

CCMs

HCO$_3^-$ → CO$_2$

Calvin cycle

Acidic stress + photorespiration
Primary production
High-CO2 grown *Phaedactylum constatum*

Lower (3-4 times) intracellular DIC

Liu et al. 2017 *Aquatic Microbial Ecology*
Rubisco → Carboxylation
Rubisco → Oxygenation

Intracellular $\text{CO}_2/\text{O}_2$ ratio

Oxygenation → Photorespiration

Photorespiration alleviates costs extra energy, releasing more CO2

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

因此，光呼吸的重要功能之一即在于光合CO2缺少时保持卡尔文循环的运转。
HC >20%

Gao et al. 2012 *Nature Climate Change*
Xu and Gao 2012  Plant Physiol.
Energetic costs: CCMs↓, acidic stress/photo-stress

CO2-fertilization: CO₂, HCO₃⁻

Low light

Enhance

Photosynthesis or Growth

High Light

Inhibit

Photorespiratory CO₂

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.
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.
The RGR of the young sporophytes *Ulva prolifera* grown under low and high CO$_2$ condition.

*Xu and Gao 2012  Plant Physiol.*
From the same batch of conchospores

50 individuals each treatment
Responses

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

- Changes with Increased $pCO_2$
- Physiological tipping point
- Acidification

<table>
<thead>
<tr>
<th>Growth</th>
<th>Maintenance</th>
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<tr>
<td>8.1</td>
<td>7.9</td>
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<tr>
<td>8.0</td>
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<tr>
<td>7.5</td>
<td>7.3</td>
</tr>
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<td>7.4</td>
<td>7.2</td>
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</table>
Respiration rate in phytoplankton under OA and control

**Lab**

<table>
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<th></th>
<th>HC</th>
<th>LC</th>
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</thead>
<tbody>
<tr>
<td>Respiration rate (fmoles O₂ per cell per h)</td>
<td>16</td>
<td>11</td>
</tr>
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</table>

*130% increase*

*Emiliania huxleyi*

**Mesocosm**

<table>
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<tr>
<th></th>
<th>HC</th>
<th>LC</th>
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<tbody>
<tr>
<td>Respiratory carbon loss rate (µg C (µg Chl a)⁻¹ d⁻¹)</td>
<td>150</td>
<td>75</td>
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</table>

*160% increase*

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

- **Mesocosm**: 4000L
- **Microcosm**: 30L
- **Lab**: 1L
- **Phytoplankton assemblages**: Single species
- **Mixed phytoplankton species**
Protein analysis

Sample preparation

Protein separation

Image acquisition

Image analysis

TOF 5800 Proteomics Analyzer

Protein spotting

Protein lyse

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

<table>
<thead>
<tr>
<th>Spot Id.</th>
<th>Protein identity</th>
<th>GI number</th>
<th>Protein score C.I. (%)</th>
<th>Total Ion C.I. %</th>
<th>Protein score (peptides)</th>
<th>MW/pI</th>
<th>Fold change</th>
<th>Function</th>
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<td>3</td>
<td>Propionyl CoA synthase</td>
<td>239994558</td>
<td>100</td>
<td>100</td>
<td>357(14)</td>
<td>69708.5/5.51</td>
<td>2.33</td>
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<td>4</td>
<td>Serine protein kinase</td>
<td>239995429</td>
<td>100</td>
<td>99.946</td>
<td>177(15)</td>
<td>74347.3/5.31</td>
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<td>9</td>
<td>Hypothetical protein AmacA_2</td>
<td>223994739</td>
<td>100</td>
<td>100</td>
<td>805(22)</td>
<td>51069.6/5.61</td>
<td>2.01</td>
<td>1.00</td>
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<td>11</td>
<td>Hypothetical protein MDMS009_211</td>
<td>254489880</td>
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<td>100</td>
<td>440(11)</td>
<td>447891.1/4.87</td>
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<td>12</td>
<td>Methane/ phenol/ toluene hydroxylase</td>
<td>148260382</td>
<td>100</td>
<td>100</td>
<td>238(5)</td>
<td>39315.7/5.76</td>
<td>3.40</td>
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<td>13</td>
<td>Acyl-CoA dehydrogenase family protein</td>
<td>83943662</td>
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<td>100</td>
<td>438(18)</td>
<td>44108.4/5.55</td>
<td>1.00</td>
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<td>14</td>
<td>Chloroplast glyceraldehyde-3-phosphate dehydrogenase</td>
<td>77024139</td>
<td>100</td>
<td>100</td>
<td>336(7)</td>
<td>44096.1/5.2</td>
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<td>15</td>
<td>Conserved hypothetical protein (bacterium S5)</td>
<td>288797257</td>
<td>100</td>
<td>99.996</td>
<td>166(7)</td>
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<td>2.50</td>
<td>1.00</td>
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<td>17</td>
<td>Enoyl-CoA hydratase</td>
<td>83955054</td>
<td>99.996</td>
<td>98.89</td>
<td>115(8)</td>
<td>28178.9/5.51</td>
<td>3.82</td>
<td>1.00</td>
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<td>Adenylate kinase</td>
<td>239993306</td>
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<td>600(16)</td>
<td>23693/4.99</td>
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<td>TRAP-T family protein transporter periplasmic binding protein</td>
<td>83943788</td>
<td>100</td>
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<td>811(17)</td>
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<td>24</td>
<td>Nucleoside diphosphate kinase</td>
<td>114765301</td>
<td>100</td>
<td>100</td>
<td>352(6)</td>
<td>15293.7/4.93</td>
<td>1.00</td>
<td>2.10</td>
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</table>
Altered metabolic pathways under OA

Jin et al. 2015  *Nature Communications*
Contents of phenolic compounds in phytoplankton

Lab test

**Phaeodactylum tricornutum**

- 25% after 20 generations
- 37% after 2000 generations

**Emiliania huxleyi**

- 56% increase

Former Ph D student: Yahe Li
Former Ph D student: Peng Jin

Jin et al. 2015 *Nature Communications*
• Kreb cycle and β-oxidation pathways are upregulated under OA, leading to higher contents of toxic phenolics

Jin et al. 2015 *Nature Communications*
Contents of phenolic compounds in zooplankton that were fed on phytoplankton (HC, LC) from microcosm and mesocosm systems

**Microcosm**

**Mesocosm**

Jin et al. 2015 *Nature Communications*
Ocean acidification increases the accumulation of toxic phenolic compounds across trophic levels

Peng Jin¹†, Tifeng Wang¹, Nana Liu¹, Sam Dupont², John Beardall³, Philip W. Boyd⁴, Ulf Riebesell⁵ & Kunshan Gao¹

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)
Monitored every second

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
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.
Fig. 9: (A) Diameter of the cells grown under different pH levels. +Co, cell with coccolith; -Co, cell without coccolith removed by bubbling CO2 for 30 seconds. (B) Thickness of coccolith layer derived from the difference in diameter of the cells with and without coccolith. Values are means ± S.D. (n ≥ 100)

P: PAR
PA: PAR+UVA
PAB: PAR+UVA+B
Corraline algae
Par, filled symbols

UV-induced inhibition of photosynthesis
inhibition of calcification caused by UV A and UVB

Gao and Zheng 2010 *Global Change Biol.*

**Coralline algae**

Less growth

Lower calcification

UV-induced photosynthetic inhibition

Gao and Zheng 2010 *Global Change Biol.*
UV-absorbing compounds

Photosynthetic pigments

Gao and Zheng 2010 *Global Change Biol.*
UVB (0.5-0.8% of PAR in terms of energy) results in higher inhibition than UVA (14-16% of PAR) under influence of ocean acidification.

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$^{-1}$
Rising pCO₂, “fertilization” effects?

Acid-base balance

CO₂

Seawater

Periplasmic Redox

H⁺

[CO₂]

Cell
General hypothesis

- Changes in carbonate chemistry, induced by OA, can influence **Redox** activity at the 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 was added to cultures of *P. globosa* at 1% (vol/vol) and incubated for 10 days, during which time algal growth was monitored via *in vivo* chlorophyll fluorescence. Samples from cultures in which lysis occurred were filtered through 0.2 µm pore-size cellulose acetate filters and a crude PgV lysate was obtained. The PgV lysate 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).
Fig. 2 Effects of ocean acidification on the interaction of *P. globosa* with its virus. HpH represents pH_{8.07}; LpH, pH_{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 CO2 grown cells, the stimulation of respiration following infection was 57.4%, but in the high CO2 grown cells, the stimulation after infection was 36.8%.

Chen et al. 2015 *Global Change Biology*
• 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 β-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

<table>
<thead>
<tr>
<th>Regions</th>
<th>Low pH</th>
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</thead>
<tbody>
<tr>
<td>The Bohai Sea (<em>Chinese Science Bulletin 2012</em>)</td>
<td>7.64</td>
</tr>
<tr>
<td></td>
<td>H⁺ rise by 220%</td>
</tr>
<tr>
<td>The Yellow Sea (<em>Biogeosciences 2014</em>)</td>
<td>7.80</td>
</tr>
<tr>
<td>The East China Sea (<em>Biogeosciences 2013</em>)</td>
<td>7.80</td>
</tr>
<tr>
<td>The Northern South China Sea (<em>JGR 2011</em>)</td>
<td>7.9</td>
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</tbody>
</table>

Ocean acidification is occurring in the Chinese waters.

“中国海洋环境公报 2012”
China marine Environ Report
Chlorophyll North vs. South

Chl. a

Ozone

N=Northern hemisphere, S=Southern hemisphere

1997         2003

N=Northern hemisphere, S=Southern hemisphere

NASA
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 biomolecules/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.
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. Bottles are incubated under PAR and UVR conditions with Quartz or Glass, and PC for Marine Primary productivity investigation protocols have neglected UV radiation.