Ocean Acidification International Coordination Centre
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UNIVERSITY OF GOTHENBURG

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THE ROYAL SWEDISH ACADEMY OF SCIENCES

Basic training course on ocean acidification

EVT1804704

14-19 March 2022

How to manipulate the chemistry

Preliminary considerations

- ✓ Ocean acidification is a multistressor change
  What parameter(s) matter for my organism/ecosystem?

- ✓ Do I want to keep the tested parameter (e.g. pH) constant or fluctuating?

- ✓ Is my experiment *realistic* (mimicking ocean acidification) *or mechanistic* (testing physiological hypothesis)?

What is a realistic ocean acidification carbonate chemistry change?

	$pCO_{2\,sw}$ (μatm)	pH_T (–)	$[H^+]$ (a)	TA (b)	DIC (b)	$[CO_2]$ (b)	$[HCO_3^-]$ (b)	$[CO_3^{2-}]$ (b)	Ω_c (–)	Ω_a (–)
Year 2007	384	8.065	8.6	2325	2065	12.8	1865	187	4.5	2.9
Year 2100	793	7.793	16.1	2325	2191	26.4	2055	110	2.6	1.7
	↑	↓		=			↑	↓	↓	↓

Part 1: Seawater carbonate chemistry

2 Approaches and tools to manipulate the carbonate chemistry

Jean-Pierre Gattuso[1,2], Kunshan Gao[3], Kitack Lee[4], Björn Rost[5] and Kai G. Schulz[6]

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Important to use water with the same properties than the sampling site

Take home message

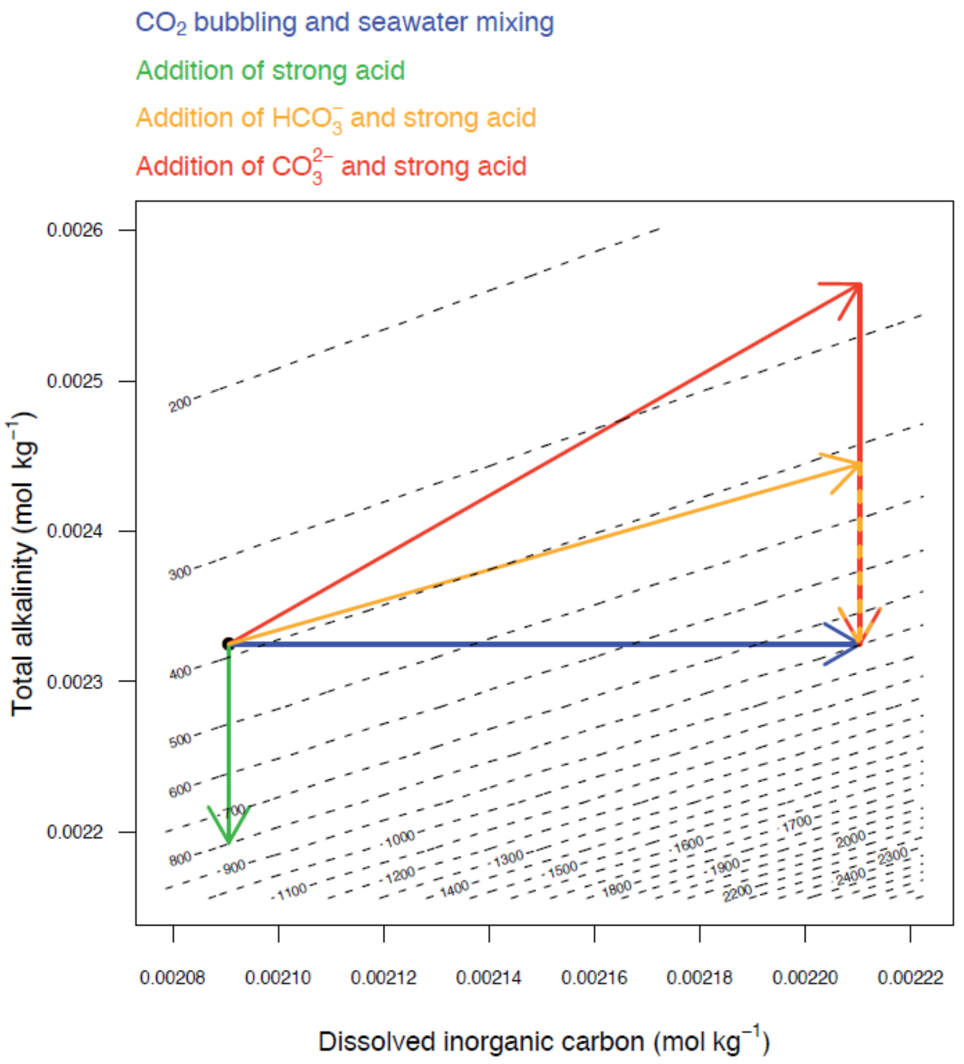
- ✓ Many methods are available to manipulate the carbonate chemistry for an experiment
- ✓ Whatever laboratory and equipment you have, there is a method for you

NON-best practice methods

- ✓ Add strong acid (e.g. HCl)
- ✓ Add HCO_3^-, CO_3^{2-}

	$pCO_{2\,sw}$ (μatm)	pH_T (–)	$[H^+]$ (a)	TA (b)	DIC (b)	$[CO_2]$ (b)	$[HCO_3^-]$ (b)	$[CO_3^{2-}]$ (b)	Ω_c (–)	Ω_a (–)
Year 2007	384	8.065	8.6	2325	2065	12.8	1865	187	4.5	2.9
Year 2100	793	7.793	16.1	2325	2191	26.4	2055	110	2.6	1.7
Addition of CO_3^{2-} and HCO_3^-; closed sys.	793	7.942	11.4	3406	3146	26.4	2901	218	5.2	3.4
Addition of CO_3^{2-} and HCO_3^-; open sys.	384	8.207	6.2	3406	2950	12.8	2580	357	8.5	5.5
Acid addition; closed sys.	793	7.768	17.1	2184	2065	26.4	1940	98	2.3	1.5
Acid addition; open sys.	384	8.042	9.1	2184	194	12.8	1767	167	4	2.6

Add strong acid, HCO_3^- and CO_3^{2-}



Add strong acid, HCO_3^- and CO_3^{2-}

	$pCO_{2\,sw}$ (μatm)	pH_T (–)	$[H^+]$ (a)	TA (b)	DIC (b)	$[CO_2]$ (b)	$[HCO_3^-]$ (b)	$[CO_3^{2-}]$ (b)	Ω_c (–)	Ω_a (–)
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Addition of:										
CO_3^{2-} and HCO_3^-; closed sys.	400	8.073	8.4	2467	2191	13.3	1977	201	4.8	3.1
followed by acid addition; closed sys.	793	7.793	16.1	2325	2191	26.4	2055	110	2.6	1.7

Precise, cheap, easy (e.g. field) to prepare water with desire chemistry

No compensation for biology and atmosphere, manual changes

Mix High CO_2 water

Seawater
(filtered, aerated
384µatm; pH 8.1)

Heavily bubble
with pure CO_2 for
2 minutes
(pH ~5.5)

Seawater
(pH 8.1)

Mix till reach the
desire pH/pCO_2

Mix High CO_2 water

	pCO_{2sw} (μatm)	pH_T (–)	$[H^+]$ (a)	TA (b)	DIC (b)	$[CO_2]$ (b)	$[HCO_3^-]$ (b)	$[CO_3^{2-}]$ (b)	Ω_c (–)	Ω_a (–)
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Addition of high-CO_2 seawater	792	7.793	16.1	2325	2191	26.4	2055	110	2.6	1.7

Precise, cheap, easy (e.g. field) to prepare water with desire chemistry

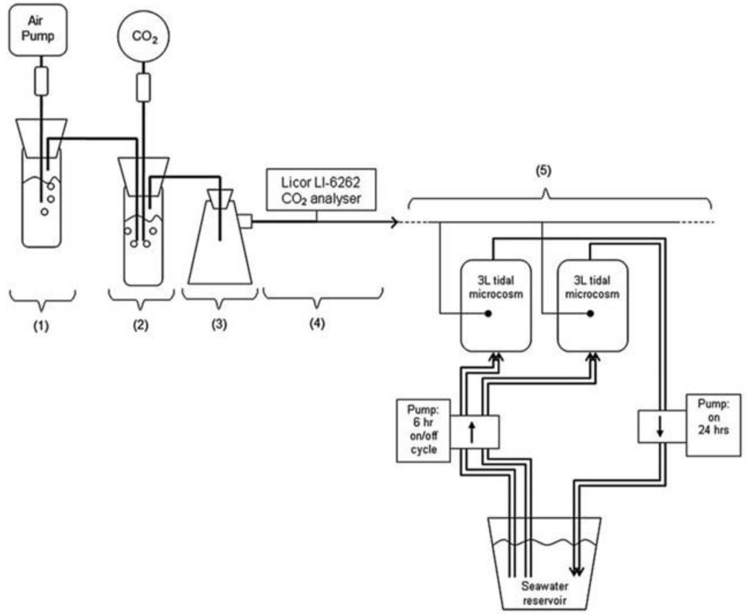
No compensation for biology and atmosphere, manual changes

Bubbling with CO_2

- ✓ Bubble with CO_2 at the target concentration (ppm)
  - Buy pre-mixed gas (expensive)

Bubbling with CO_2

✓ Bubble with CO_2 at the target concentration (ppm)
  - Buy pre-mixed gas (expensive)
  - Gas mixer (manual)
  - Gas mixer (automatic)

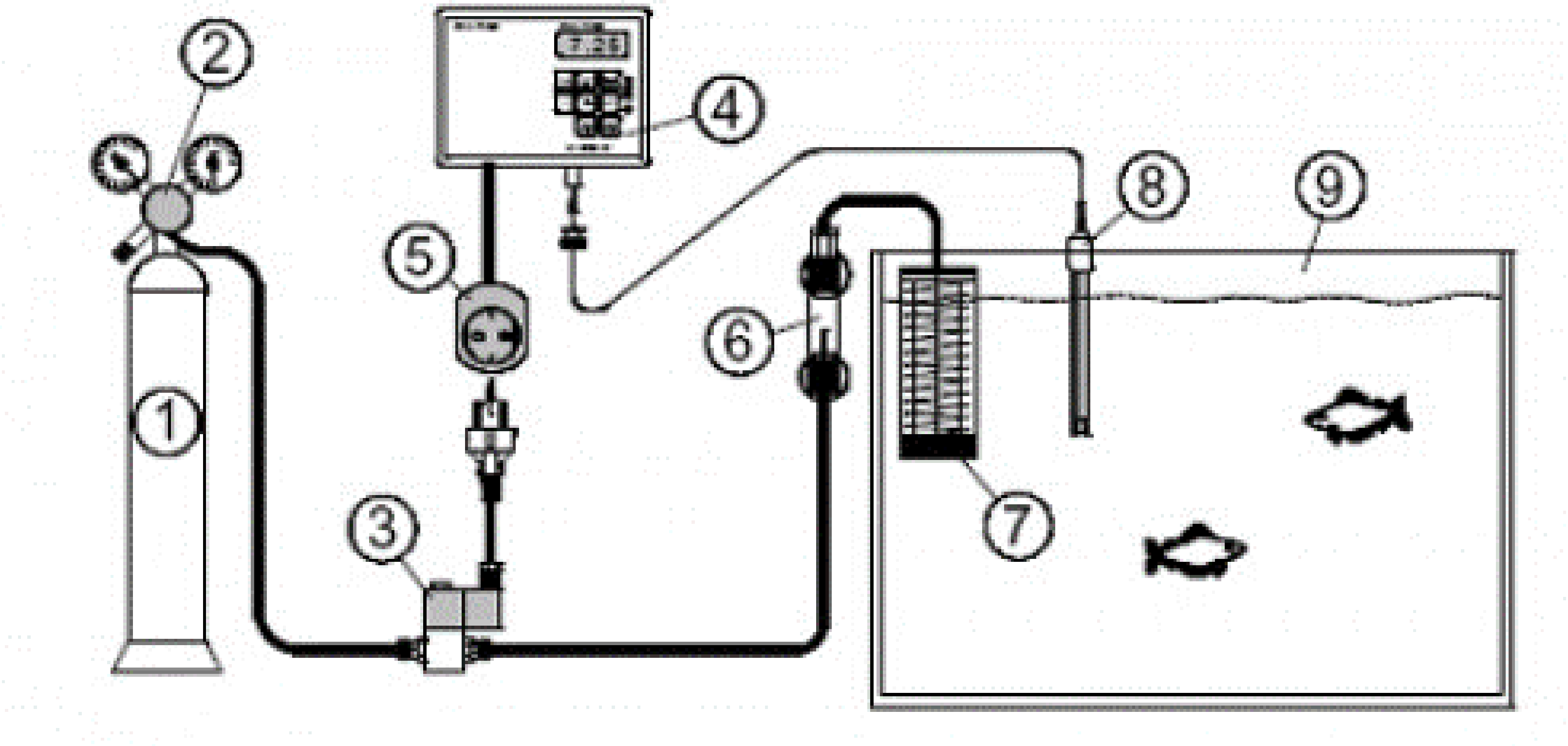


(e.g. Findlay et al. 2008)

Bubbling with CO_2

- ✓ Bubble with CO_2 at the target concentration (ppm)
  - Buy pre-mixed gas (expensive)
  - Gas mixer (manual)
  - Gas mixer (automatic)
- ✓ Bubble with pure CO_2
  - pH stats

pH stat



Bubbling with CO_2

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Gas bubbling	793	7.793	16.1	2325	2191	26.4	2055	110	2.6	1.7

→ Precise, more or less easy, compensation for respiration/photosynthesis, dynamic control

More expensive (equipment, gas), may limit replication (e.g. pH stats)

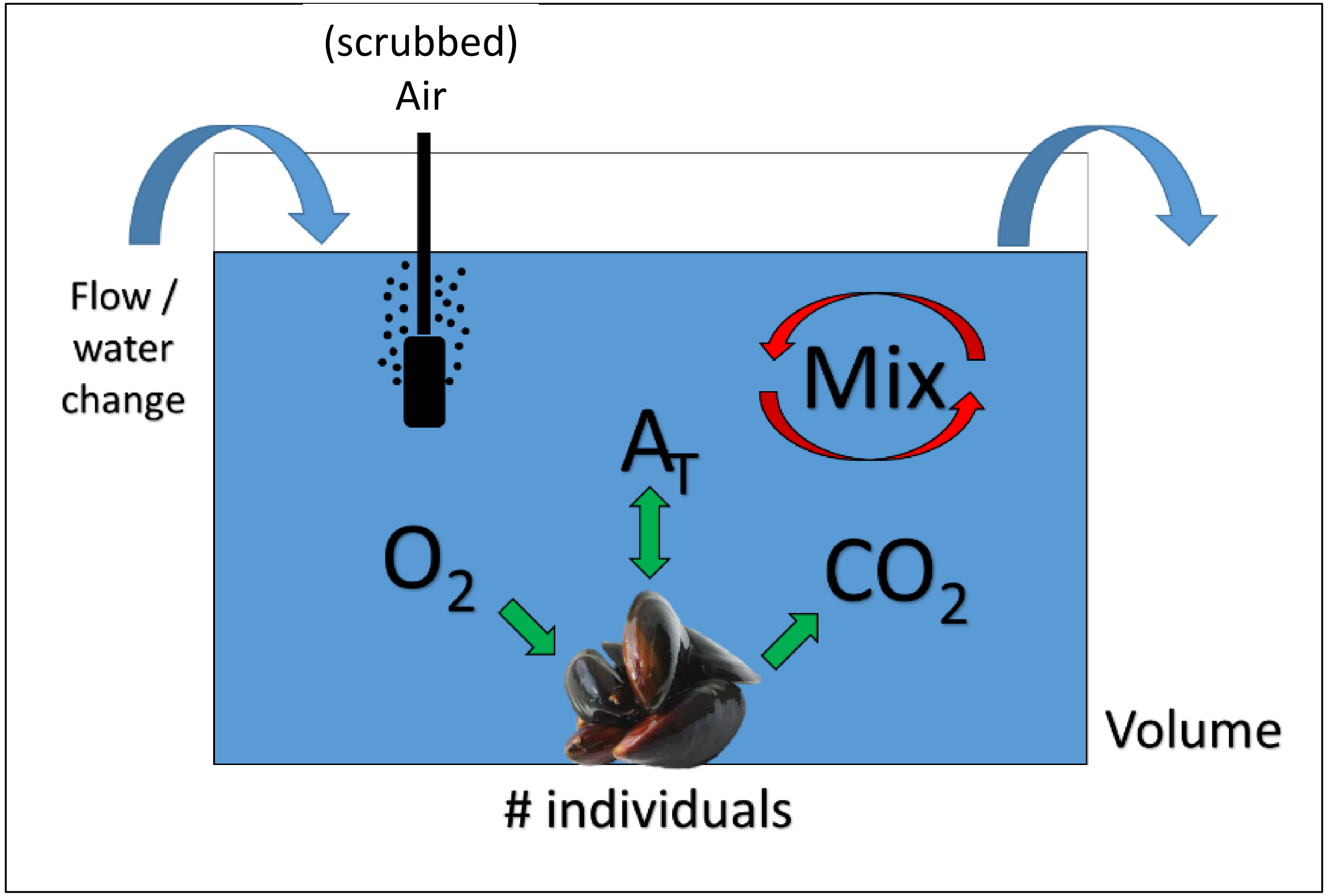
Summary: 3 best practice methods

- ✓ Add strong acid, HCO_3^- and CO_3^{2-}
- ✓ Mix High CO_2 waters
- ✓ Bubble CO_2
  - Keep CO_2 constant
  - Keep pH constant

Batch of seawater

Dynamic control

What to consider to keep the chemistry constant?



Sometime, you need to filter the water (NOT autoclave)

Example: manually made seawater, little biology, closed system

No contact with air

Example: manually made seawater, little biology, closed system

No contact with air

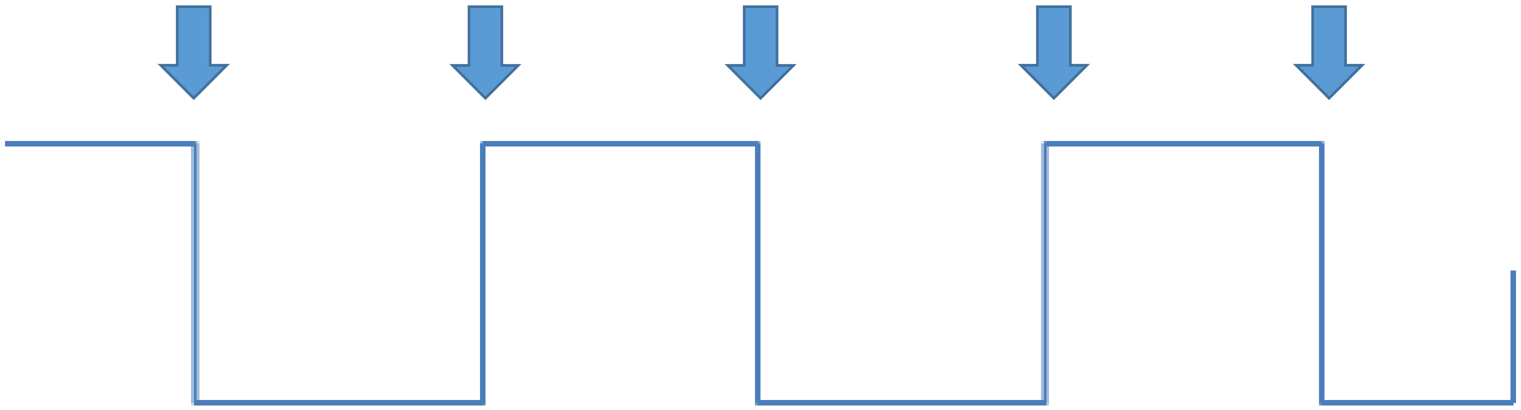
Fluctuating chemistry

- ✓ Chemistry is rarely stable in the field. It can be desirable to include variability into experimental design:
  - Realistic (mimicking field)
  - Mechanistic

Methods to make chemistry fluctuate

- ✓ Manual water change
- ✓ Creative use of pH or pCO_2 stats

Water change or
Alternate between 2 pHstats



Methods to make chemistry fluctuate

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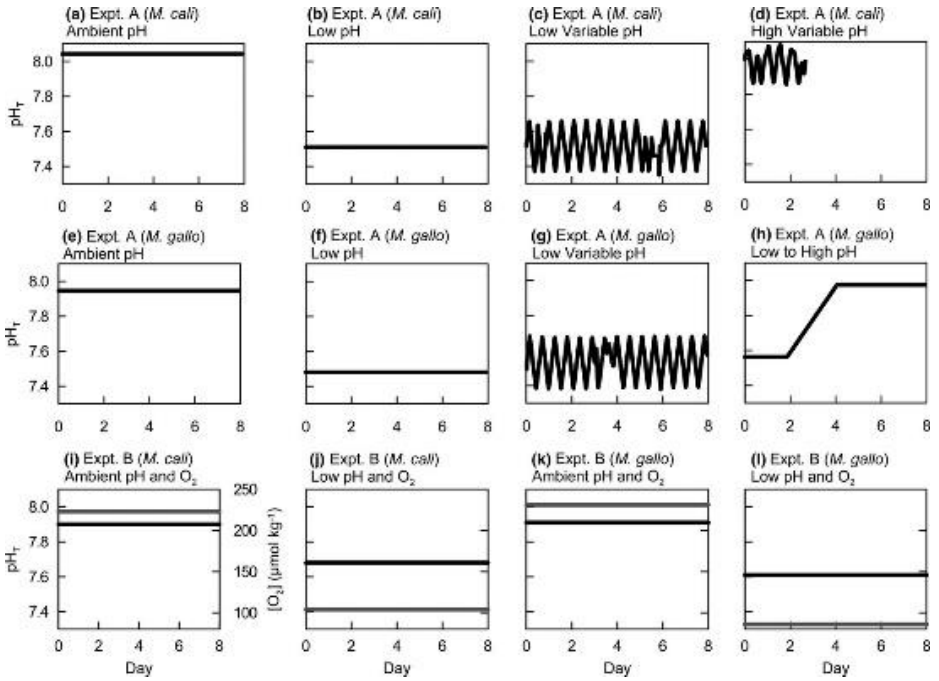


Global Change Biology

Primary Research Article | Full Access

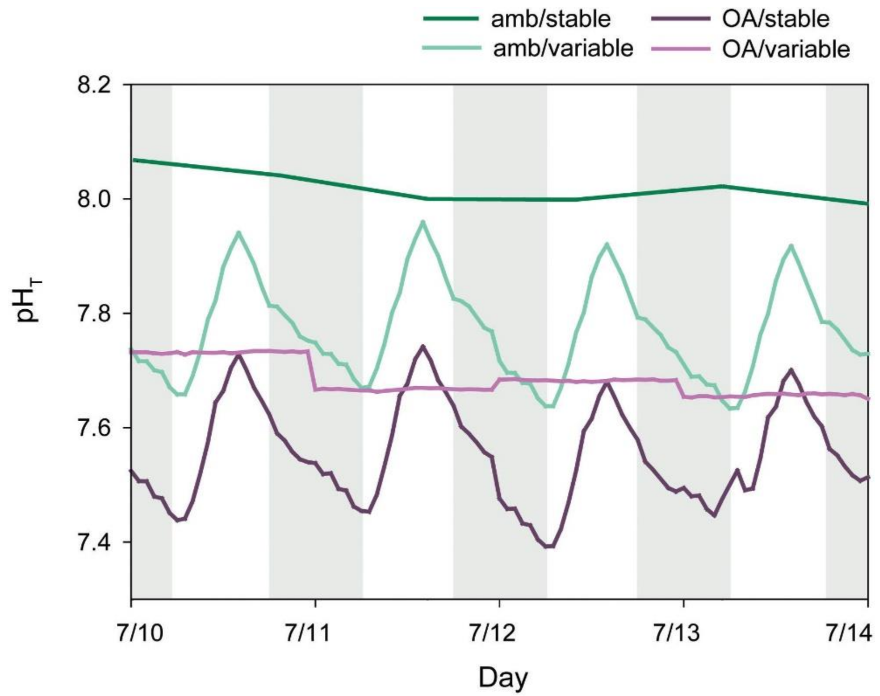
Can variable pH and low oxygen moderate ocean acidification outcomes for mussel larvae?

Christina A. Frieder, Jennifer P. Gonzalez, Emily E. Bockmon, Michael O. Navarro, Lisa A. Levin

First published: 16 December 2013 | https://doi.org/10.1111/gcb.12485 | Citations: 76

Methods to make chemistry fluctuate

- ✓ Manual water change
- ✓ Creative use of pH stats
- ✓ Automatic control (e.g. offset)



pH Variability Exacerbates Effects of Ocean Acidification on a Caribbean Crustose Coralline Alga

Maggie D. Johnson[1,2,3]*, Lucia M. Rodriguez Bravo[1], Shevonne E. O'Connor[4], Nicholas F. Varley[5] and Andrew H. Altieri[1,6]

Methods to make chemistry fluctuate

- ✓ Manual water change
- ✓ Creative use of pH stats
- ✓ Automatic control (e.g. offset)
- ✓ Biologically-driven variability

Day: light
= Photosynthesis + respiration

O_2 ↑ CO_2 ↓ pH ↑

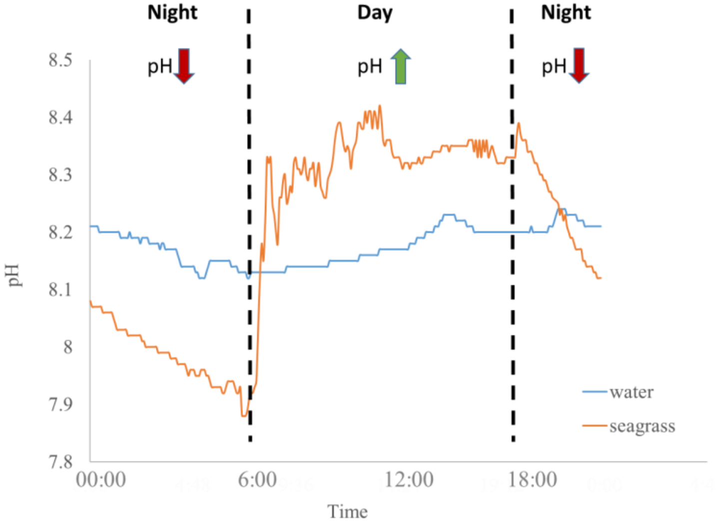
Night: dark
= respiration

O_2 ↓ CO_2 ↑ pH ↓

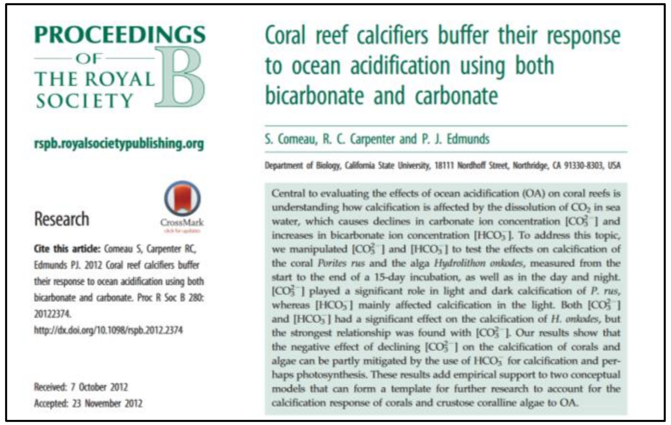
Methods to make chemistry fluctuate

Seawater — pH 8.0

CO_2 → Header tank — pH 7.7



“Unrealistic” seawater chemistry to test specific hypotheses



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Research

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Coral reef calcifiers buffer their response to ocean acidification using both bicarbonate and carbonate

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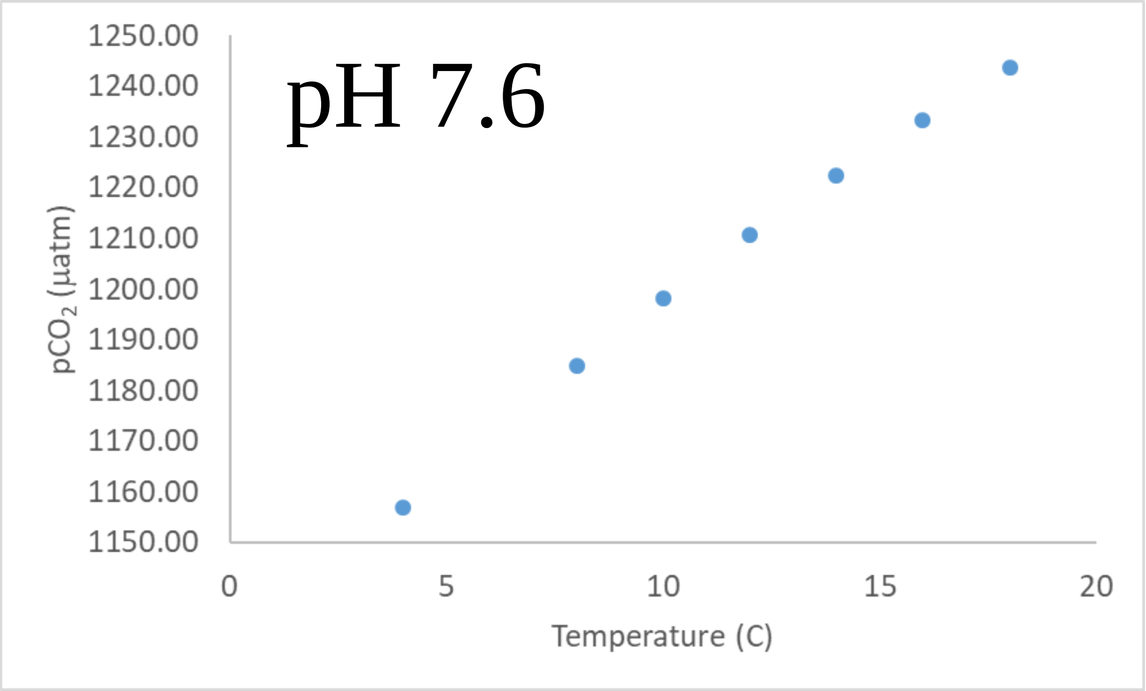
Central to evaluating the effects of ocean acidification (OA) on coral reefs is understanding how calcification is affected by the dissolution of CO_2 in sea water, which causes declines in carbonate ion concentration [CO_3^{2-}] and increases in bicarbonate ion concentration [HCO_3^-]. To address this topic, we manipulated [CO_3^{2-}] and [HCO_3^-] to test the effects on calcification of the coral *Porites rus* and the alga *Hydrolithon onkodes*, measured from the start to the end of a 15-day incubation, as well as in the day and night. [CO_3^{2-}] played a significant role in light and dark calcification of *P. rus*, whereas [HCO_3^-] mainly affected calcification in the light. Both [CO_3^{2-}] and [HCO_3^-] had a significant effect on the calcification of *H. onkodes*, but the strongest relationship was found with [CO_3^{2-}]. Our results show that the negative effect of declining [CO_3^{2-}] on the calcification of corals and algae can be partly mitigated by the use of HCO_3^- for calcification and perhaps photosynthesis. These results add empirical support to two conceptual models that can form a template for further research to account for the calcification response of corals and crustose coralline algae to OA.

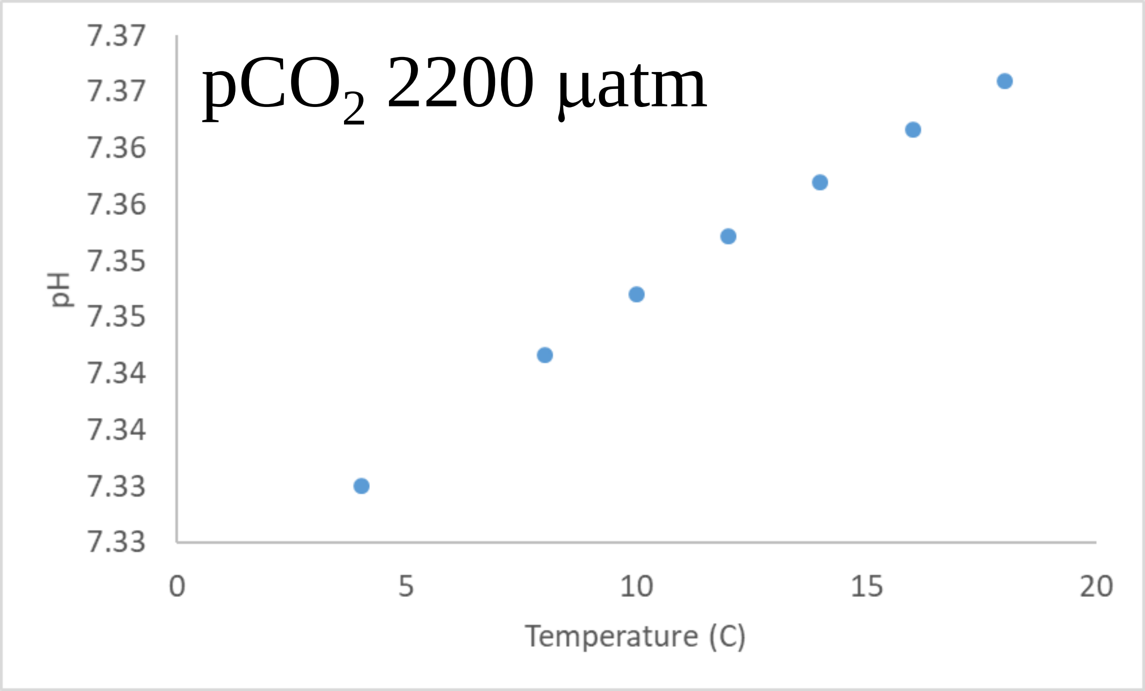
Table S6. Mean parameters of the carbonate chemistry over the two experimental trials. The concentration of bicarbonate ions [HCO_3^-], the concentration of carbonate ions [CO_3^{2-}], the concentration of aqueous CO_2 [CO_2], the partial pressure of CO_2 (pCO_2) and the aragonite saturation state (Ω_a) were derived from pH_T, total alkalinity, salinity and temperature.

CO_3^{2-} conditions	HCO_3^- conditions	[HCO_3^-] (µmol kg^{-1})	[CO_3^{2-}] (µmol kg^{-1})	[CO_2] (µmol kg^{-1})	pCO_2 (µatm)	Ω_a	A_T (µmol kg^{-1})	pH_T	Tempera (°C)
Low CO_3^{2-}	High HCO_3^-	2243 ± 8	75 ± 2	56 ± 3	2108 ± 86	1.20 ± 0.03	2424 ± 6	7.44 ± 0.01	27.7 ± (
	Med HCO_3^-	1695 ± 12	85 ± 2	27 ± 1	1047 ± 29	1.35 ± 0.03	1910 ± 13	7.62 ± 0.01	27.7 ± (
	Low HCO_3^-	1025 ± 32	82 ± 5	13 ± 3	503 ± 125	1.32 ± 0.08	1258 ± 42	7.80 ± 0.03	27.5 ± (
Mediun CO_3^{2-}	High HCO_3^-	2287 ± 19	223 ± 7	19 ± 1	733 ± 22	3.58 ± 0.12	2814 ± 14	7.91 ± 0.01	27.7 ± (
	Med HCO_3^-	1731 ± 7	227 ± 3	11 ± 0.2	401 ± 7	3.65 ± 0.05	2289 ± 6	8.04 ± 0.01	27.7 ± (
	Low HCO_3^-	1069 ± 21	203 ± 7	5 ± 0.4	188 ± 15	3.26 ± 0.11	1612 ± 21	8.19 ± 0.02	27.8 ± (
High CO_3^{2-}	High HCO_3^-	2334 ± 17	384 ± 5	11 ± 0.2	435 ± 8	6.17 ± 0.09	3224 ± 24	8.13 ± 0.01	27.8 ± (
	Med HCO_3^-	1802 ± 13	381 ± 5	7 ± 0.2	257 ± 8	6.11 ± 0.08	2712 ± 12	8.25 ± 0.01	27.4 ± (
	Low HCO_3^-	1195 ± 14	365 ± 5	3 ± 0.1	120 ± 5	5.82 ± 0.08	2114 ± 8	8.41 ± 0.01	27.5 ± (

Specific HCO_3^- and CO_3^{2-} concentration using CO_2, HCl, NaOH and Na_2CO_3

Caution: need some serious design for multiple drivers experiment with parameters interacting with the carbonate chemistry

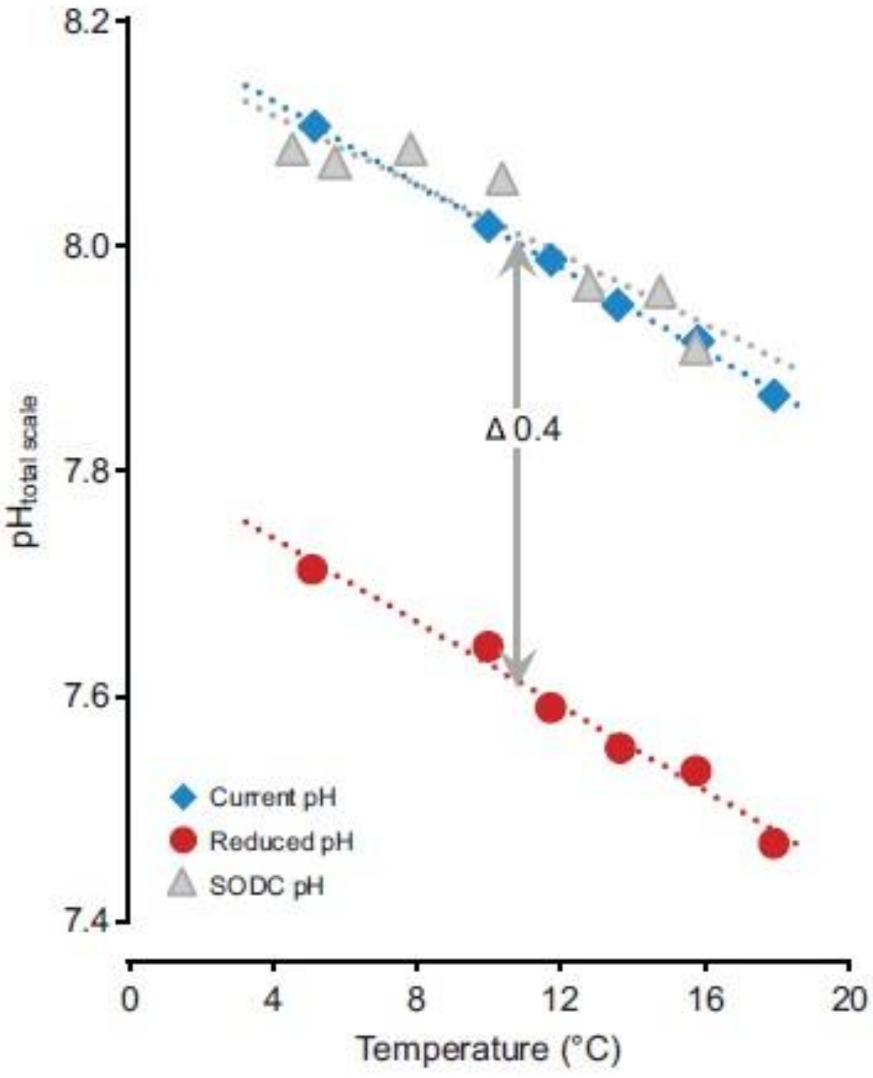




Same pH = different pCO2

Same pCO2 = different pH

One solution: offset natural pH



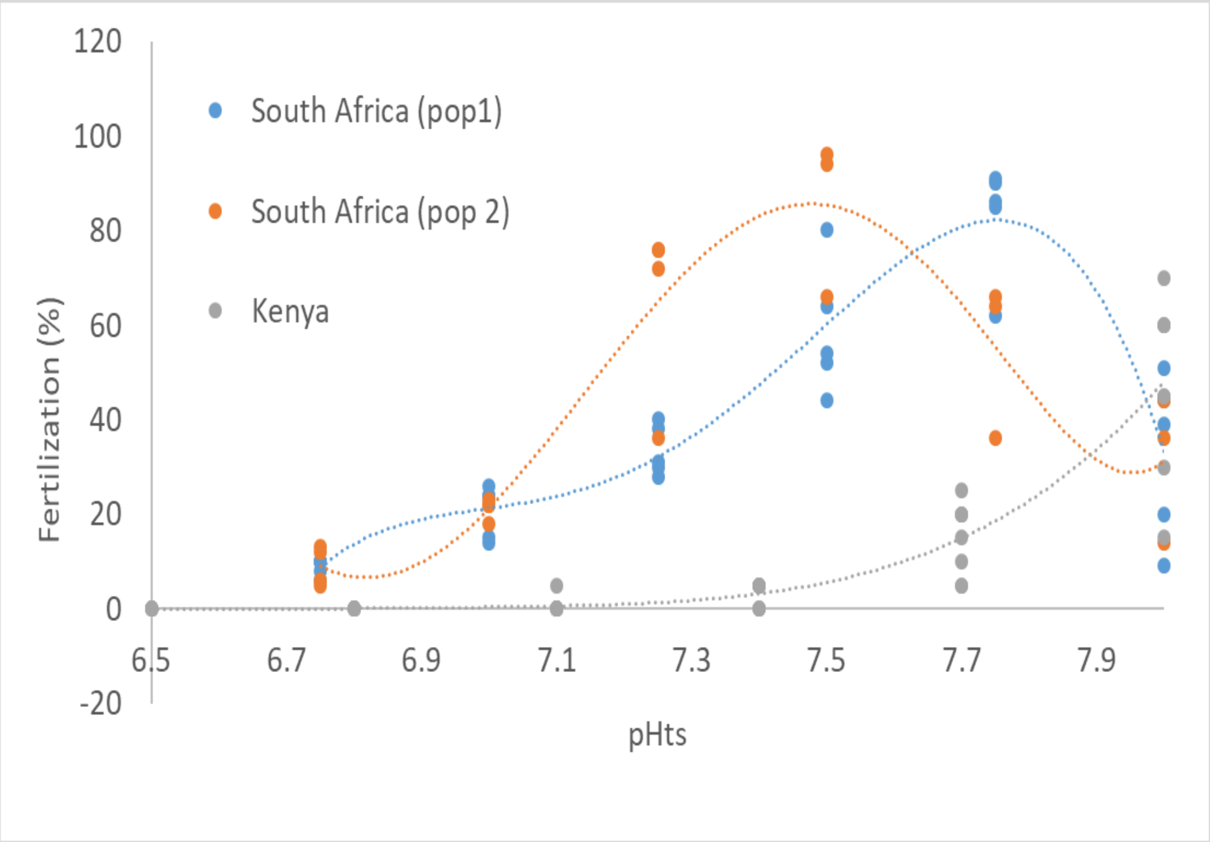
Different combination of pH / temperature for each temperature

(Grans et al. 2014)

Take home message

- ✓ Many methods are available to manipulate the carbonate chemistry for an experiment
- ✓ Use the best approach for your question (or you question based on what you can do)
- ✓ Make pilot experiments to optimize your system
- ✓ Whatever laboratory and equipment you have, there is a method for you

You don’t need fancy equipment to make a nice experiment if you have a good question



- ✓ Manual CO_2 manipulation
- ✓ Multi-well plates
- ✓ Microscope, pipettes
- ✓ pH meter, sampling alkalinity
- ✓ Fertilization assay (2h)