Ocean Acidification International Coordination Centre

IAEA OA-ICC

OA ALLIANCE — INTERNATIONAL ALLIANCE TO COMBAT OCEAN ACIDIFICATION

Basic Training Course on Ocean Acidification

9 - 13 September 2024

EVT2205463

hosted by

United Methodist University (UMU)

How to study biological impacts of ocean acidification

1. General consideration on experimental design
2. How to manipulate the carbonate chemistry
3. What scenario to test

1. **General consideration on experimental design**
2. How to manipulate the carbonate chemistry
3. What scenario to test

Take home messages

Every experiment is an abstraction of reality

George E. P. Box

There is nothing like a perfect experiment !

"Essentially, all models are wrong,
but some are useful"

Essentially, all experiments are wrong,
but most are useful

Be aware and honest about your limitations

How to design your experiment

1. What is your question? Your hypothesis?

2. How can I test this?

   - What are my limitations?

   - What is the best model?

   - What are the best endpoints?

   - What are the best design/stats?

   - What are my controls?

   - etc.

Can I REALLY answer my question with the collected data?

How to design your experiment

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Do the right thing

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- etc.

Do the things right

Can I REALLY answer my question with the collected data?

Trade-offs

Realism

[duration, tested parameter, environment, etc.]

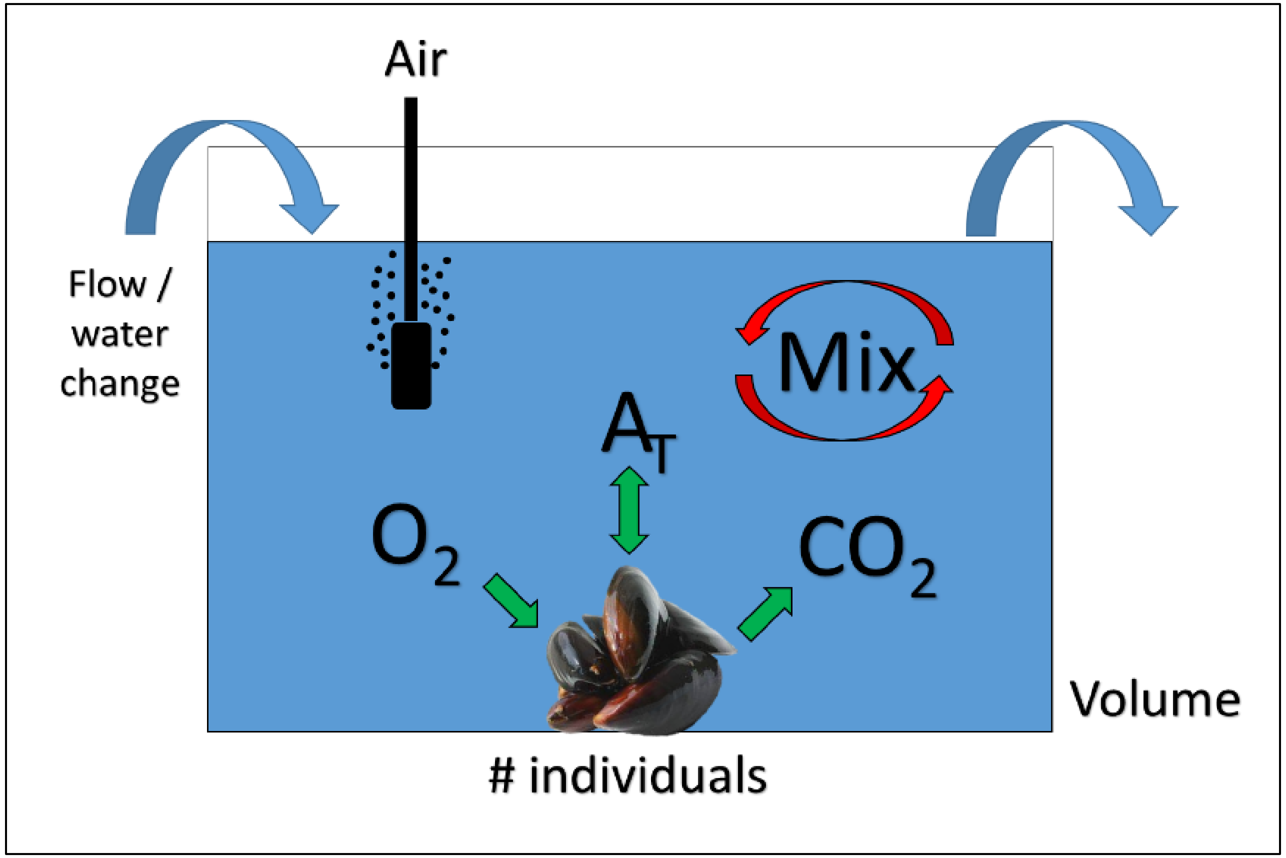
vs.

Feasibility

[manpower, money, space, time]

The aquarium system

Depend on the species and stage/size/density/species specificities



The aquarium system

Depend on the species

- ✓ Type and amount of food
- ✓ Physico-chemical conditions
- ✓ Behaviour (escape, cannibalism, etc.)
- ✓ Etc.

If not well designed, can lead to confounding factors

Need pilot experiment

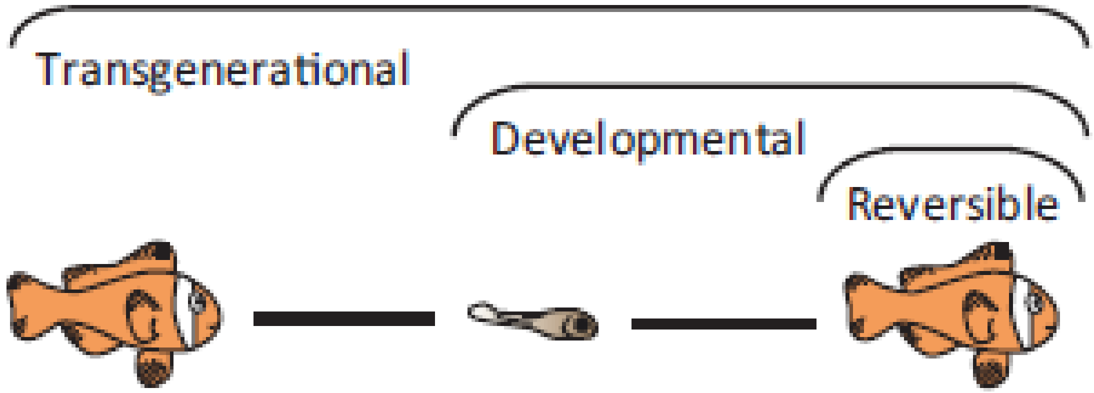
Duration

Depend on the species and question

Effect of ocean acidification on fecundity

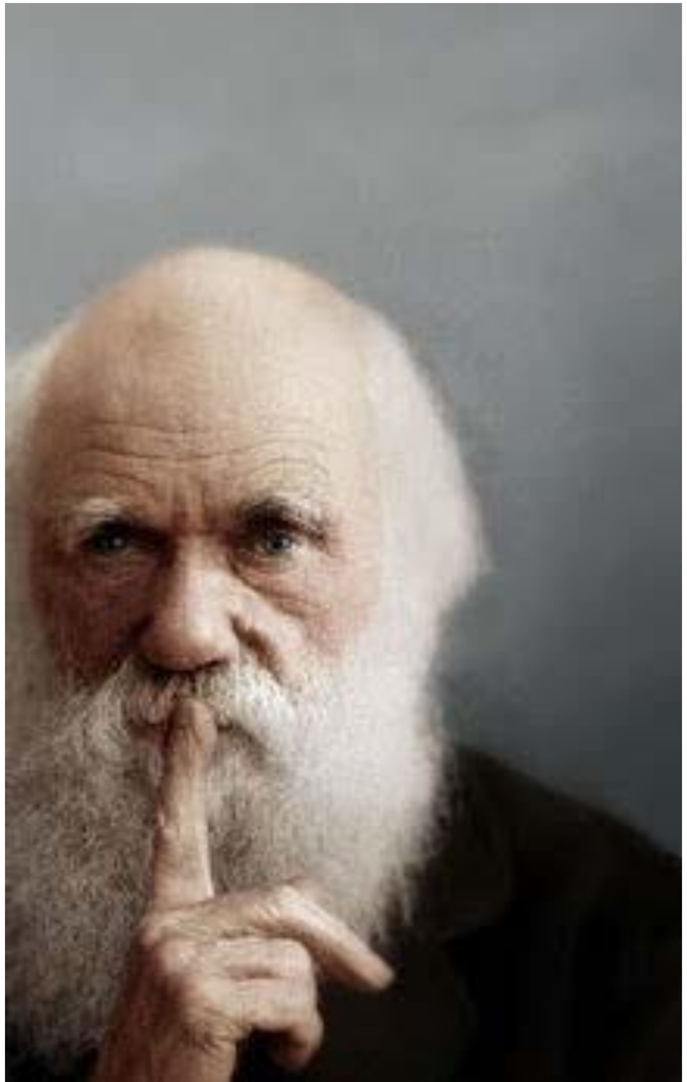
… but can be long

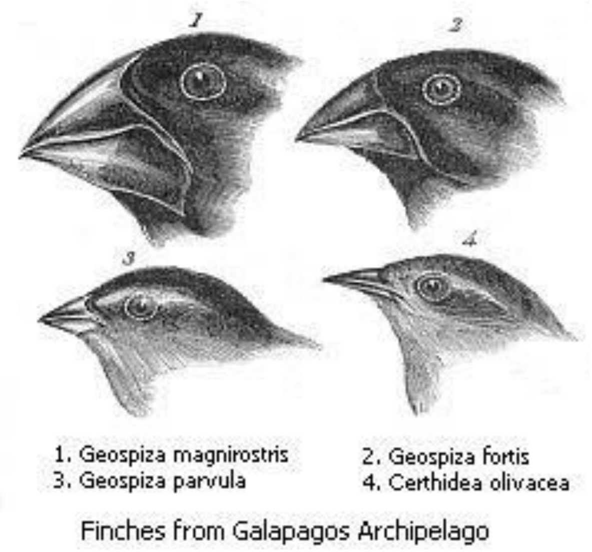
Duration - stages



More relisient offsprings to OA

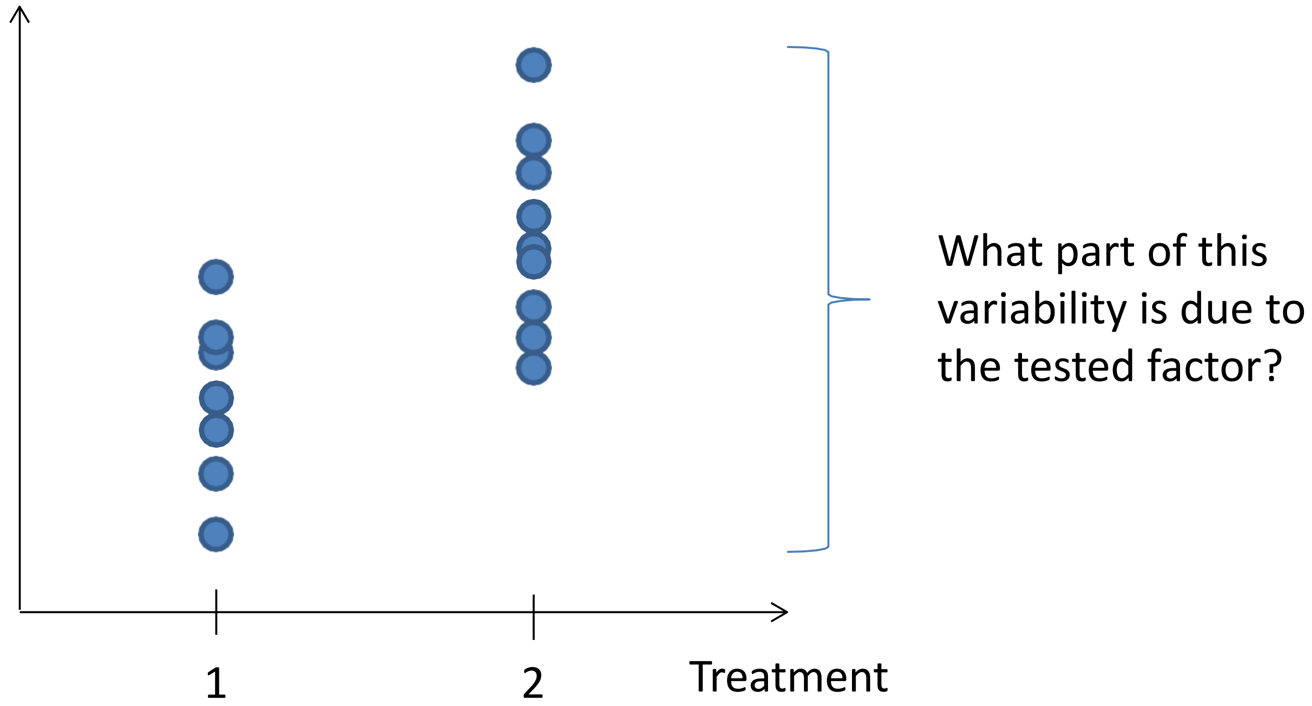
Variability





Explain variability

Sources of variability



”biologically relevant” vs.
”technical/confounding factors”

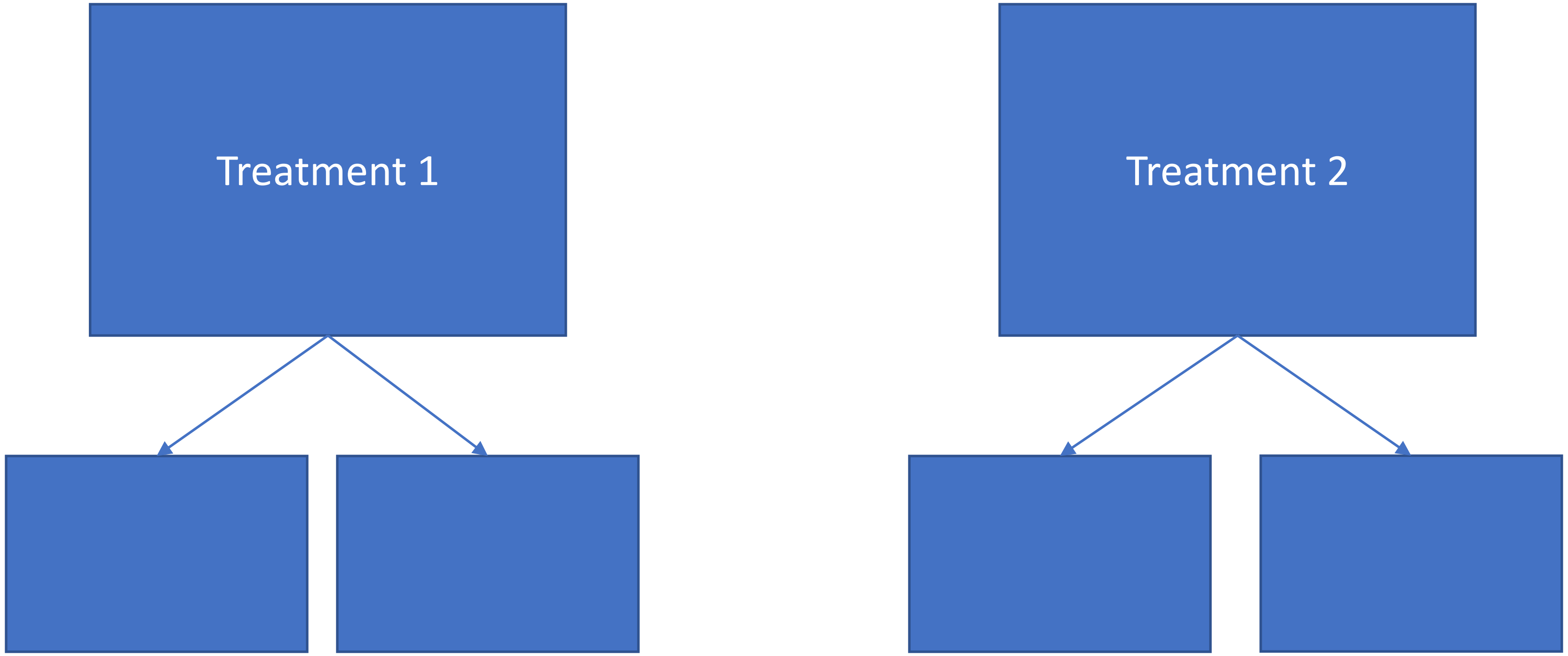
Confounding factors – need for replication

e.g. position in the room
dirt in aquarium
etc.

-> Observed effect not attributed to the treatment

Solution: replication + randomization

Pseudo-replication



Sources of variability

Unwanted variability may ”hide” real variability

Higher the variability, the more data you need to see effects

Variability depends on endpoints

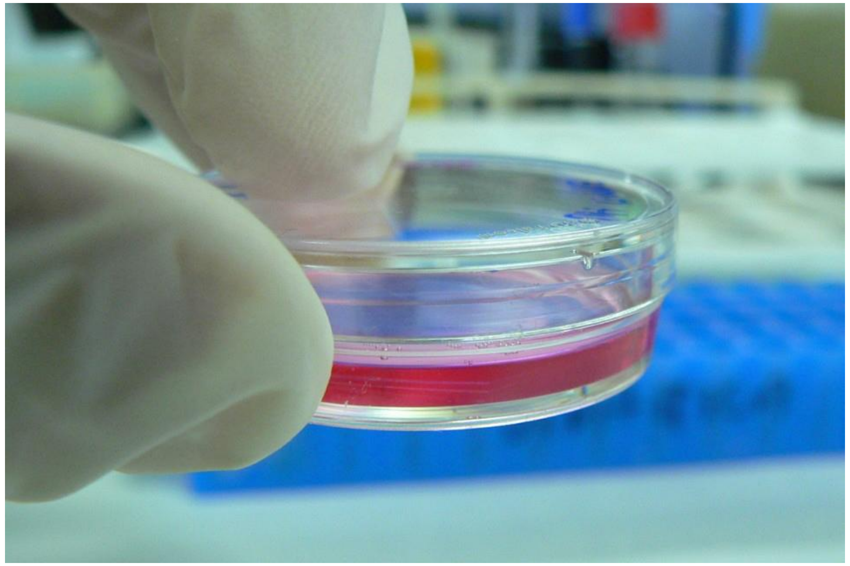
-> Minimize unwanted variability

-> Pilot studies + power analyse (number of replicates / samples)

Replication

# Parameters		# Treatments		# replicates		# tanks
1	x	2	x	2	=	4
1	x	2	x	4	=	8
1	x	4	x	4	=	16
2	x	4	x	4	=	32
3	x	4	x	4	=	48

Practical limitations



Practical aspects - Summary

- ✓ Aquarium system
  (water quality, stability, etc.)
- ✓ Duration
- ✓ Source of variability / Frequency
- ✓ True replication
- ✓ Randomization

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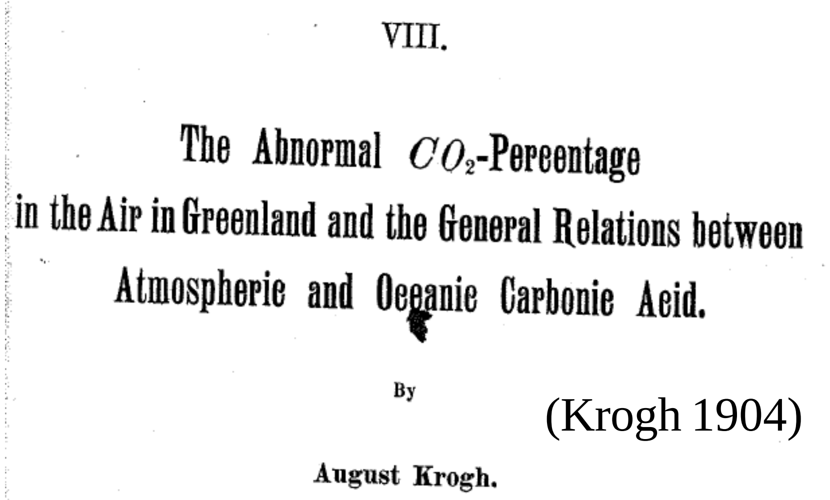
    - etc.

Can I REALLY answer my question with the collected data?

August Krogh



VIII.

The Abnormal CO_2-Percentage in the Air in Greenland and the General Relations between Atmospheric and Oceanic Carbonic Acid.

By

August Krogh.

(Krogh 1904)

Krogh's principle

"For such a large number of problems there will be some animal of choice, or a few such animals, on which it can be most conveniently studied"

The top model

- Biological feature (e.g. life cycle, generation time)

- Ecological / Economical importance

- Tools available (e.g. functional methods, genome)

- Charismatic species

- etc.

What fits your question?

- Size / Weight

- Age

- Life-history stages

- Etc.

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Endpoints?

Fitness (e.g. survival, growth, reproduction)

Physiology – energy budget
(e.g. respiration, feeding, excretion, calcification)

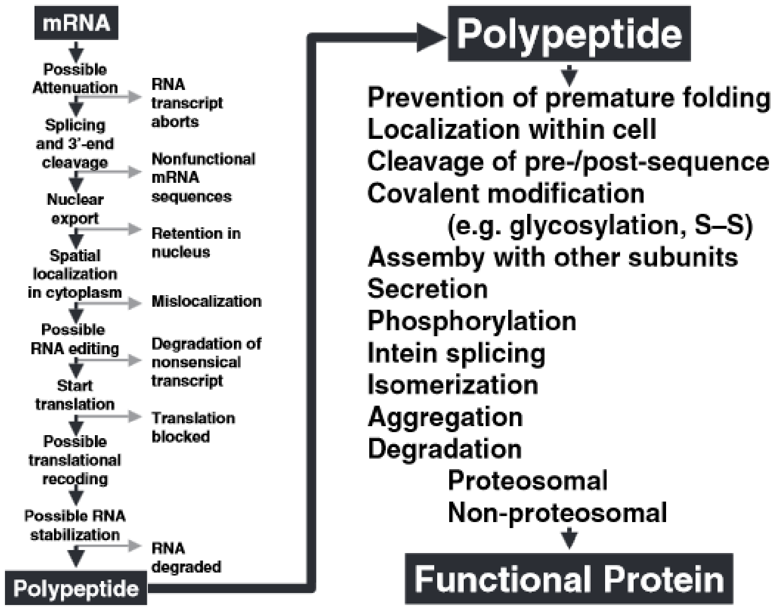
Etc. etc.

Best endpoints?

- Not the “coolest” method

- Not the most familiar method

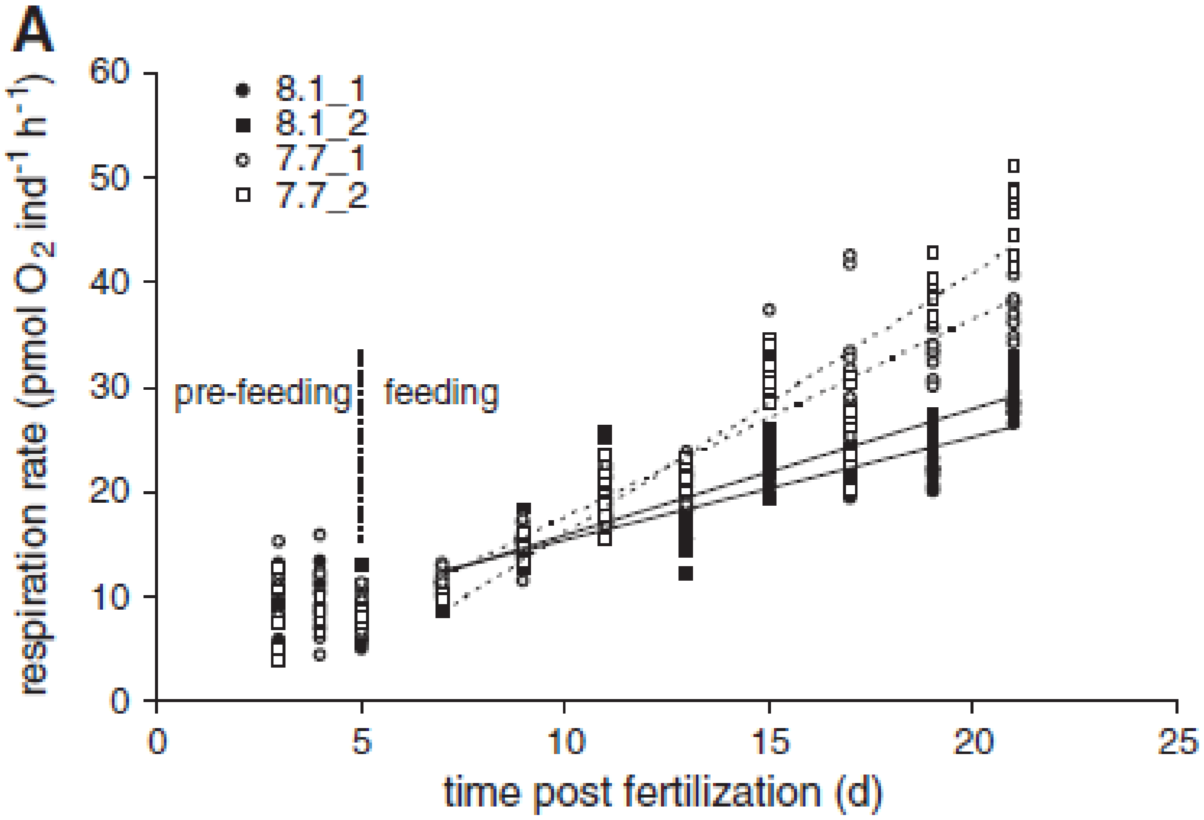
- As close to function as possible (e.g. fitness)



Feder & Walser 2005

How often?

Frequency (more = more chance to identify effects & interactions)



Changes ≠ bad

We like bad news

Negative effect:	9.8 citations / year
Positive/neutral effect:	6.2 citations / year

A change in your proxy ≠ change in fitness

How to design your experiment

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- etc.

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How to design your experiment

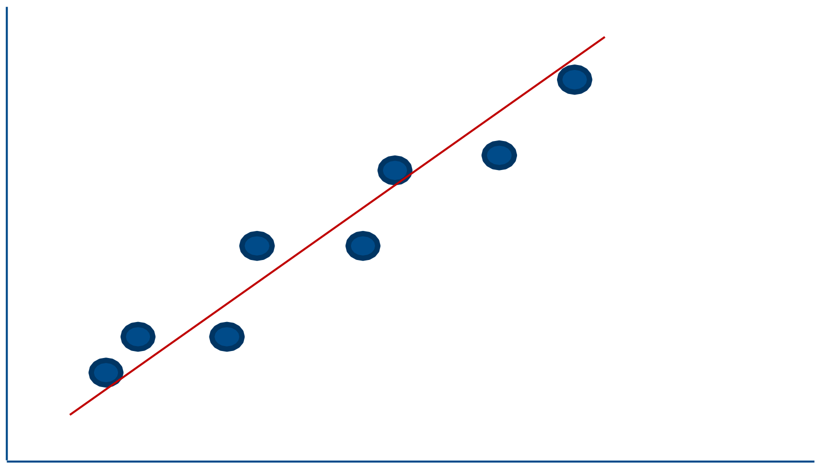
“ANOVA” design

Little predictive power

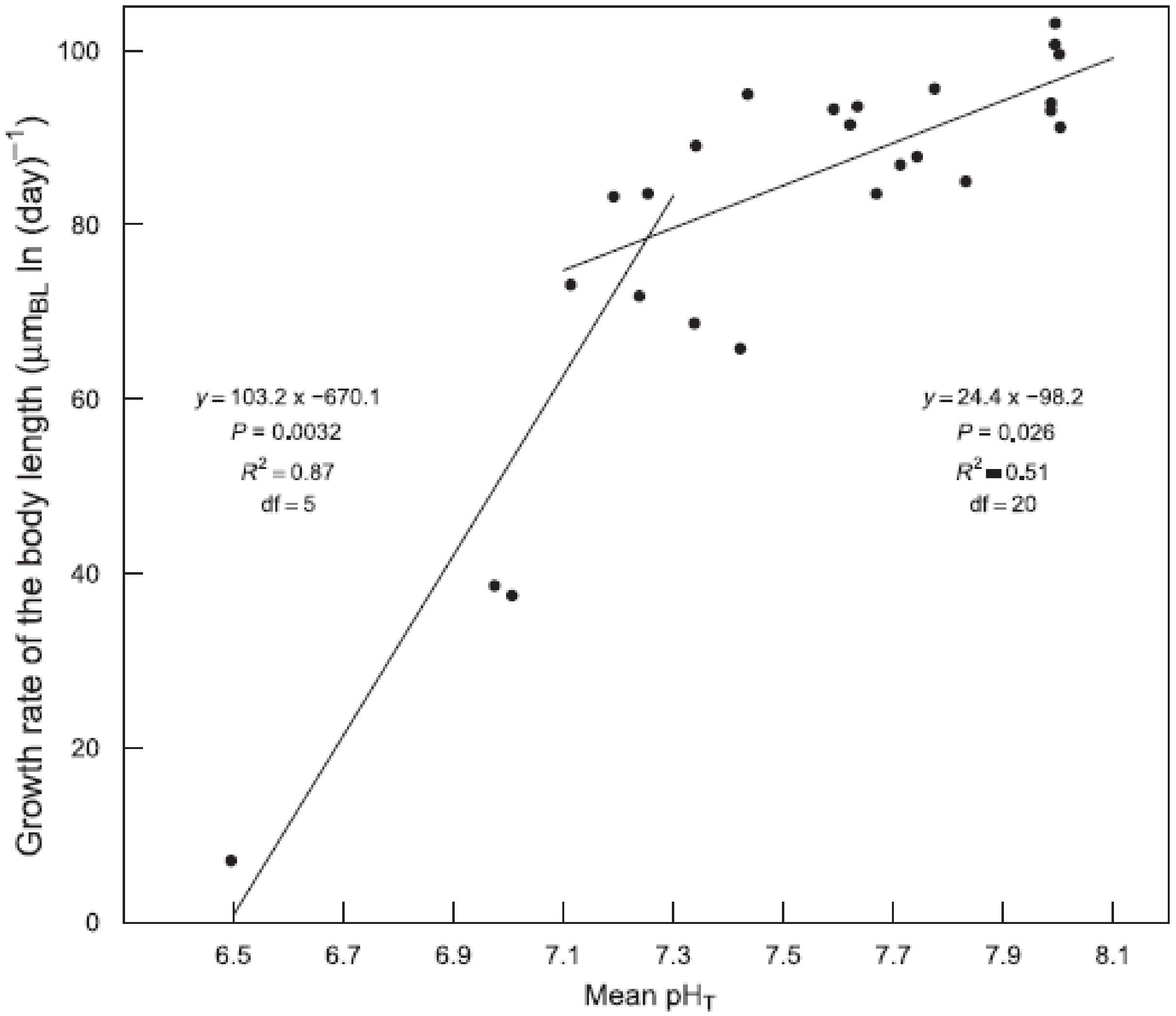
Scenario 1	Scenario 2	Scenario 3
n=x	n=x	n=x

“Regression” design

Need to have a relationship



ANOVA vs. Regression



(Dorey et al. 2013)

How to design your experiment

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    - What are the best design/stats?

    - What are my controls?

    - etc.

Can I REALLY answer my question with the collected data?

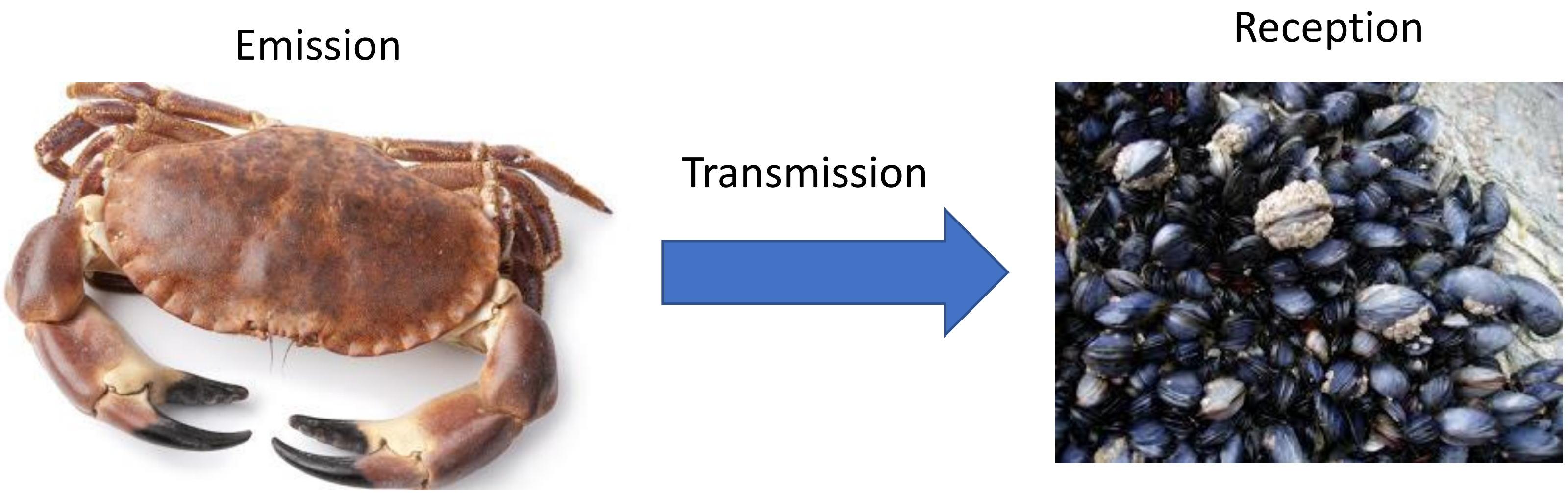
Test the right scenarios

Take species niche, behaviour & variability into your thinking

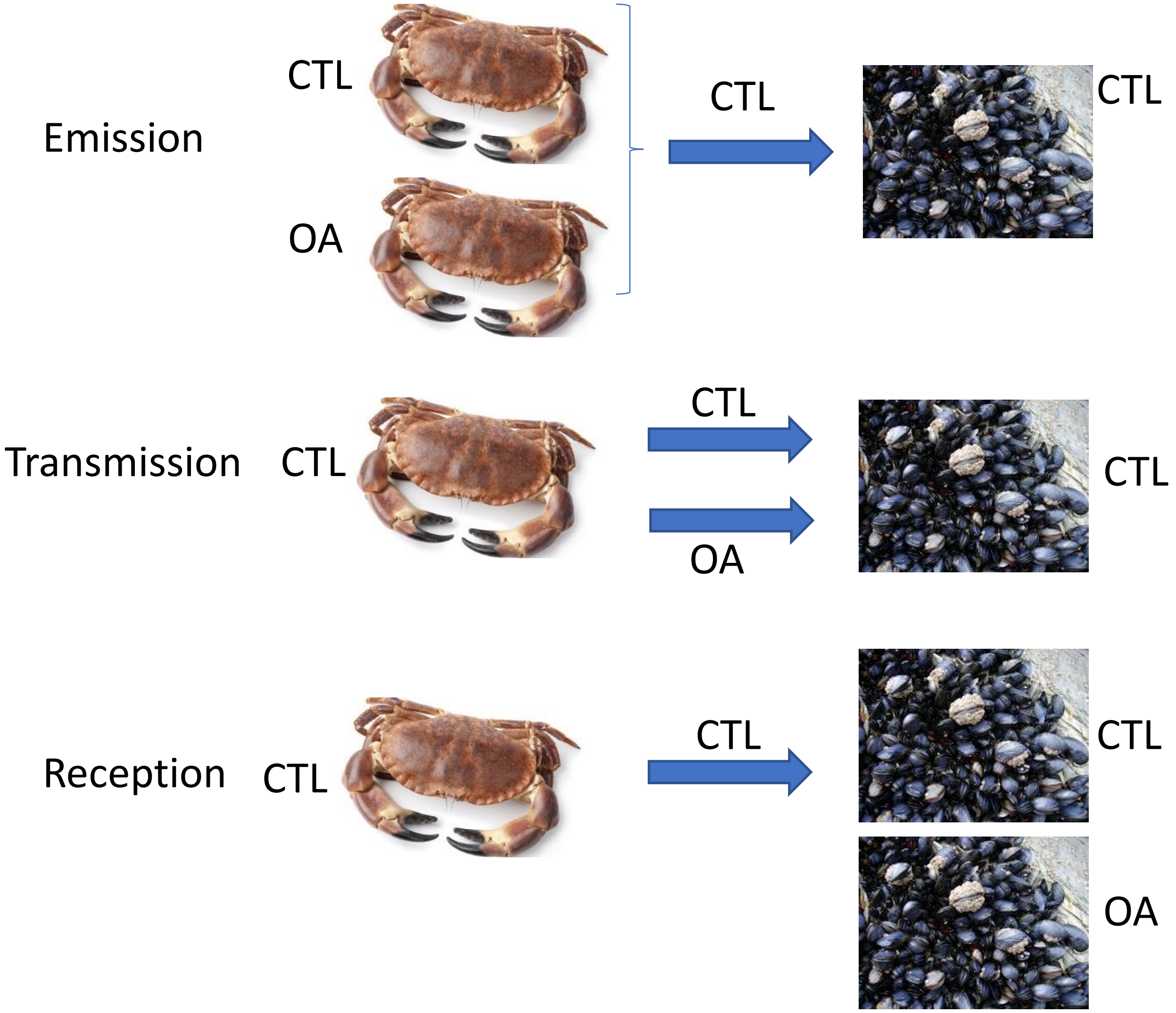
Example: impact of ocean acidification on chemical communication

Two temperature: CTL vs OA

Example: impact of ocean acidification on chemical communication



Example: impact of ocean acidification on chemical communication



+ interactions

2 emissions x
2 transmissions x
2 receptions
= 8 treatments x replicates

Reminder: you need to measure the carbonate chemistry

- ✓ 2 parameters of the carbonate chemistry (e.g. pH, TA) + temperature, salinity

- ✓ Chemistry is time consuming and expensive.
  - ✓ Think about the methods / quality
  - ✓ Do not over do it (quality, quantity)

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   - etc.

Can I REALLY answer my question with the collected data?

1. General consideration on experimental design
2. **How to manipulate the carbonate chemistry**
3. What scenario to test

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]

[1]Laboratoire d'Océanographie, CNRS, France
[2]Observatoire Océanologique, Université Pierre et Marie Curie-Paris 6, France
[3]State Key Laboratory of Marine Environmental Science, Xiamen University, China
[4]Pohang University of Science and Technology, South Korea
[5]Alfred Wegener Institute for Polar and Marine Research, Germany
[6]Leibniz Institute of Marine Sciences (IFM-GEOMAR), Germany

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

Take home message

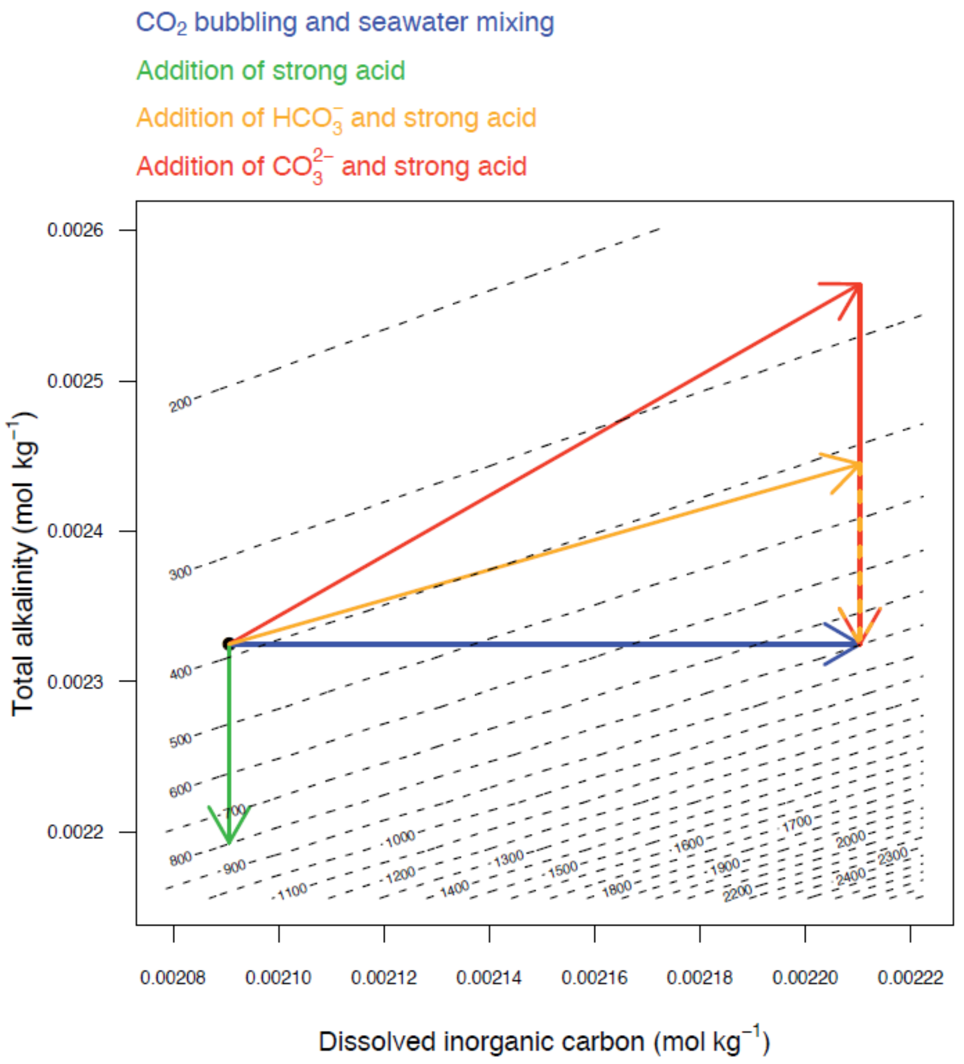
- ✓ Many methods are available to manipulate the carbonate chemistry for an experiment
- ✓ Whatever laboratory and equipment you have, there is a method for you

NON-best practice methods

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

	pCO_{2sw} (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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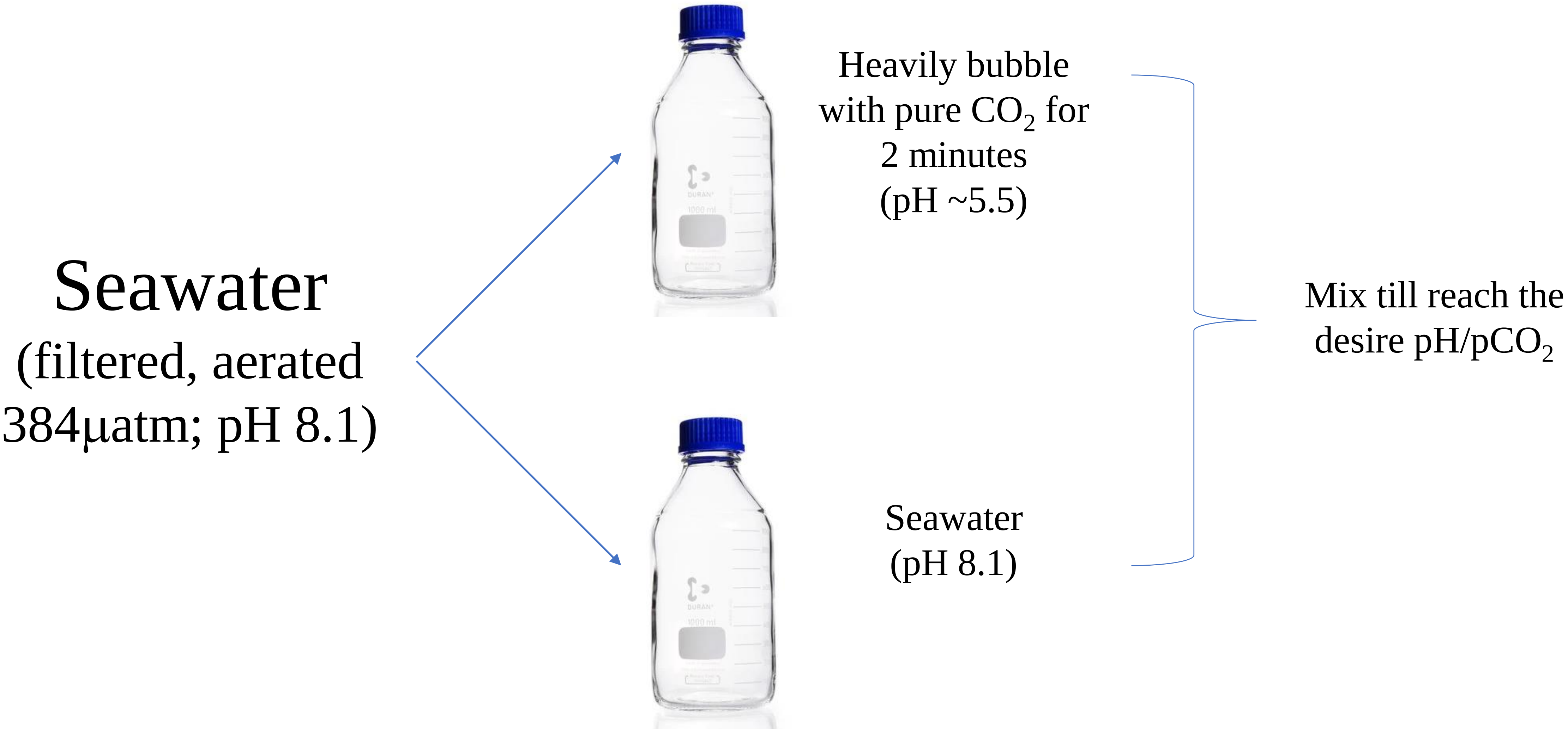
Year 2100	793	7.793	16.1	2325	2191	26.4	2055	110	2.6	1.7
Addition of:										
CO_3^{2-} and HCO_3^-; closed sys.	400	8.073	8.4	2467	2191	13.3	1977	201	4.8	3.1
followed by acid addition; closed sys.	793	7.793	16.1	2325	2191	26.4	2055	110	2.6	1.7

→ 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



Mix High CO_2 water

	$pCO_{2\,sw}$ (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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Year 2100	793	7.793	16.1	2325	2191	26.4	2055	110	2.6	1.7
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

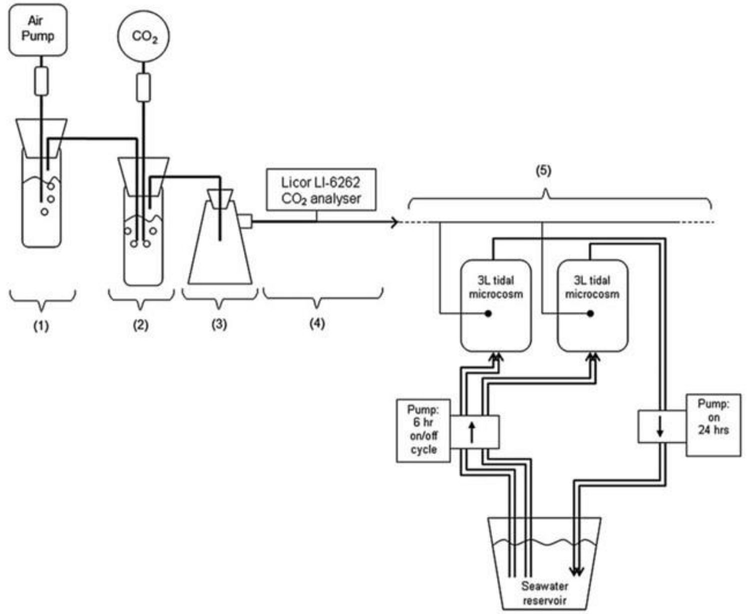
No compensation for biology and atmosphere, manual changes

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)

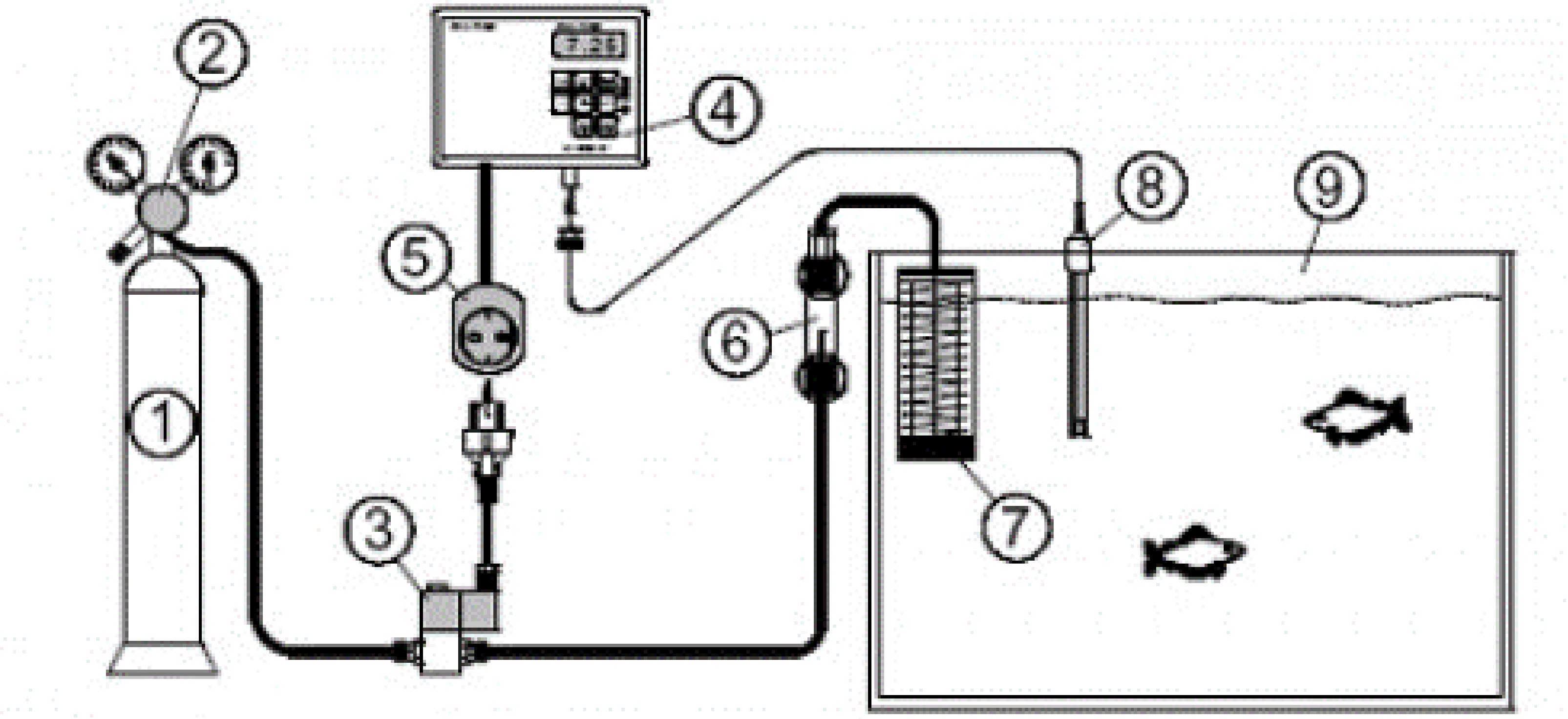


(e.g. Findlay et al. 2008)

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)

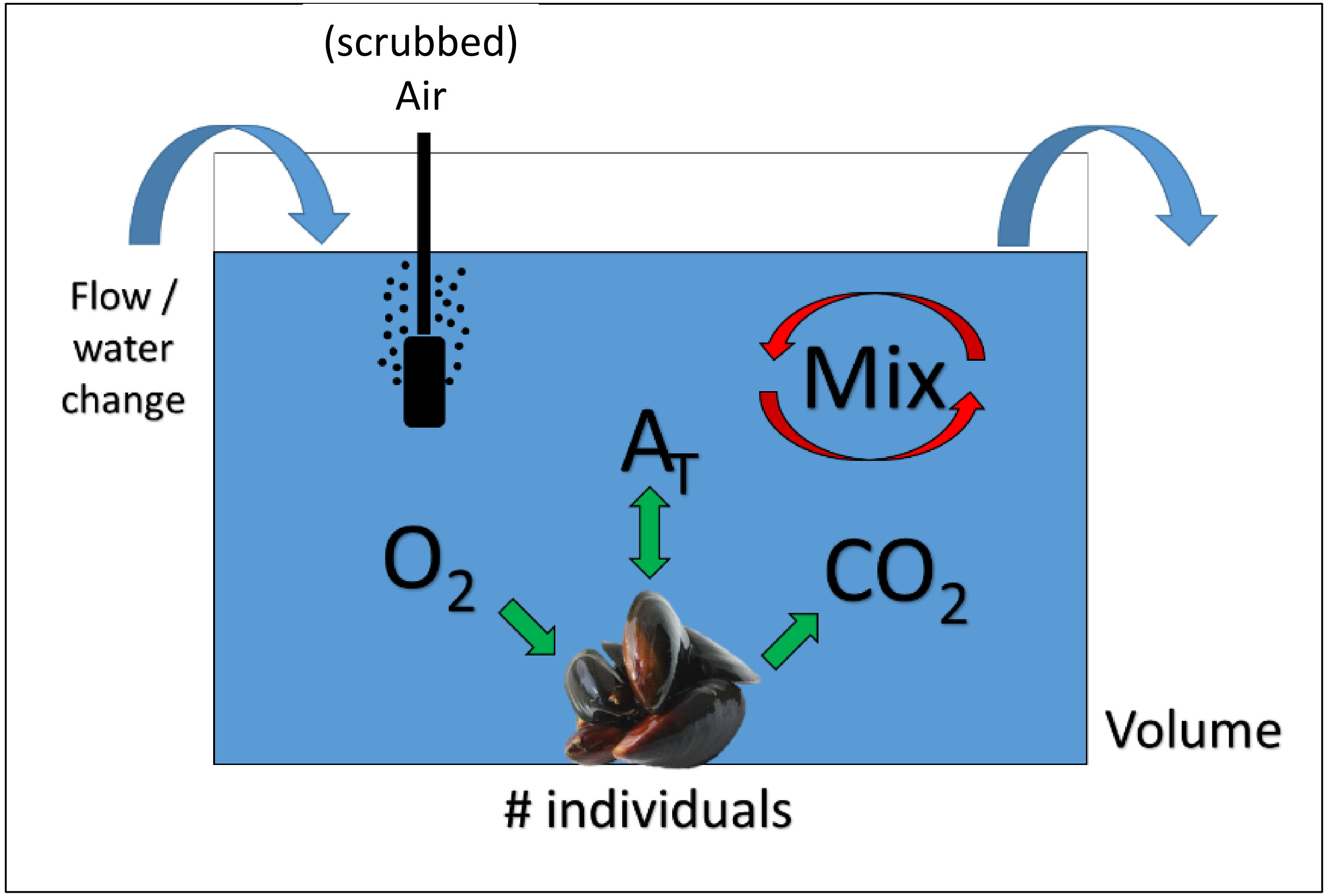
Summary: 3 best practice methods

- ✓ 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

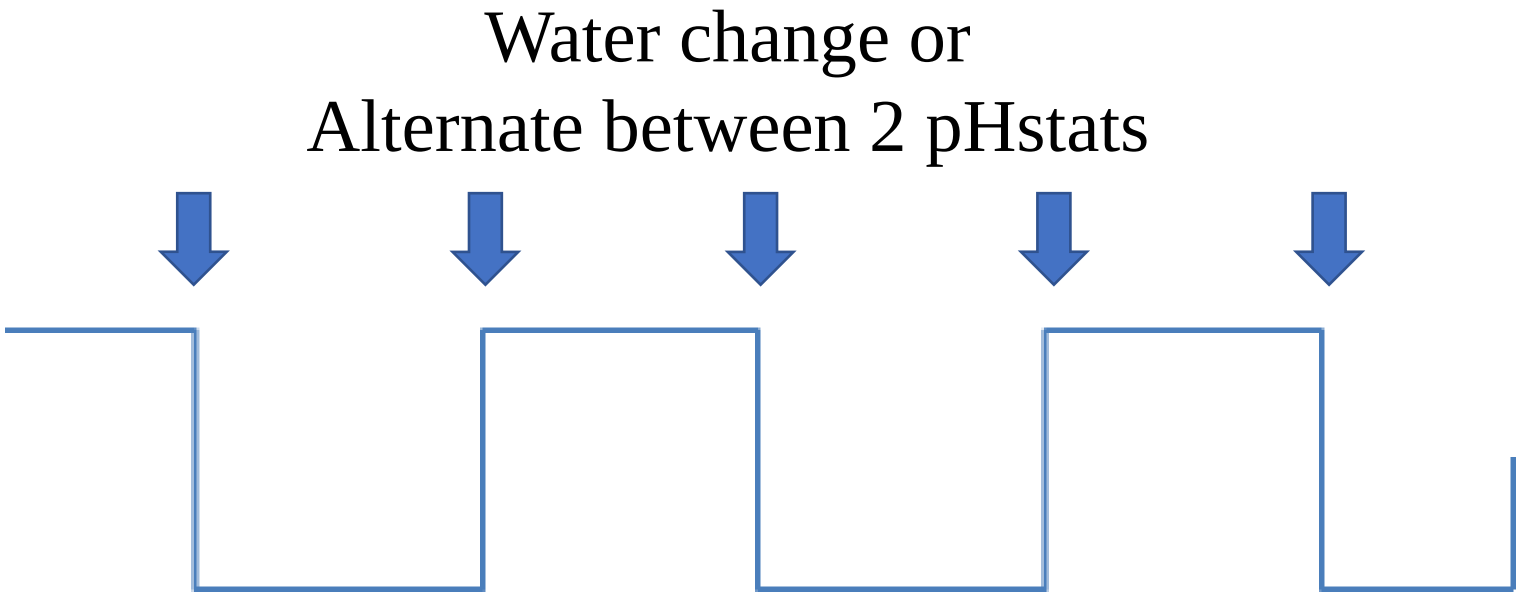
No contact with air

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



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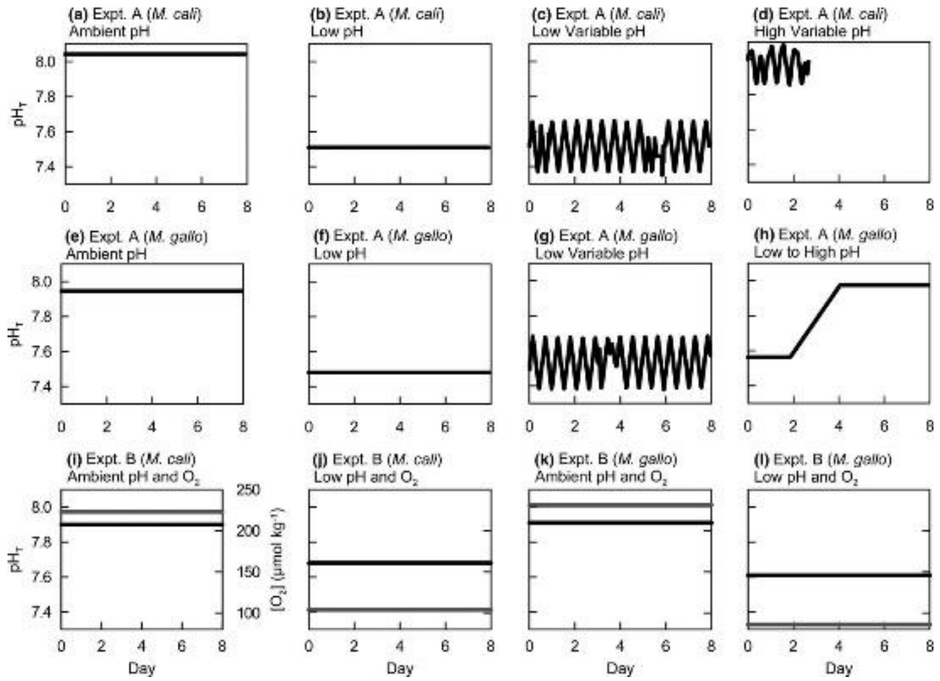


Global Change Biology

Primary Research Article | Full Access

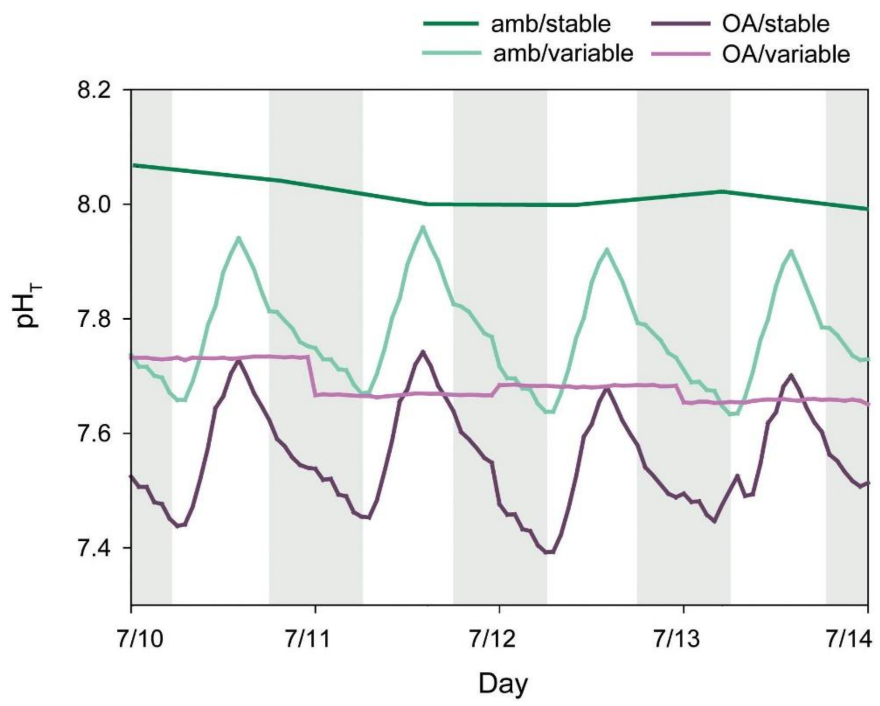
Can variable pH and low oxygen moderate ocean acidification outcomes for mussel larvae?

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

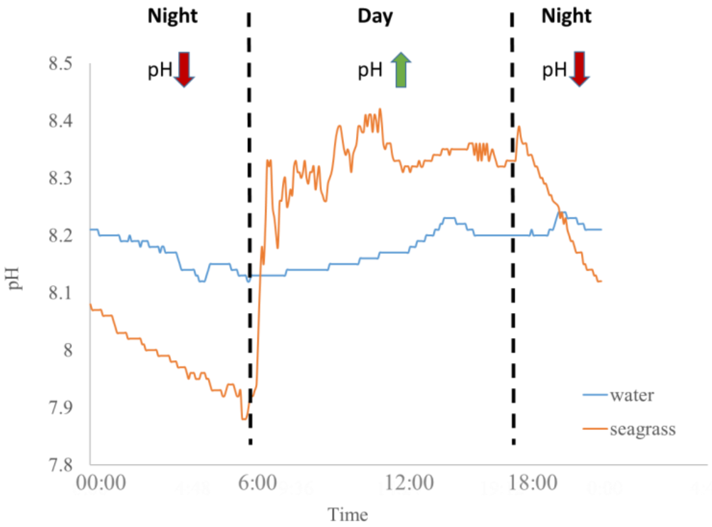
Seawater

pH 8.0

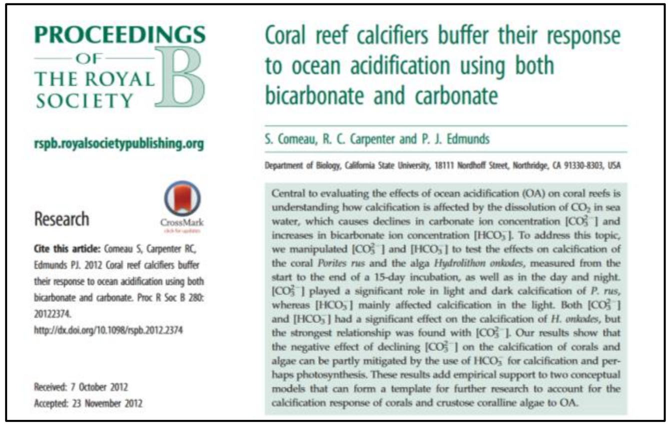
CO_2

Header tank

pH 7.7



“Unrealistic” seawater chemistry to test specific hypotheses



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Research

Cite this article: Comeau S, Carpenter RC, Edmunds PJ. 2012 Coral reef calcifiers buffer their response to ocean acidification using both bicarbonate and carbonate. Proc R Soc B 280: 20122374. http://dx.doi.org/10.1098/rspb.2012.2374

Received: 7 October 2012
Accepted: 23 November 2012

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

S. Comeau, R. C. Carpenter and P. J. Edmunds

Department of Biology, California State University, 18111 Nordhoff Street, Northridge, CA 91330-8303, USA

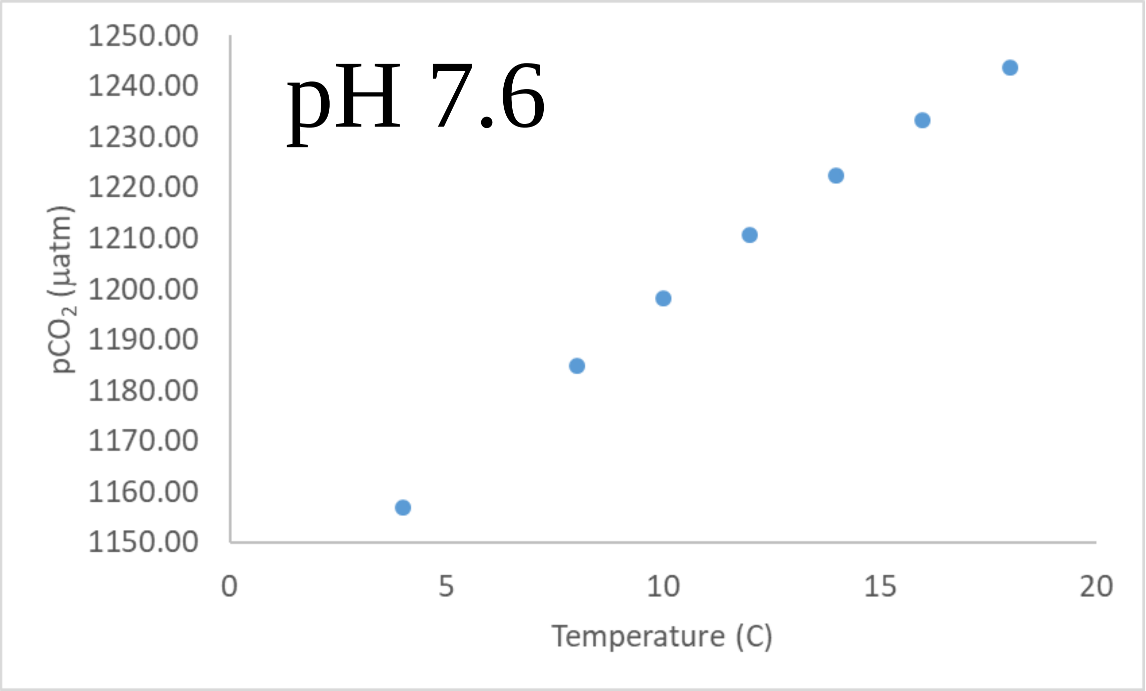
Central to evaluating the effects of ocean acidification (OA) on coral reefs is understanding how calcification is affected by the dissolution of CO_2 in sea water, which causes declines in carbonate ion concentration [CO_3^{2-}] and increases in bicarbonate ion concentration [HCO_3^-]. To address this 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.

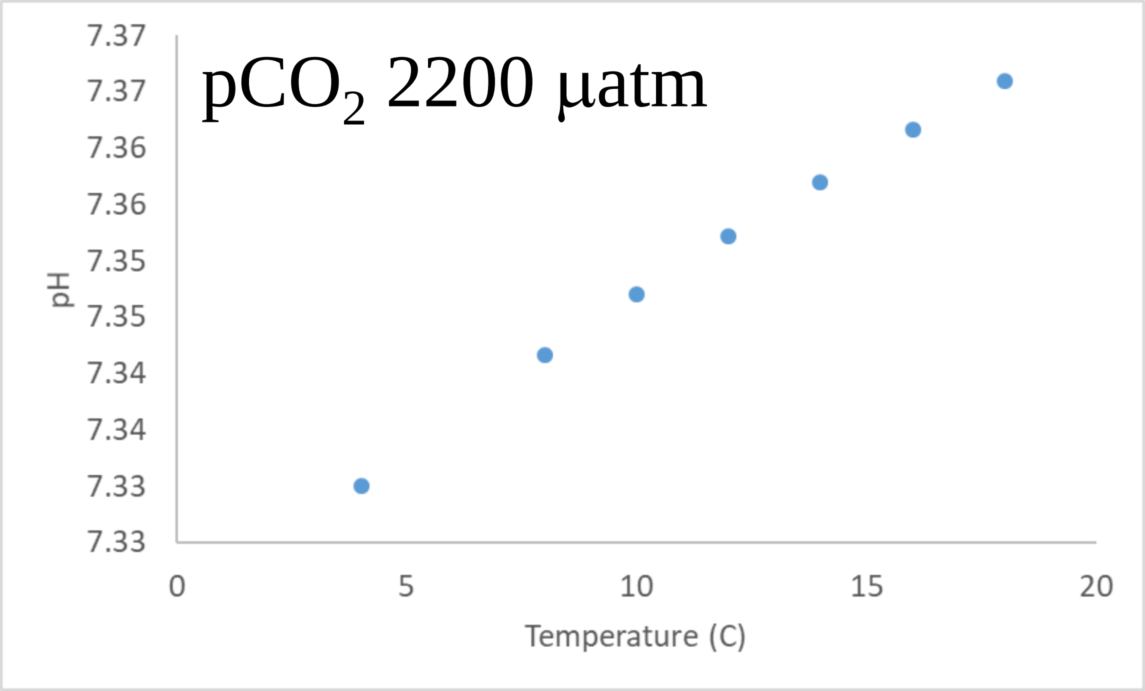
Table S6. Mean parameters of the carbonate chemistry over the two experimental trials. The concentration of bicarbonate ions [HCO_3^-], the concentration of carbonate ions [CO_3^{2-}], the concentration of aqueous CO_2 [CO_2], the partial pressure of CO_2 (pCO_2) and the aragonite saturation state (Ω_a)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

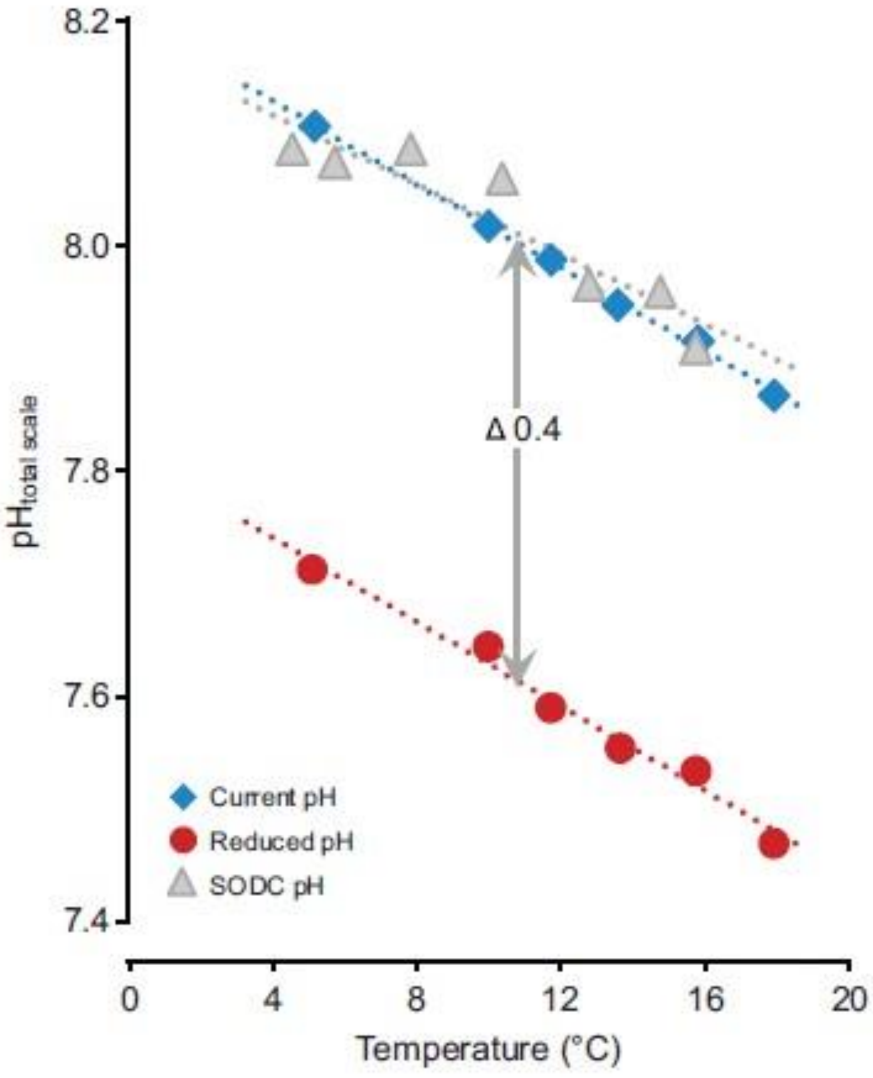




Same pH = different pCO2

Same pCO2 = different pH

One solution: offset natural pH



Different combination of pH / temperature for each temperature

(Grans et al. 2014)

Take home message

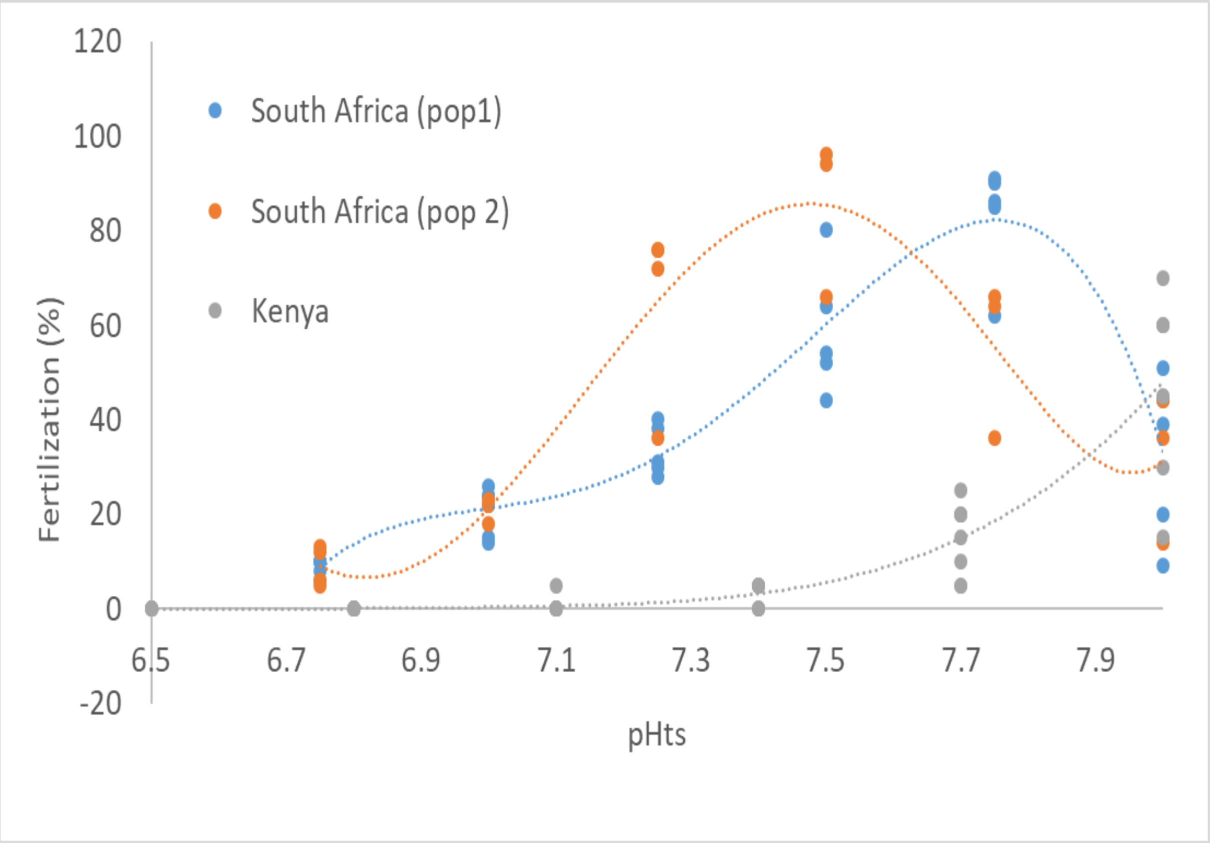
- ✓ Many methods are available to manipulate the carbonate chemistry for an experiment

- ✓ 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)

1. General consideration on experimental design
2. How to manipulate the carbonate chemistry
3. **What scenario to test**

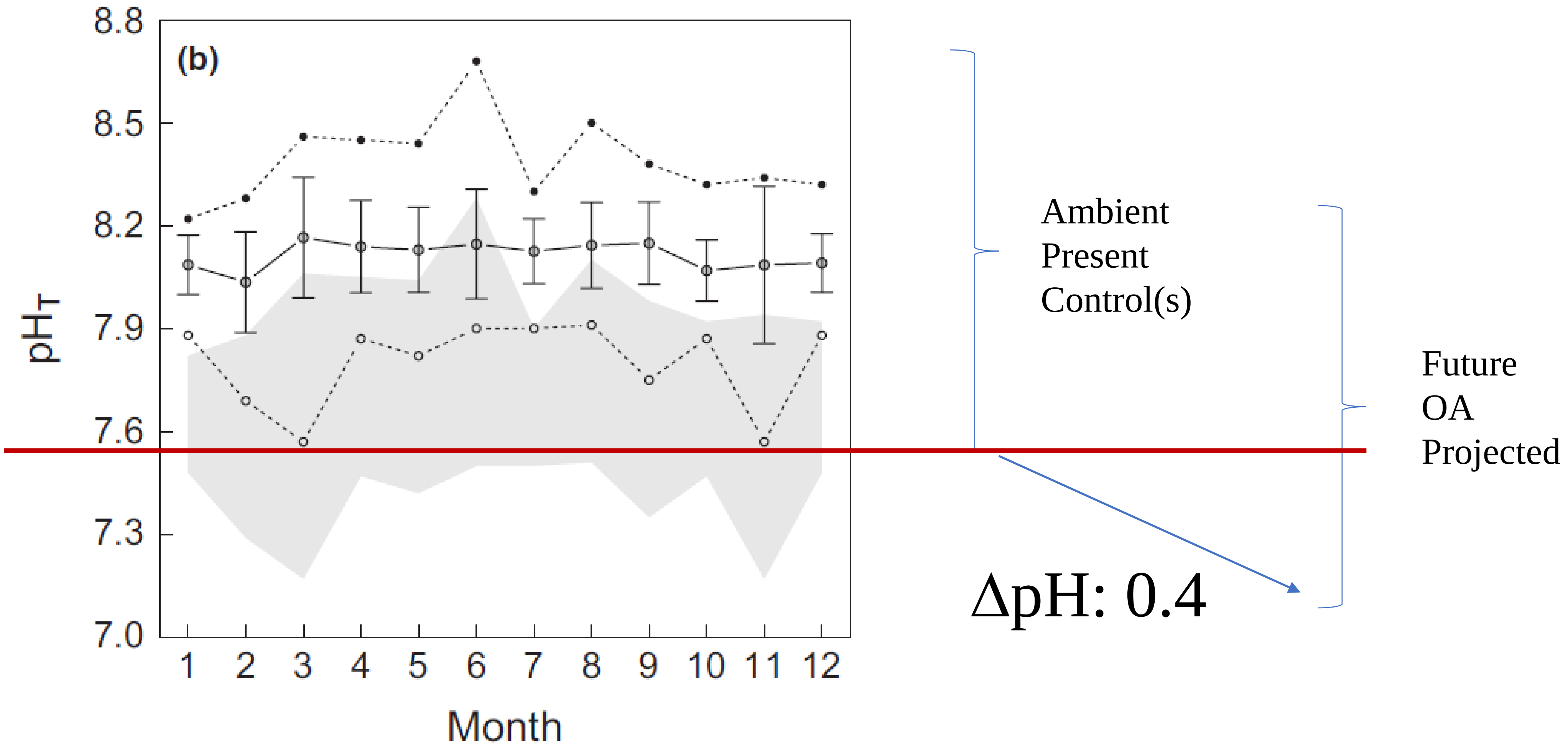
Before you start an experiment

What are the physico-chemical conditions experienced by my organism/ecosystem?

Three options:

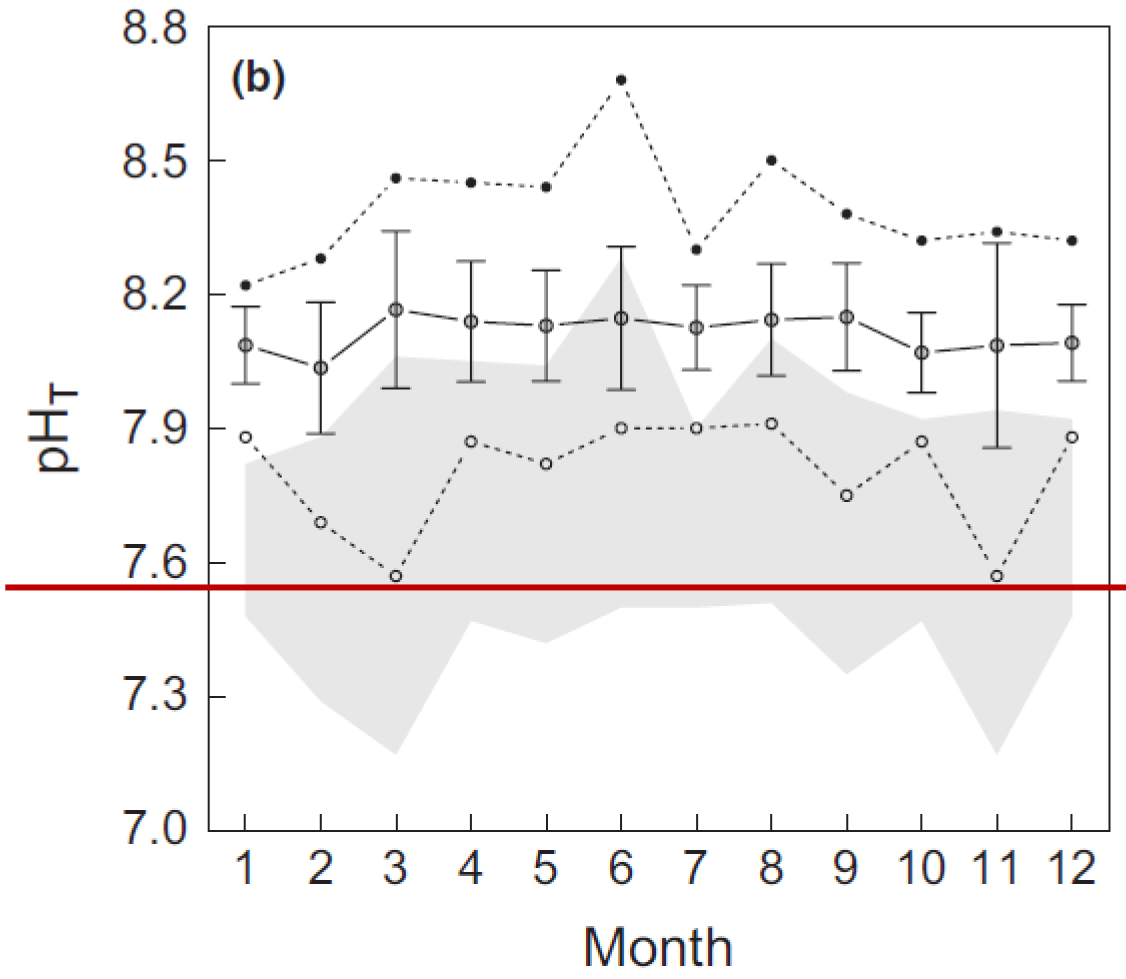
- ✓ Data are available (weather)
- ✓ Data are not available:
  - Collect some data to characterize the variability
  - Use data from a similar environment

Define your scenarios based on the present variability



(Dorey et al. 2013)

Two pH scenarios (example)

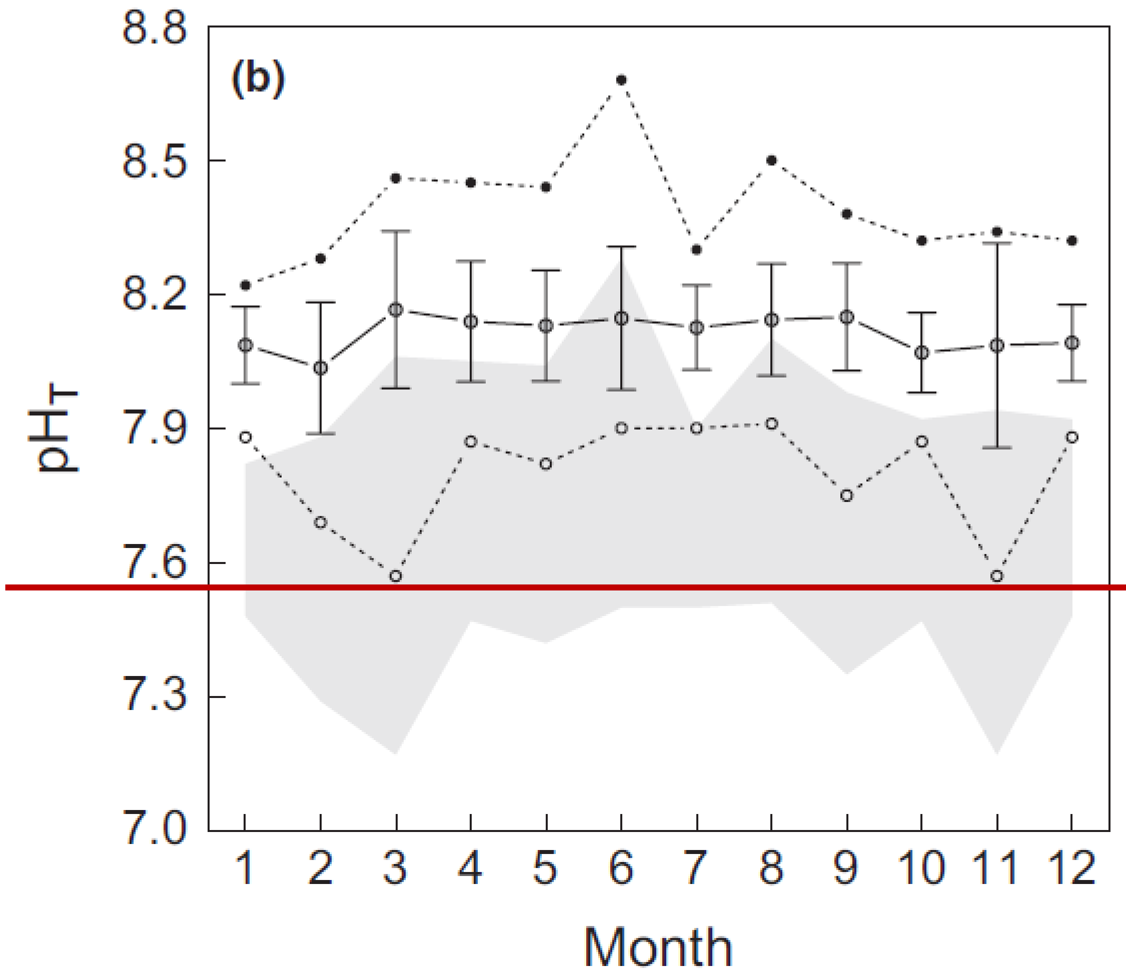


pH 8.1 (present average)

pH 7.5 (outside present range)

(Dorey et al. 2013)

Three pH scenarios (example)



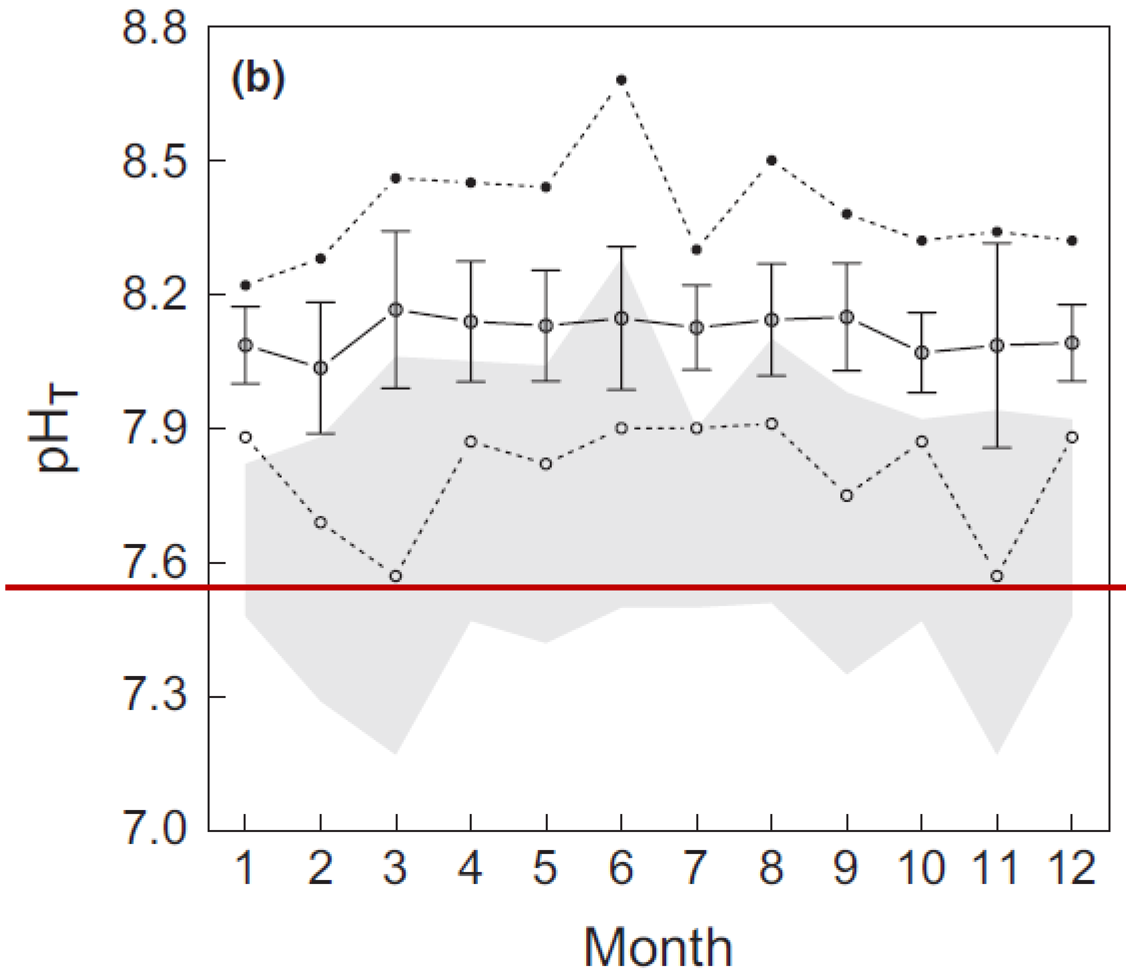
pH 8.1 (present average)

pH 7.6 (extreme of present)

pH 7.4 (outside present range)

(Dorey et al. 2013)

Range of pH scenarios (example)



pH 8.3

pH 8.1

pH 7.9

pH 7.7

pH 7.5

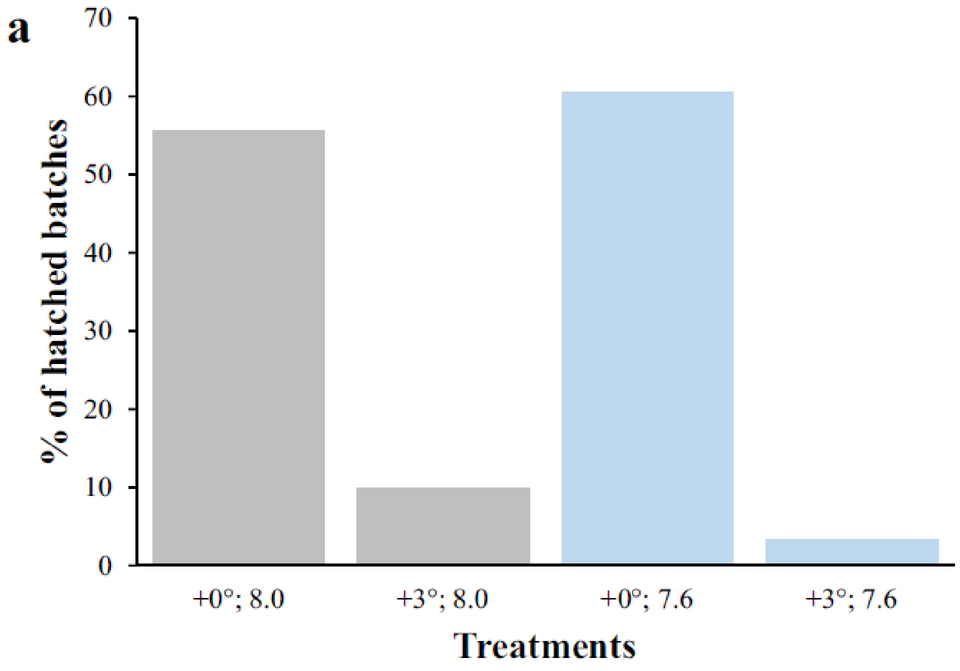
pH 7.3

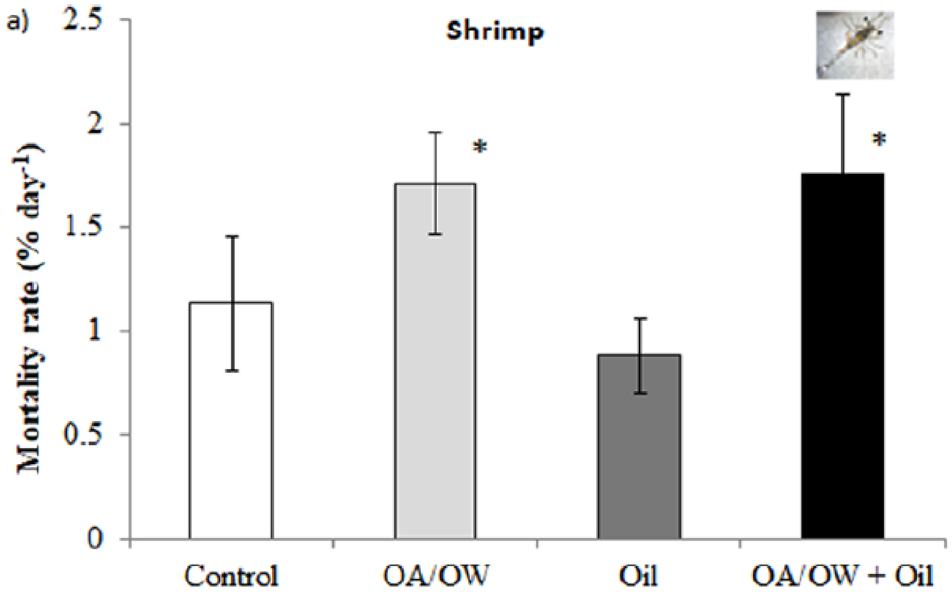
(Dorey et al. 2013)

Recommendations (writing)

- ✓ Use “pH” values in your manuscript as a given pH can be relevant in the context of present natural variability and OA
- ✓ Put tested pH into context of natural variability in the Methods
- ✓ Use the terminology “ocean acidification” in the Discussion

Recommendations (writing)





Other parameters

- ✓ All other parameters (not manipulated) should be kept as close as possible to the field (except if testing specific hypotheses)
  e.g. alkalinity, salinity, temperature, food, oxygen, etc.
  Key if you are not using seawater from the sampling site

- ✓ Be careful with interactions !

(Bad) example

Tested factor: temperature

High density
Closed aquarium

Confounding factors: O_2, CO_2

Often require a pilot experiment or working with experts