Sam Dupont
Researcher, Associate Professor
University of Gothenburg

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Assistant Professor
University of Hong Kong

Visiting scholar
University of California, Berkeley

Experimental design & Physiological endpoints

On the menu

1. How to design a relevant biological experiment?

2. Physiological endpoints (case: larval stages)

On the menu

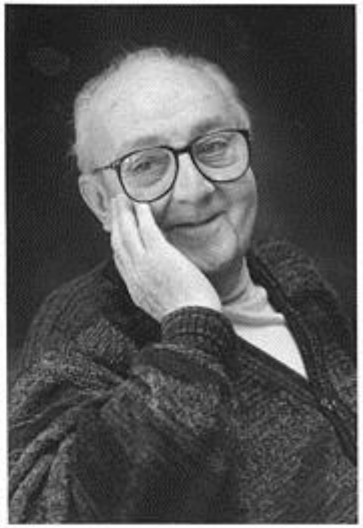
1. How to design a relevant biological experiment?

2. Physiological endpoints (case: larval stages)

Take home messages

Every experiment is an abstraction of reality

There is nothing like a perfect experiment !



George E. P. Box

"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

Trade-offs

Realism

[duration, tested parameter, environment, etc.]

VS.

Feasibility

[manpower, money, space, time]

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

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?

What is your question?

Read the literature ! or ask experts

… not only OA literature…

[Theoretical background, methods, etc.]

Standing on the shoulders of giants

Key challenges in OA

Evolution | Ecology | Multiple drivers

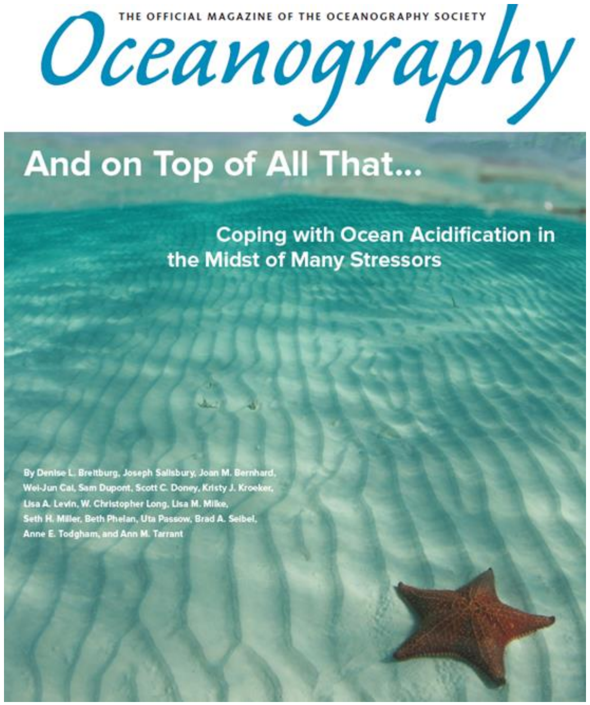
Review

Evolution in an acidifying ocean

Jennifer M. Sunday[1,2], Piero Calosi[3], Sam Dupont[4], Philip L. Munday[5,6], Jonathon H. Stillman[7,8], and Thorsten B.H. Reusch[9]

[1] Department of Biological Sciences, Simon Fraser University, Burnaby, British Columbia, V5A 1S6, Canada
[2] Biodiversity Research Centre, University of British Columbia, Vancouver, British Columbia, V6T 1Z4, Canada

Variability



ESA CENTENNIAL PAPER

Ecology, 96(1), 2015, pp. 3–15
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Ocean acidification through the lens of ecological theory

Brian Gaylord,[1,14] Kristy J. Kroeker,[1] Jennifer M. Sunday,[2] Kathryn M. Anderson,[2] James P. Barry,[3] Norah E. Brown,[2] Sean D. Connell,[4] Sam Dupont,[5] Katharina E. Fabricius,[6] Jason M. Hall-Spencer,[7] Terrie Klinger,[8] Marco Milazzo,[9] Philip L. Munday,[10] Bayden D. Russell,[4] Eric Sanford,[1] Sebastian J. Schreiber,[11] Vengatesen Thiyagarajan,[12] Megan L. H. Vaughan,[2] Steven Widdicombe,[13] and Christopher D. G. Harley[2]

What is your question?

Read the literature ! (or ask experts)

… not only OA literature…

[Theoretical background, methods, etc.]

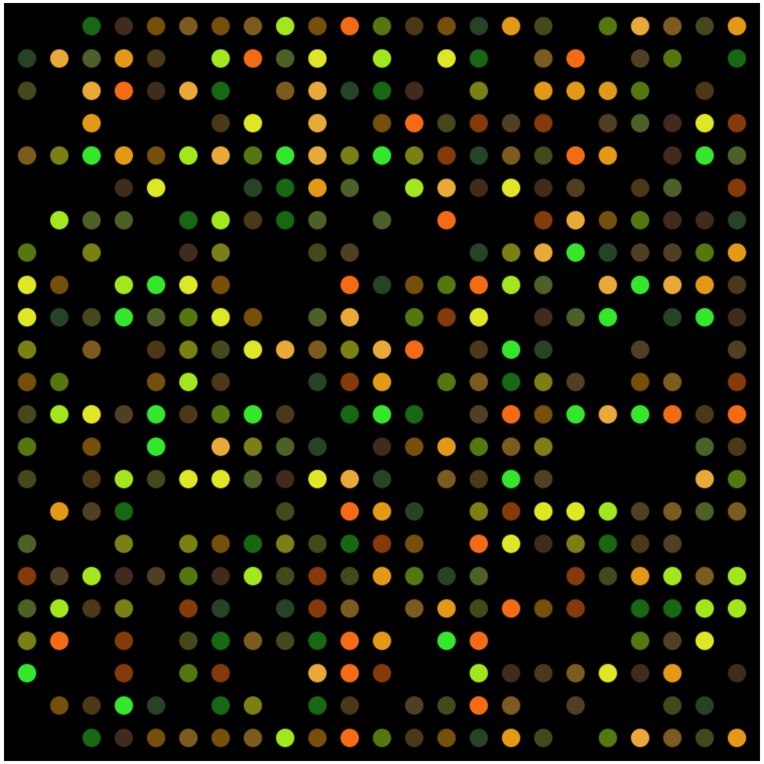
Exploration is fine BUT do not "stamp collect"

Do not base your experiment on a technique
[e.g. "-omic revolution"]

Impact of OA on sea urchin larvae

CONTROL vs ACIDIFICATION

1 time point



Technically sound

Conclusion???

How to design your experiment

1. What is your question? Your hypothesis?

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   - What are my limitations?

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   - etc.

Can I REALLY answer my question with the collected data?

e.g. TRUE replication

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ICES Journal of Marine Science

ICES CIEM International Council for the Exploration of the Sea / Conseil International pour l'Exploration de la Mer

ICES Journal of Marine Science; doi:10.1093/icesjms/fsv118

Experimental design in ocean acidification research: problems and solutions

Christopher E. Cornwall[1,2]* and Catriona L. Hurd[1]

[1]Institute for Marine and Antarctic Studies, University of Tasmania, Private Bag 129, Hobart, TAS 7001, Australia
[2]School of Earth and Environment and ARC Centre of Excellence in Coral Reef Studies, The University of Western Australia, 35 Stirling Highway, Crawley, WA 6009, Australia

NEWS IN FOCUS

MATERIALS SCIENCE

Stanene makes its debut

Graphene's tin cousin may conduct without heat loss.

BY CHRIS CESARE

Two years after physicists predicted that tin should be able to form a mesh just one atom thick, researchers report that they have made it[1]. The thin film is called stanene (from the Latin *stannum* meaning tin, which also gives the element its chemical symbol, Sn) and is the latest cousin of graphene, the honeycomb lattice of carbon atoms that has spurred thousands of studies into related 2D materials (see *Nature* 522, 274–276; 2015).

In theory, stanene has a talent that graphene does not: at room temperature, electrons should be able to travel along the edges of the tin mesh without colliding with other electrons and atoms as they do in most materials. This makes the film what physicists call a topological insulator, and means that it should be able to conduct electricity without losing energy as waste heat, according to predictions[2] made in 2013 by Shou-Cheng Zhang, a physicist at Stanford University in California, who is a co-author of the latest study.

A thin film of stanene might be the perfect highway along which to ferry current in electric circuits, says Peide Ye, a physicist and electrical engineer at Purdue University in West Lafayette, Indiana

Marine snails from the US West Coast show signs of shell weakening as a result of ocean acidification.

OCEAN ACIDIFICATION

Seawater studies come up short

Experiments fail to predict size of acidification's impact.

BY DANIEL CRESSEY

The past decade has seen accelerated attempts to predict what these changes in

Daily Mail .com Science

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Are climate scientists doom-mongering? Bulk of research on impacts of ocean acidification is FLAWED, new study finds

- Scientists have warned growing carbon emissions are leading to the oceans getting more acidic as carbon dioxide gas dissolves in sea water
- A review of 465 studies found just 27 used appropriate experimental design
- They say the flaws 'undermine' confidence in the impacts of acidic oceans
- It comes a month after figures revealed the Arctic ice cap regrew in 2013

By COLIN FERNANDEZ, ENVIRONMENT CORRESPONDENT FOR THE DAILY MAIL

Correspondence

Outdated listing puts species at risk

Loopholes could allow illegal wildlife traders and hunters in China to evade prosecution or to receive reduced sentencing. The problem stems from China's Protected Species List (PSL): this has not been updated since it was implemented in 1989, resulting in incongruity with newer taxonomy.

Appendices I and II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) and the International Union for Conservation of Nature (IUCN) use taxonomic classifications based on recent revisions to geographical distributions and phylogenetic relationships. Some inconsistency issue requires that all 181 signatory nations to CITES adopt unambiguous standardized and internationally coherent naming policies, following the IUCN Red List and CITES Species+ (www.speciesplus.net).

Zhao-Min Zhou* *Yunnan Public Security Bureau for Forests, Kunming, Yunnan, China.*
zhouzm81@gmail.com
**On behalf of 6 correspondents (see go.nature.com/hubzzy for full list).*

Physicists' report on EU green electricity

The European Physical Society has released a report on European Union (EU) plans for sustainable production urges Europe to continue to lead the way in cutting greenhouse-gas emissions.

Jozef Ongena *Laboratory for Plasma Physics, Royal Military Academy, Brussels, Belgium.*
Christophe Rossel *European Physical Society, Mulhouse, France.*
j.ongena@fz-juelich.de

Laboratory seawater studies are justified

In our view, your report 'Seawater studies come up short' (*Nature* 524, 18–19; 2015) fails to capture the nuances of the survey results you discuss (see C. E. Cornwall and C. L. Hurd *ICES J. Mar. Sci.* http://doi.org/68g; 2015).

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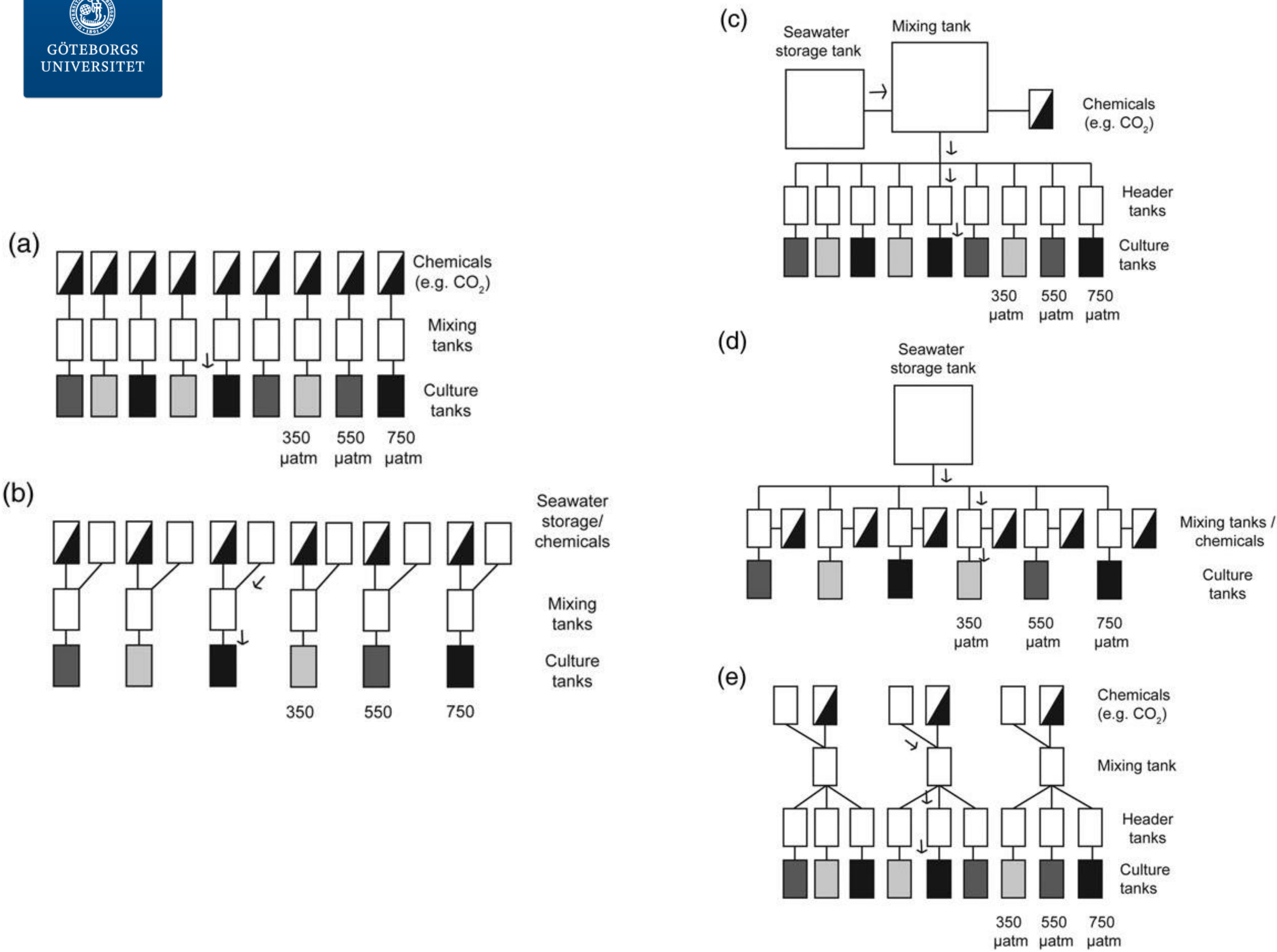
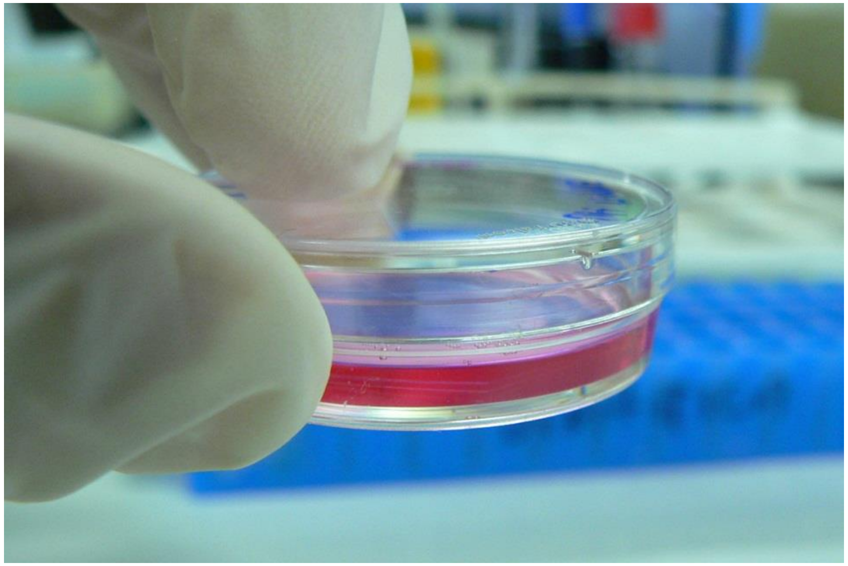


e.g. replication

# 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



How to design your experiment

1. What is your question? Your hypothesis?

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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.

The top model

(Dupont et al. 2014)

How to design your experiment

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   - etc.

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Endpoints?

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

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

Etc. etc.

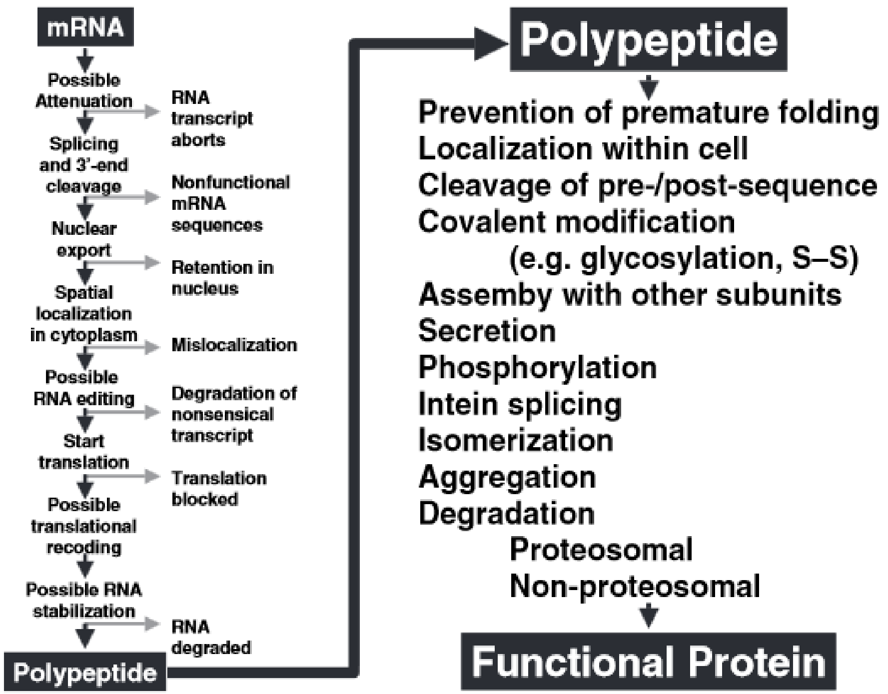
Question on methods, ask us !

Best endpoints?

- Not the “coolest” method

- Not the most familiar method

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



Feder & Walser 2005

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

Fitness is relative (interpretation)

	 Single	Multi	 Single
Control	=	=	=
-0.4	(-)		-
-0.8	---		-

How to design your experiment

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   - etc.

Can I REALLY answer my question with the collected data?

How to design your experiment

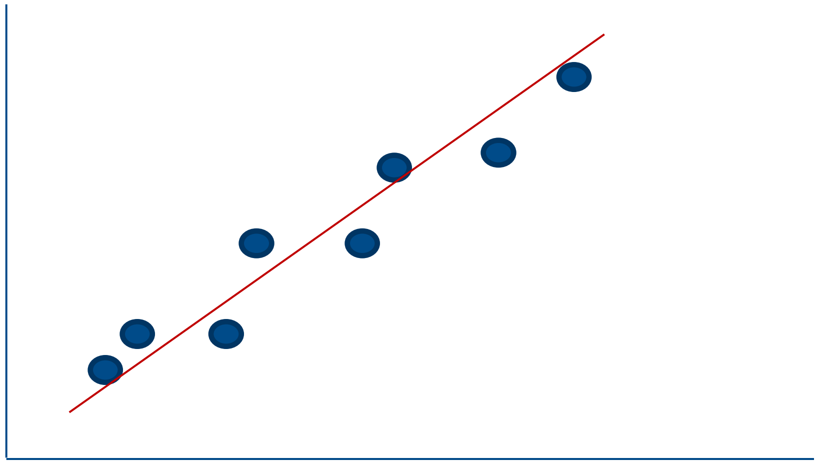
“ANOVA” design

Little predictive power

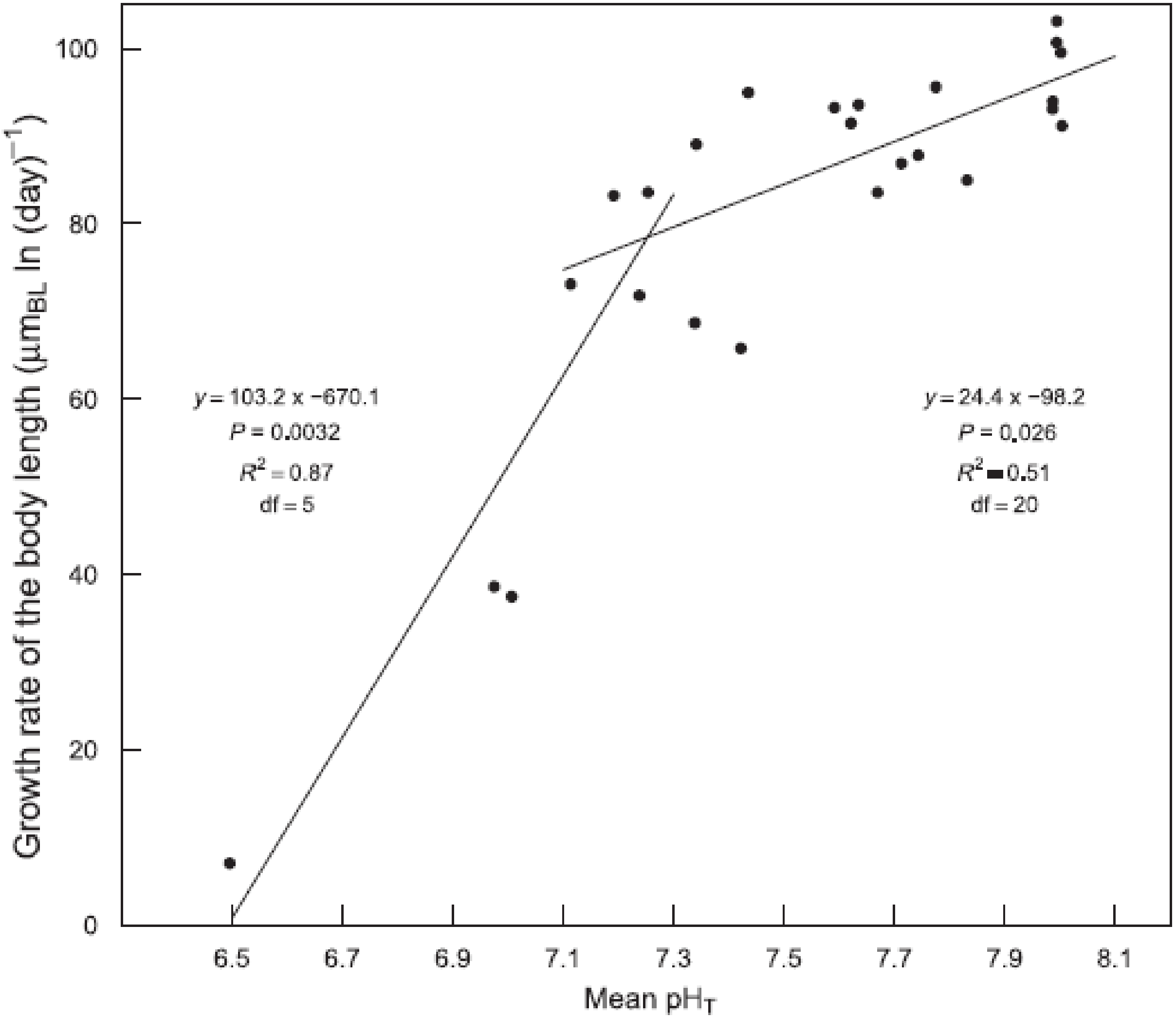
Scenario 1	Scenario 2	Scenario 3
n=x	n=x	n=x

“Regression” design

Need to have a relationship



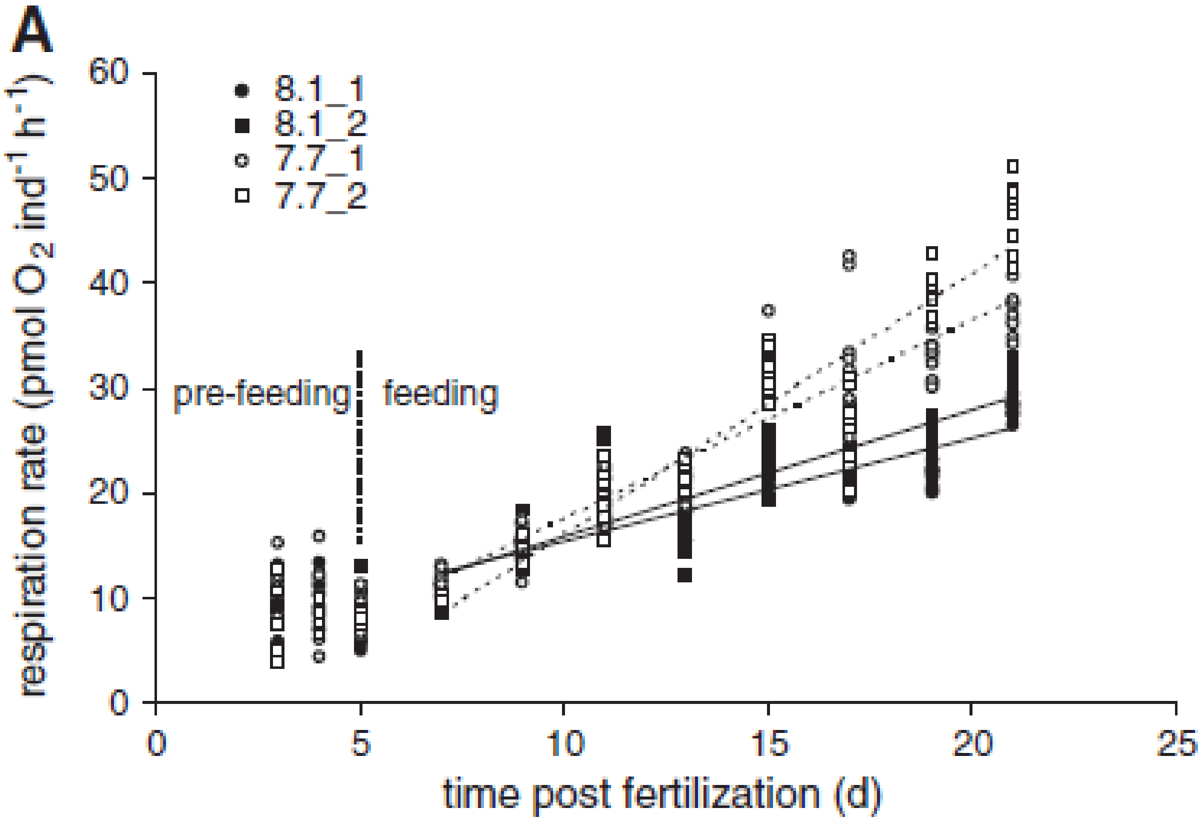
ANOVA vs. Regression



(Dorey et al. 2013)

Sampling strategy

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



(Stumpp et al. 2011)

How to design your experiment

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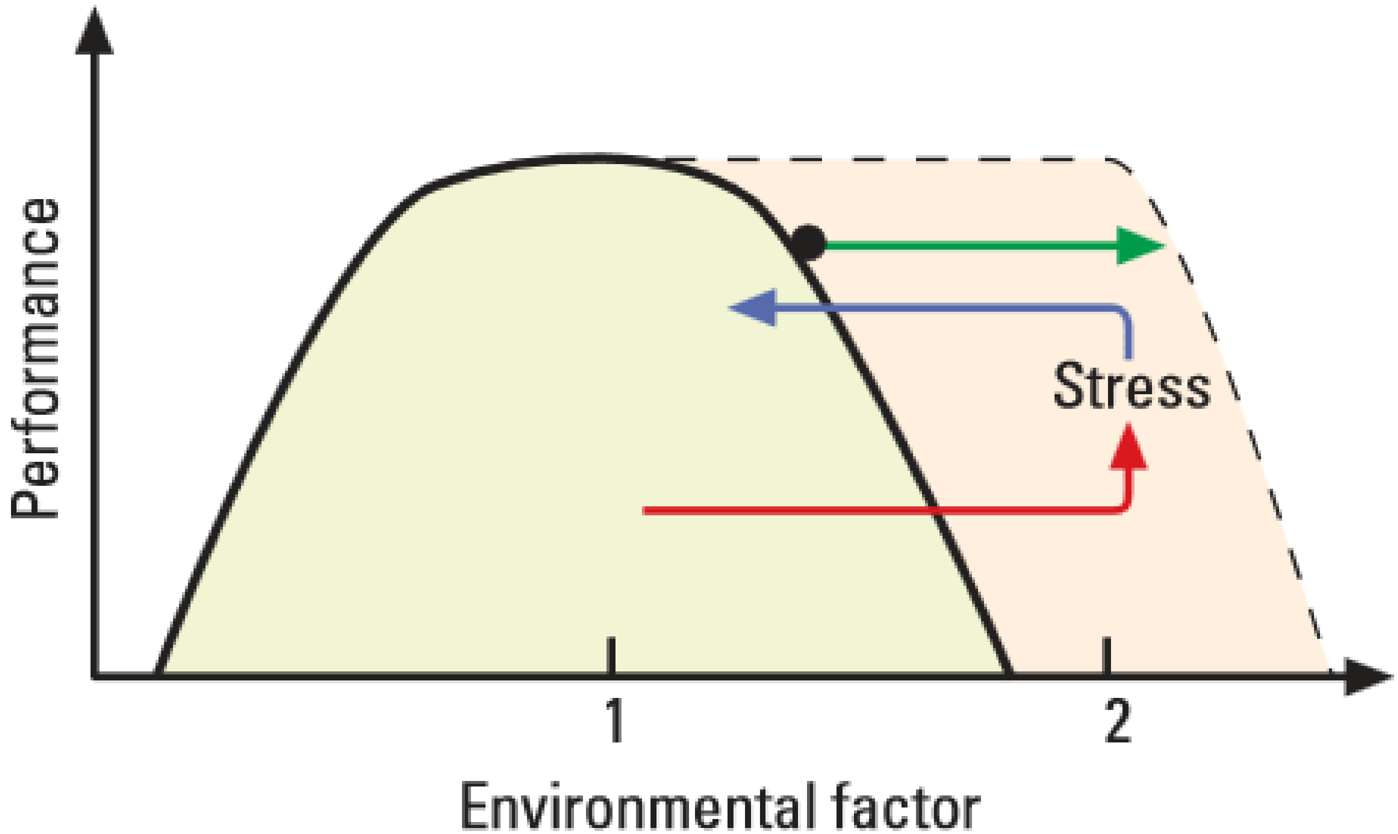
Can I REALLY answer my question with the collected data?

Stress ecology - niche



(Van Straalen 2007)

Need to understand the biology of your species

On the menu

1. How to design a relevant biological experiment?

2. **Physiological endpoints (case: larval stages)**

Example

Question: What is the impact of OA on larvae?
(multiple drivers, variability)

Model: sea urchin

Hypothesis: Interaction can be predicted by mode of action

Strategy: Monitoring, mechanistic understand, field and lab experiments, models

Societal relevance: Predictive model, mapping

An impossible task?

It is NOT possible to test ALL species/ecosystems, in ALL locally relevant conditions including LOCAL variability (today and future)

We need to understand the mechanisms

Dupont & Pörtner (2013) Nature

E.g. organism response to multiple drivers

1. Good data on local variability / future scenarios
2. Good understanding of biological response for each driver [mode of action]
3. Build models

Mix all the ingredients & test using scenarios [field, laboratory]

Importance of sorting stressors

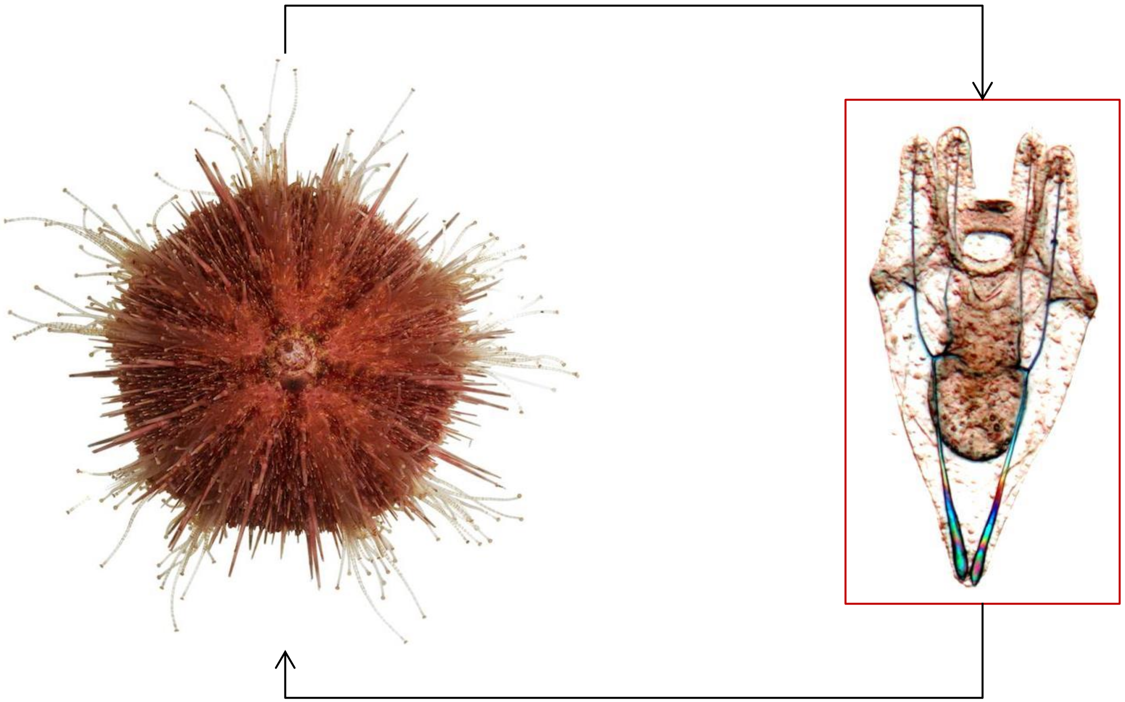
Driver A

Driver B (similar mode of actio

Driver C (different mode of actio

Sea urchin

- Keystone species
- Commercially important
- Genome available + GRN
- Functional tools
- Centuries of data (model)
- Sensitive to acidification



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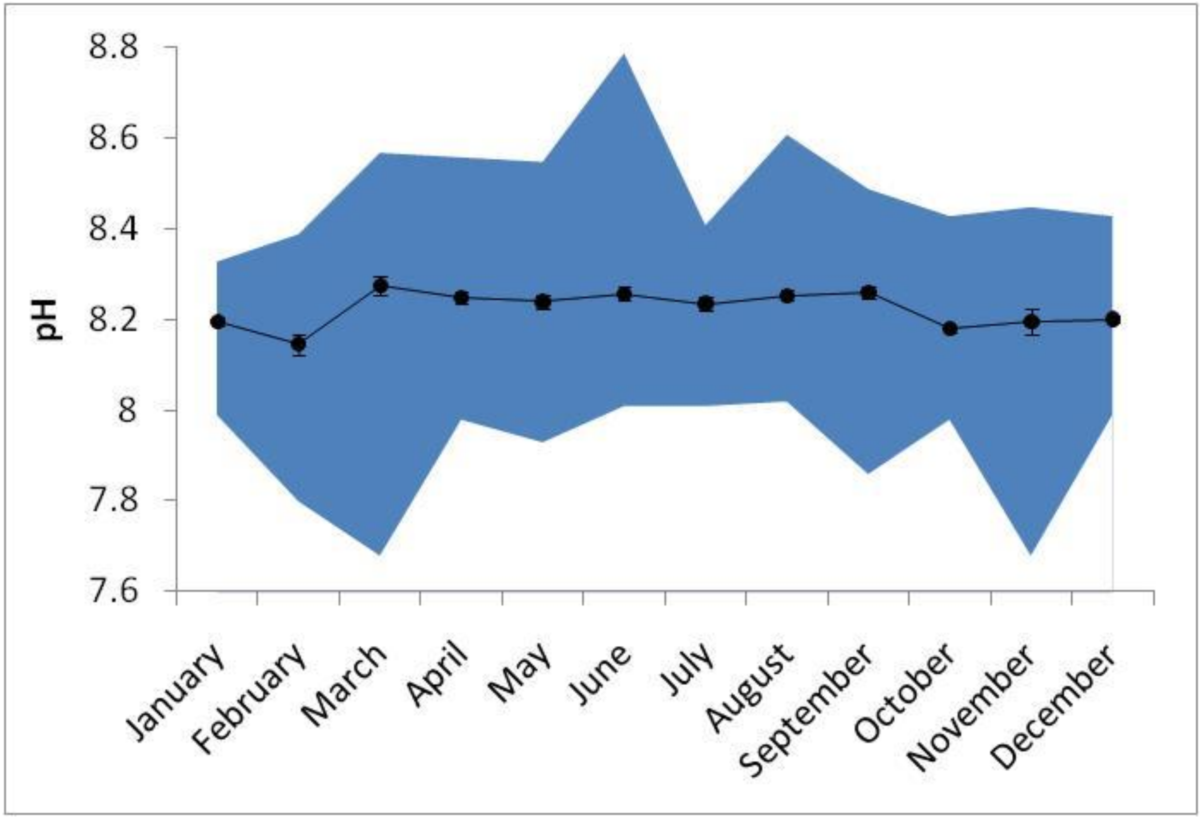
1. Good data on local variability / future scenarios



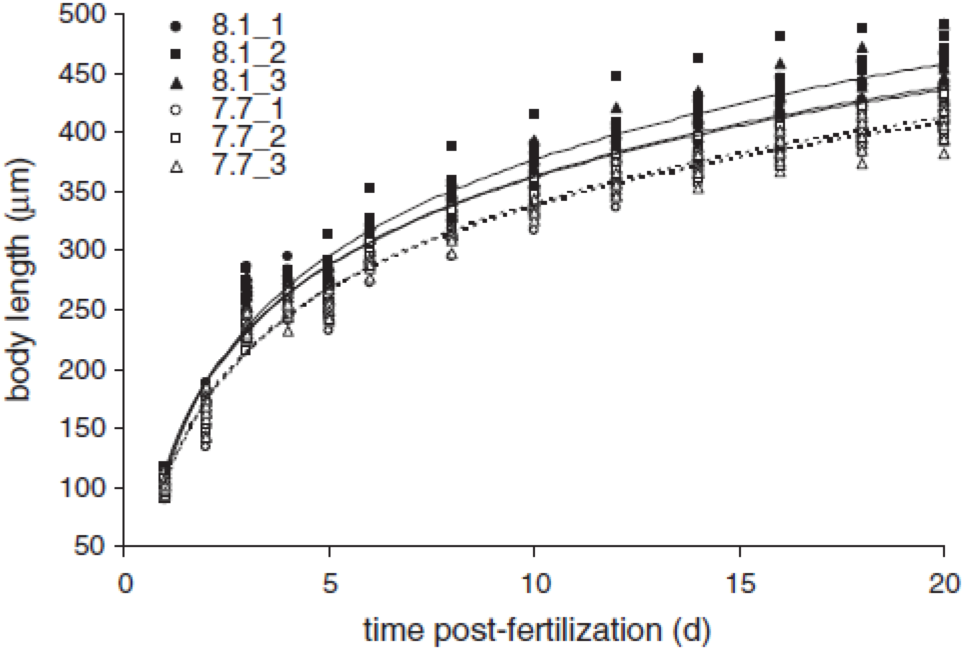
(Dorey et al. 2013)

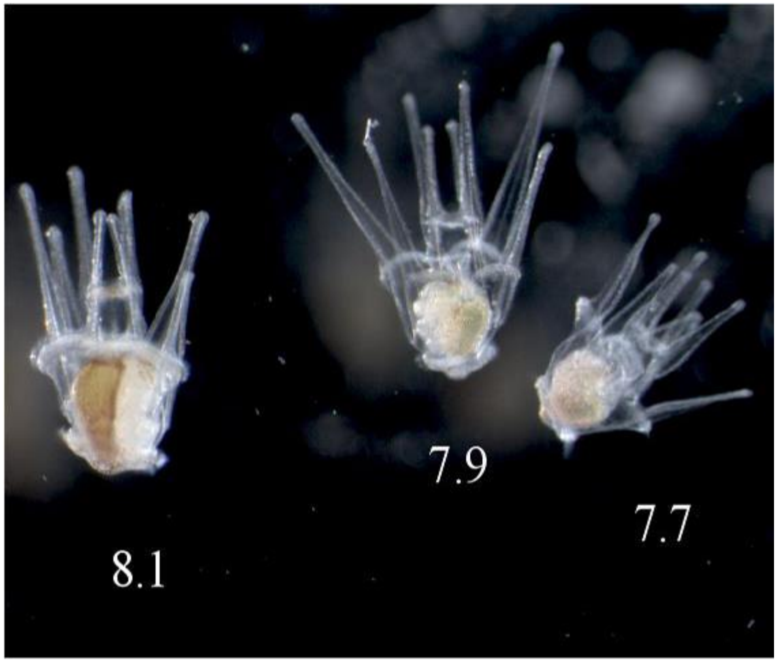
E.g. organism response to multiple drivers

1. Good data on local variability / future scenarios
2. Good understanding of biological response for each driver [mode of action]
3. Build models

Mix all the ingredients & test using scenarios [field, laboratory]

Growth

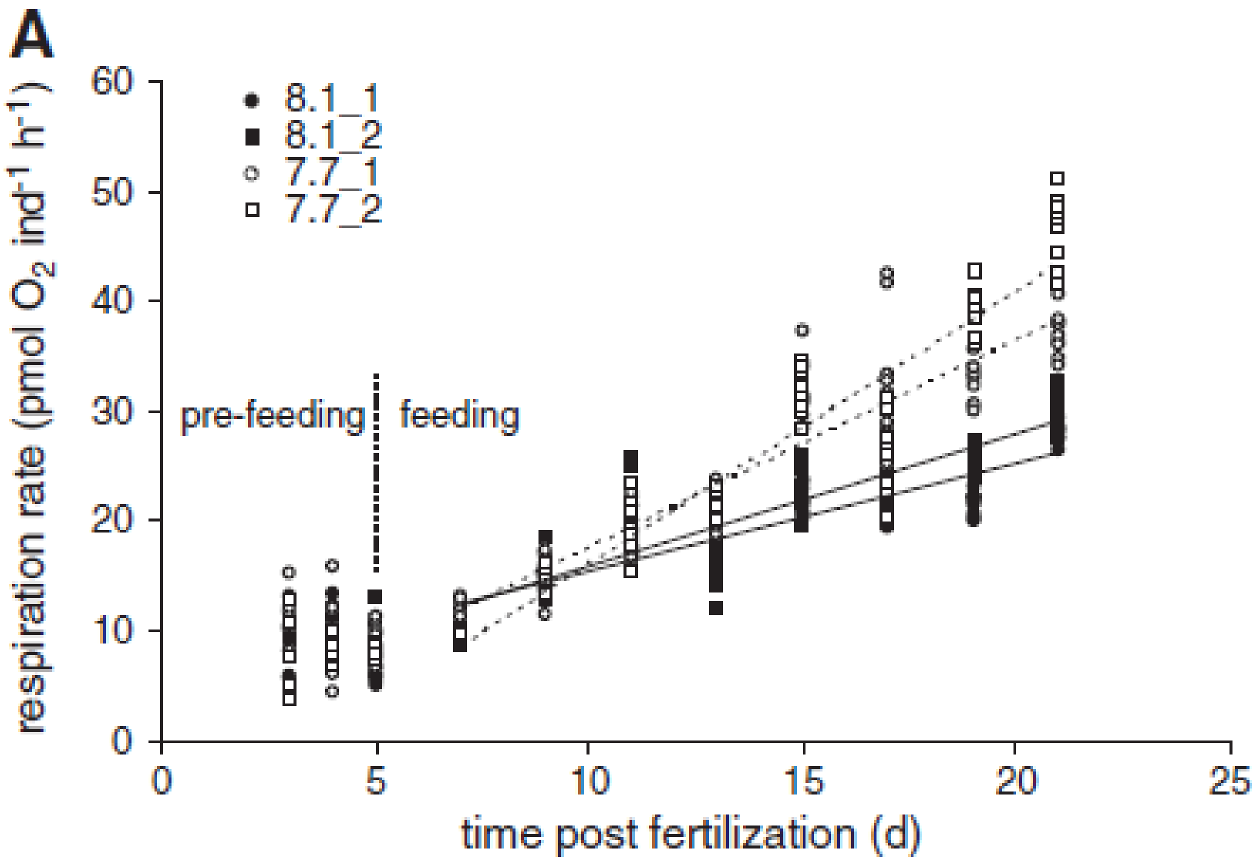




Delay in development

e.g. Martin et al. (2011) <u>J. Exp. Biol</u>; Stumpp et al. (2011a,b) <u>Comp. Biochem Physiol</u>

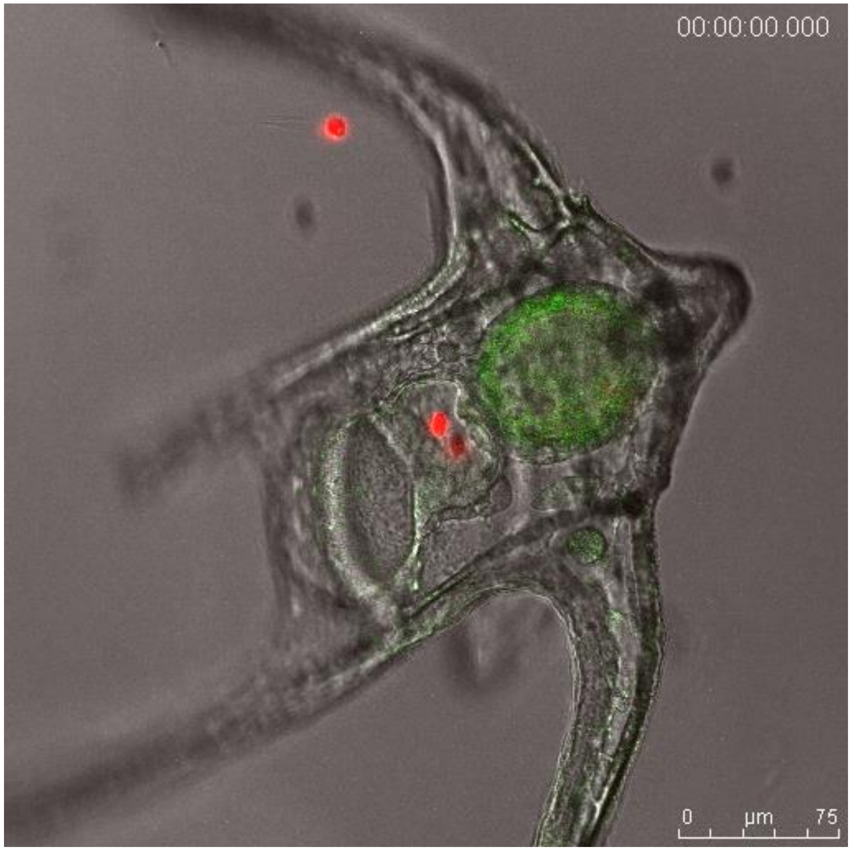
Dissecting the energy budget



Increased respiration

Stumpp et al. (2011a,b) Comp. Biochem. Physiol.

Feeding physiology



- Ingestion/Digestion rates
- pH in the digestive track
- Enzymatic activity
- Cellular structure

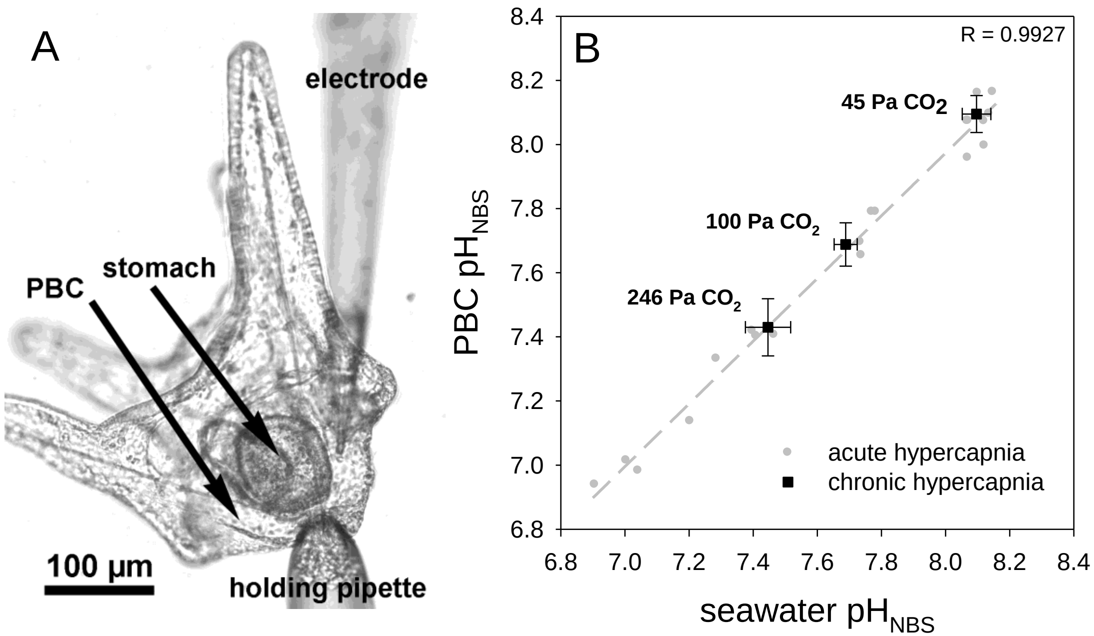
- **Stomach is alkaline**
- **Compensation mechanisms**
- **Extra costs**

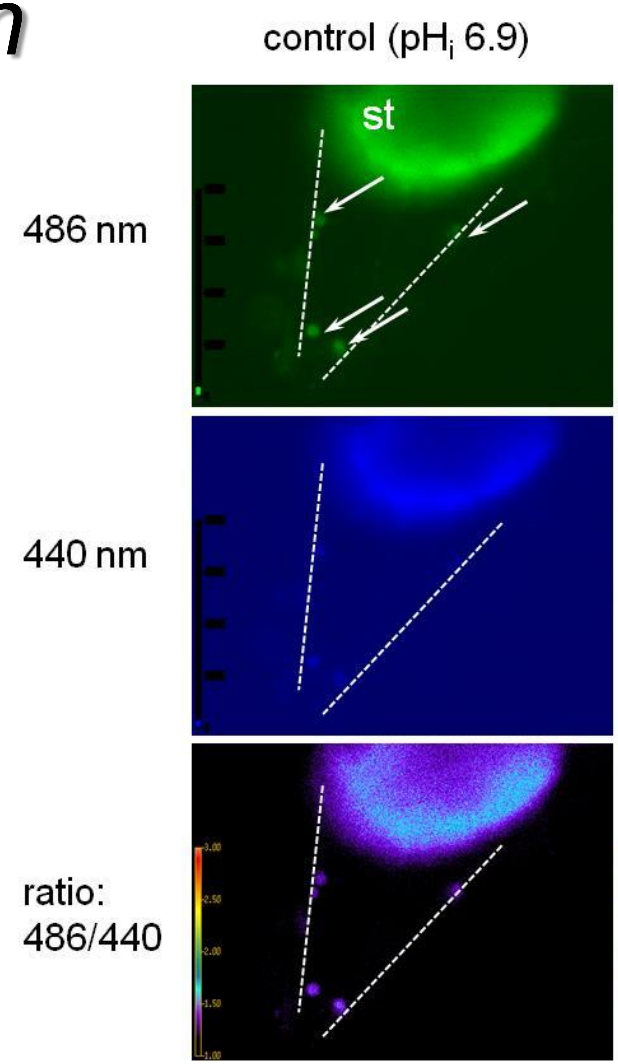
nature climate change
LETTERS
PUBLISHED ONLINE: 20 OCTOBER 2013 | DOI: 10.1038/NCLIMATE2028

Digestion in sea urchin larvae impaired under ocean acidification

Meike Stumpp[1,2,3†], Marian Hu[1,2,3†], Isabel Casties[1], Reinhard Saborowski[4], Markus Bleich[2], Frank Melzner[3] and Sam Dupont[1*]

Acid-base regulation





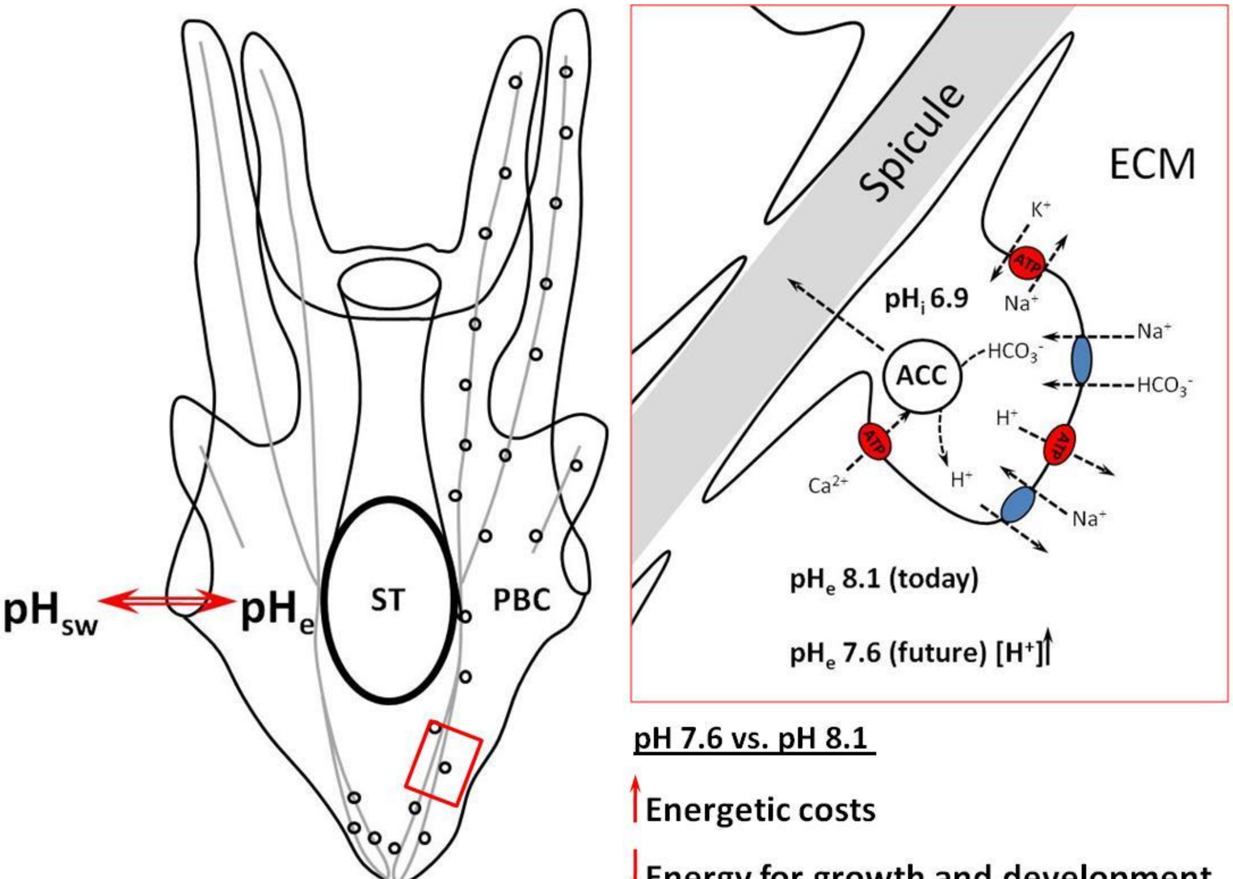
Acidified seawater impacts sea urchin larvae pH regulatory systems relevant for calcification

Meike Stumpp[a,b,c,1], Marian Y. Hu[a,b,c,1], Frank Melzner[b], Magdalena A. Gutowska[a,b], Narimane Dorey[c], Nina Himmerkus[a], Wiebke C. Holtmann[a], Sam T. Dupont[c], Michael C. Thorndyke[c], and Markus Bleich[a,2]

[a]Institute of Physiology, Christian Albrechts University Kiel, 24098 Kiel, Germany; [b]Helmholtz Centre for Ocean Research Kiel (GEOMAR), 24105 Kiel, Germany; and [c]Department of Biological and Environmental Sciences, The Sven Lovén Centre for Marine Science, University of Gothenburg, Kristineberg, 45178 Fiskebäckskil, Sweden

Edited by George N. Somero, Stanford University, Pacific Grove, CA, and approved September 19, 2012 (received for review June 22, 2012)

Acid-base regulation



<u>pH 7.6 vs. pH 8.1</u>

↑Energetic costs

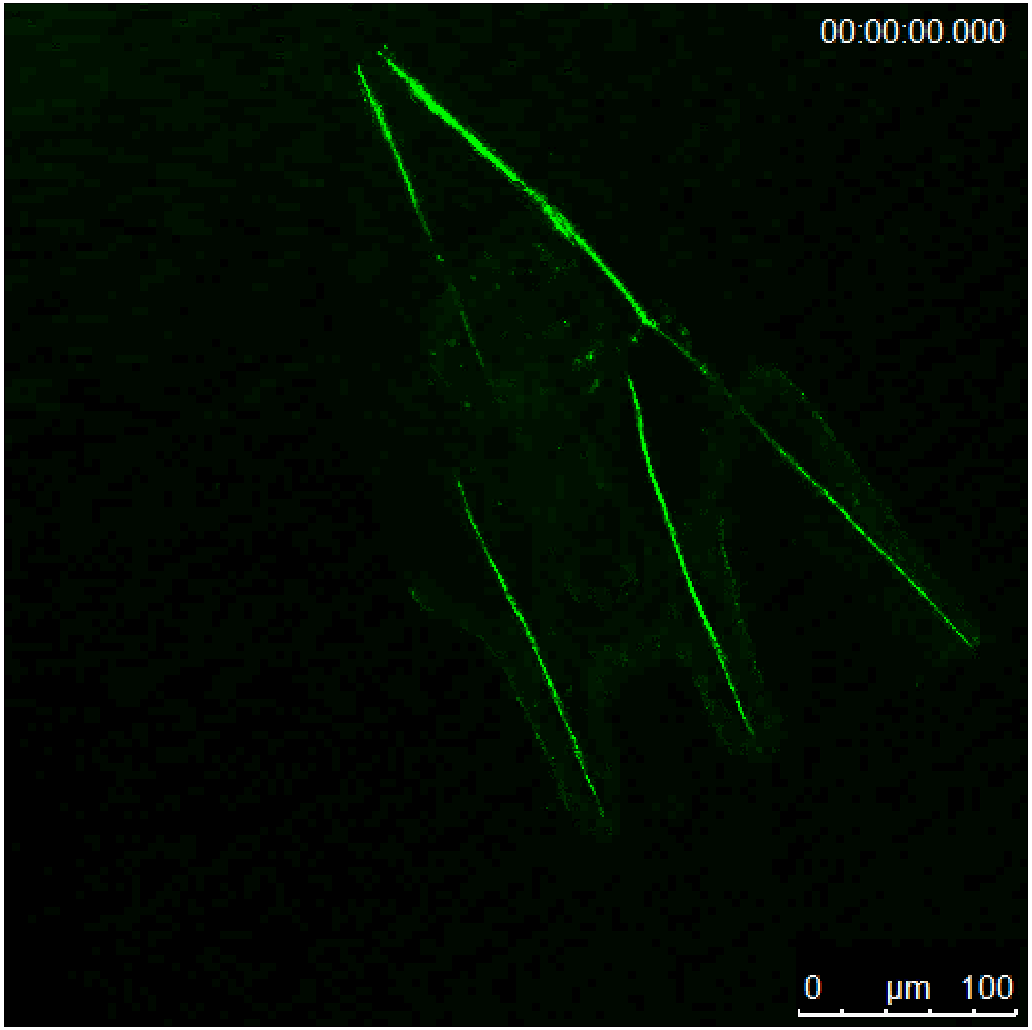
↓Energy for growth and development

↓Juvenile energy reserves

- No pHe regulation
- pHi regulation
- Role of HCO3$^-$, H$^+$-pumps
- Extra costs

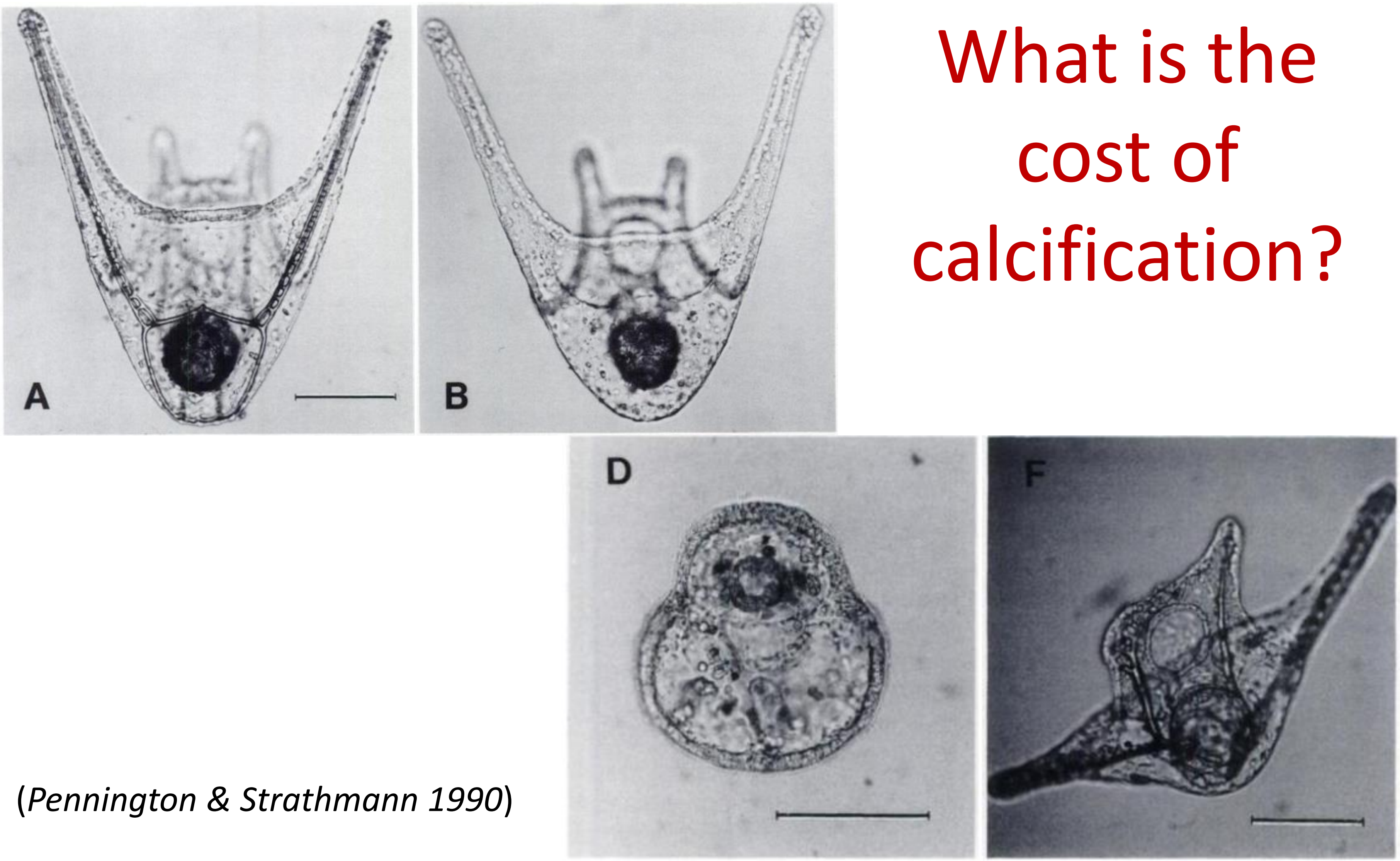
Acid-base regulation

- **Key role of H+-pumps**



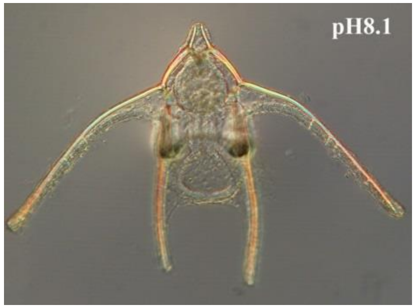
(Dupont et al., unpublished)

Cost of calcification



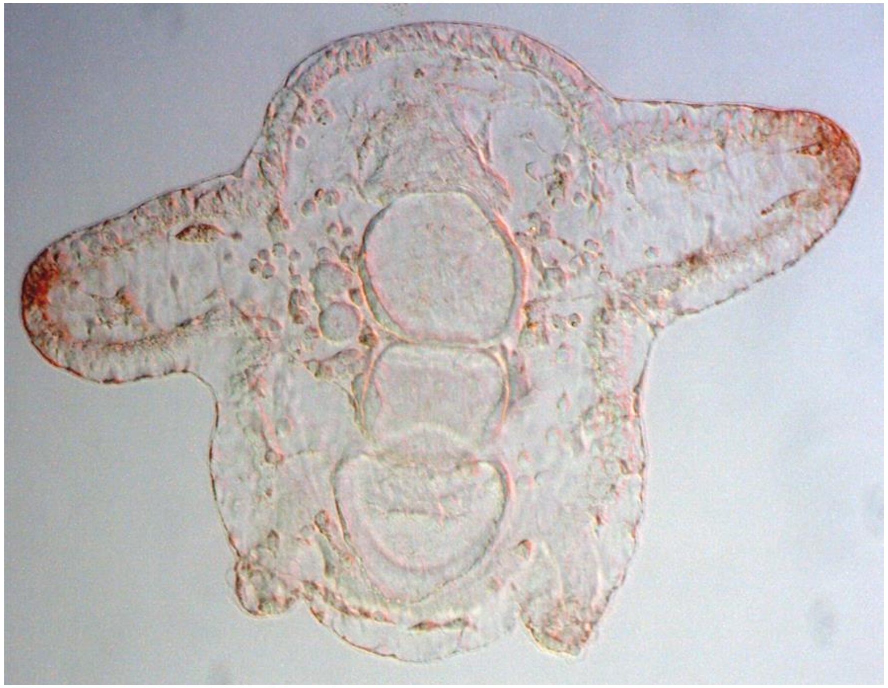
What is the cost of calcification?

(*Pennington & Strathmann 1990*)



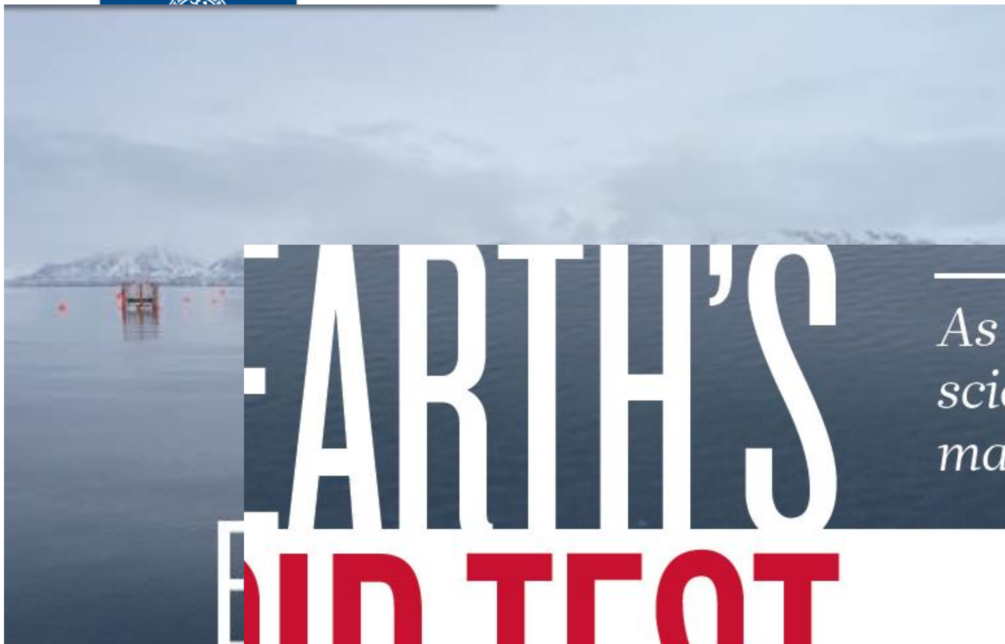
Pluteus 7d (control)

pH 5.8…3 days



7d pluteus + 3d decalcification

3d pluteus



EARTH'S ACID TEST

As the oceans rapidly grow more acid scientists are scrambling to discover h marine life is likely to react.

BY QUIRIN SC CHIERMEIER

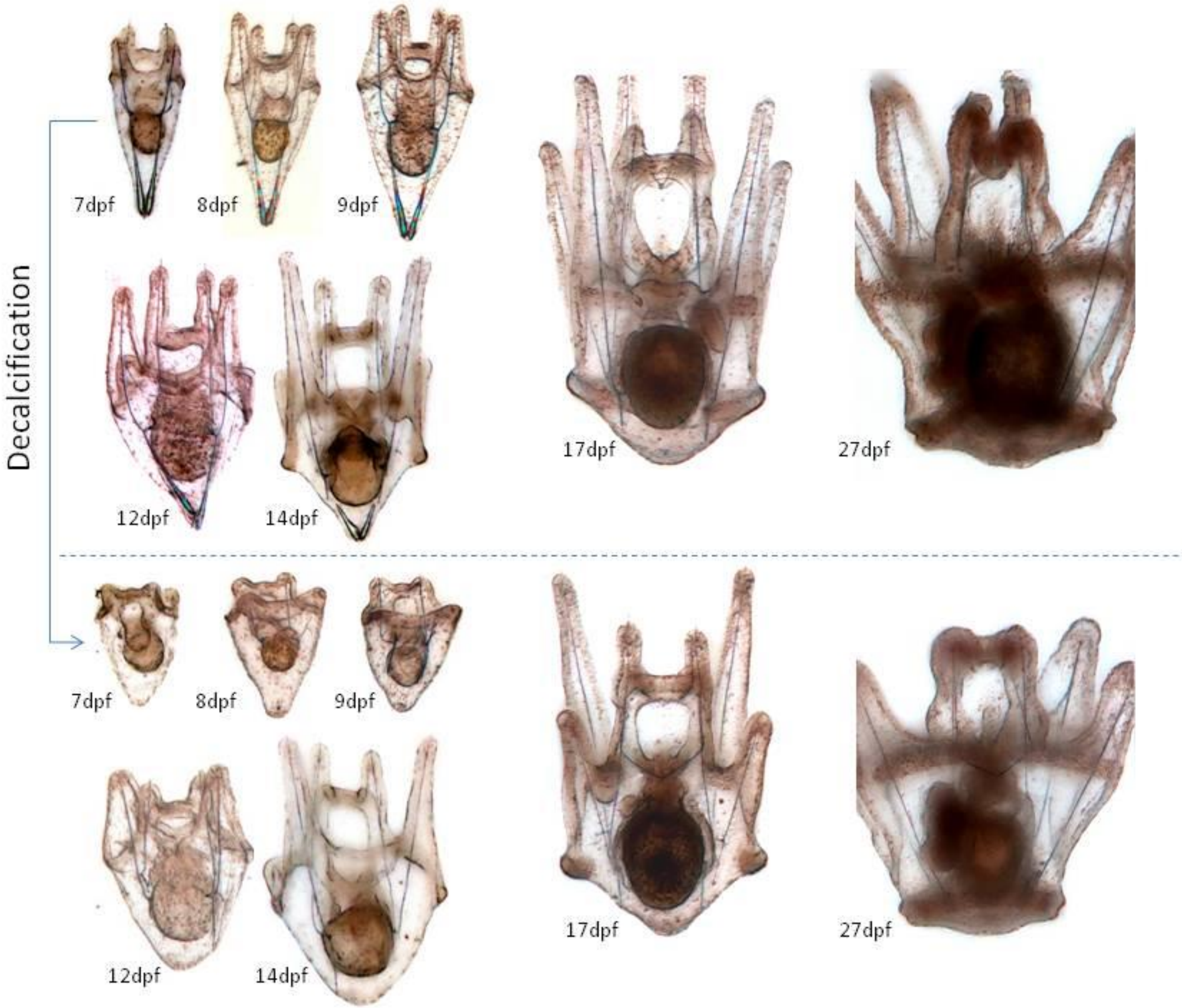
The Friday night beers made Sam Dupont forget all about his sea urchins. Earlier that day, in April 2010, the young Belgian eco-physiologist had put a batch of urchin larvae into a bath of highly acidic water to see how their skeletons would fare. When nothing obvious happened after a few hours, Dupont decided to join some friends at the pub and check on the experiment later

J.-P. GATTUSO/CNRS

climbed by 30% over the past 150 years, and some regions have already become corrosive enough to inhibit the growth of corals and other species for part of the year. According to projections, most creatures with calcium carbonate shells, such as mussels and snails, could run into problems within a few decades. By the end of this century, the acidification could even impede the growth of important groups of plankton, thus endangering entire marine ecosystems, from fisheries to coral reefs.

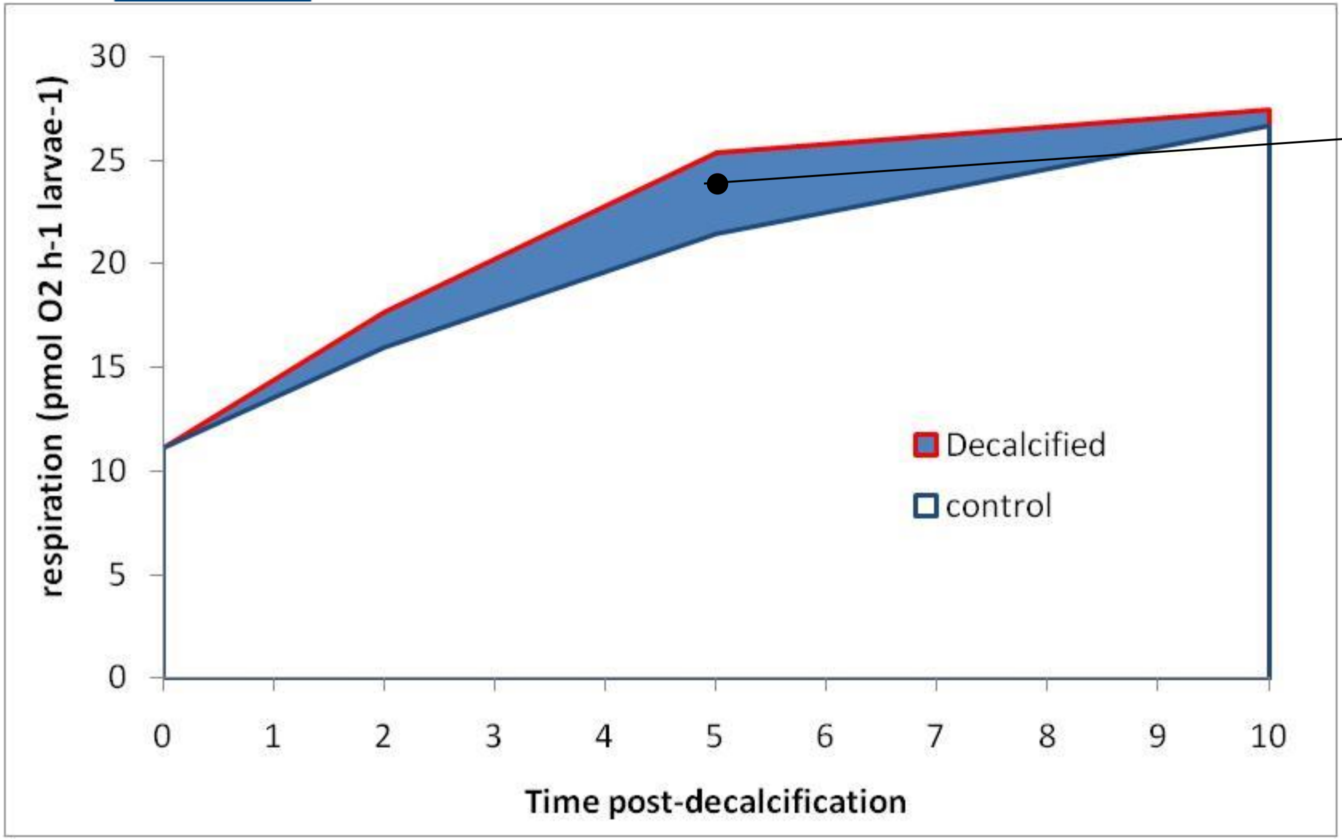
Although the urchin experiment hints that some organisms are able to survive brief exposures to highly acidic water, other studies are revealing unexpected problems that might threaten even creatures without hard shells, such as fin fish. Preliminary work suggests that responses could be highly variable, depending on factors such as water temperature, a creature's evolutionary history and the availability and quality of food.

An experiment off the coast of Spitsbergen tests the effects of elevated carbon dioxide concentrations on marine life.



Calcification = >10% of energy budget

Cost of re-calcification



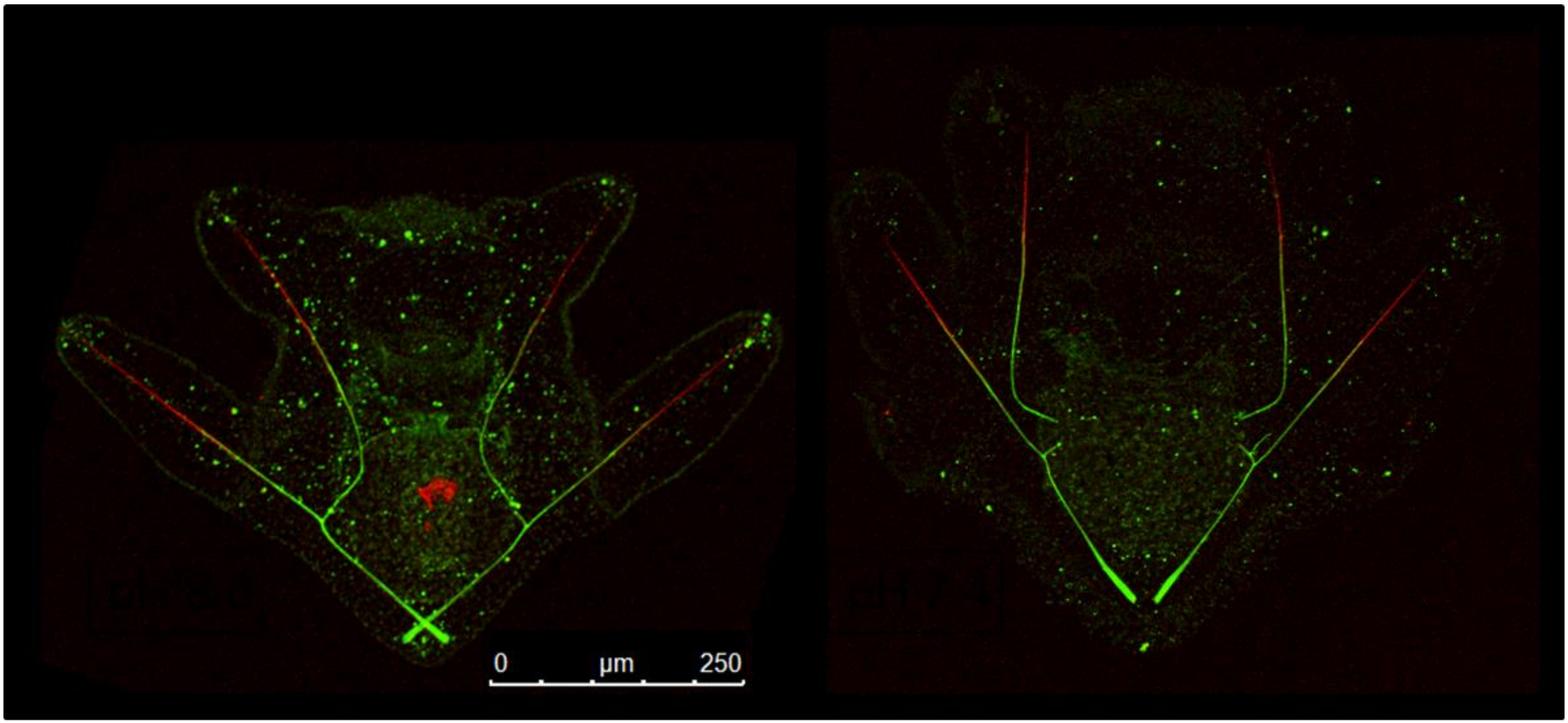
9.66% extra respiration to build a full new skeleton

Cost of calcification < 10 % (control conditions)

Under low pH conditions: 22%

➡ *2x increased cost to build/keep skeleton*

Dissolution



Control

OA

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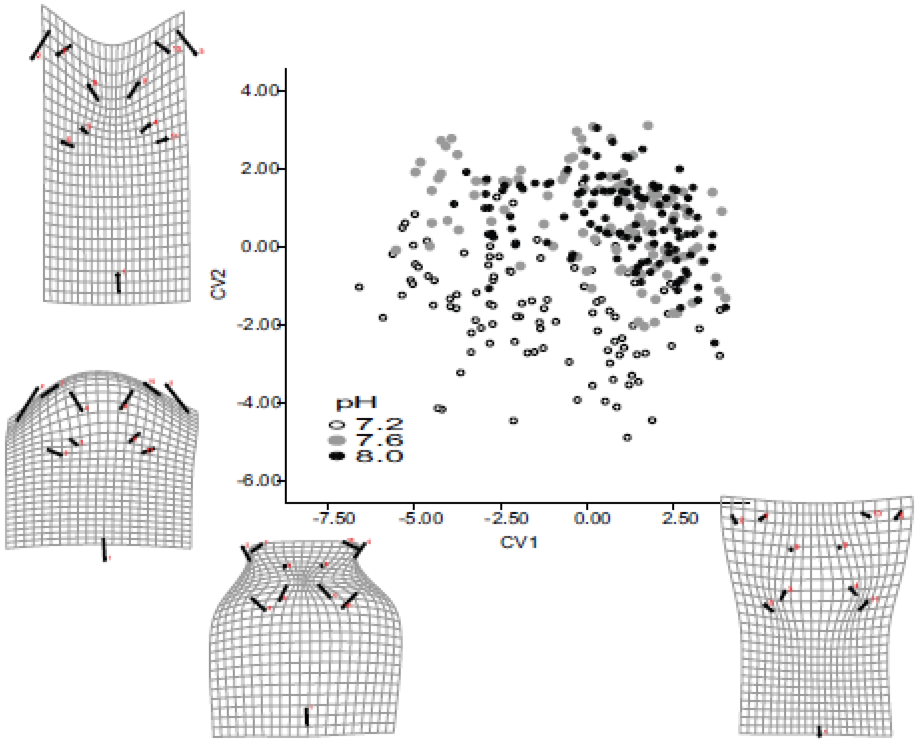
Swimming



- Delay in development
- Morphological changes
- Maintained swimming performance at a given time

SCIENTIFIC REPORTS

OPEN

SUBJECT AREAS:
CLIMATE CHANGE
ECOLOGY

Acidification reduced growth rate but not swimming speeds of larval sea urchins

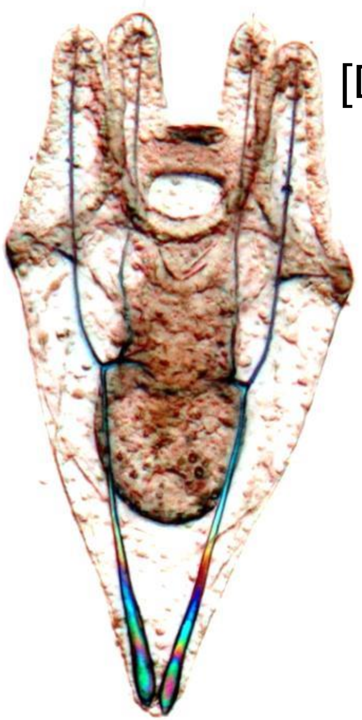
Kit Yu Karen Chan[1,2], Eliseba García[3] & Sam Dupont[4]

Mechanistic understanding

Settle
[Dorey et al. In prep]

Growing
[Dorey et al. 2013]

Surviving
[Dorey et al. 2013; Dupont et al. 2012]



Swimming
[Chan et al. In prep]

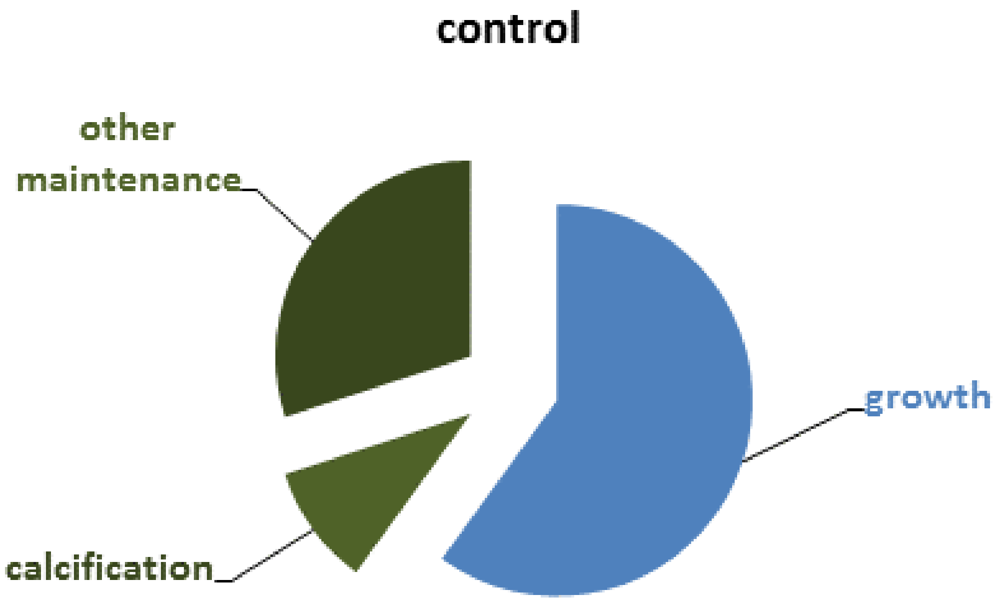
Calcifying
[Dupont et al. In prep]

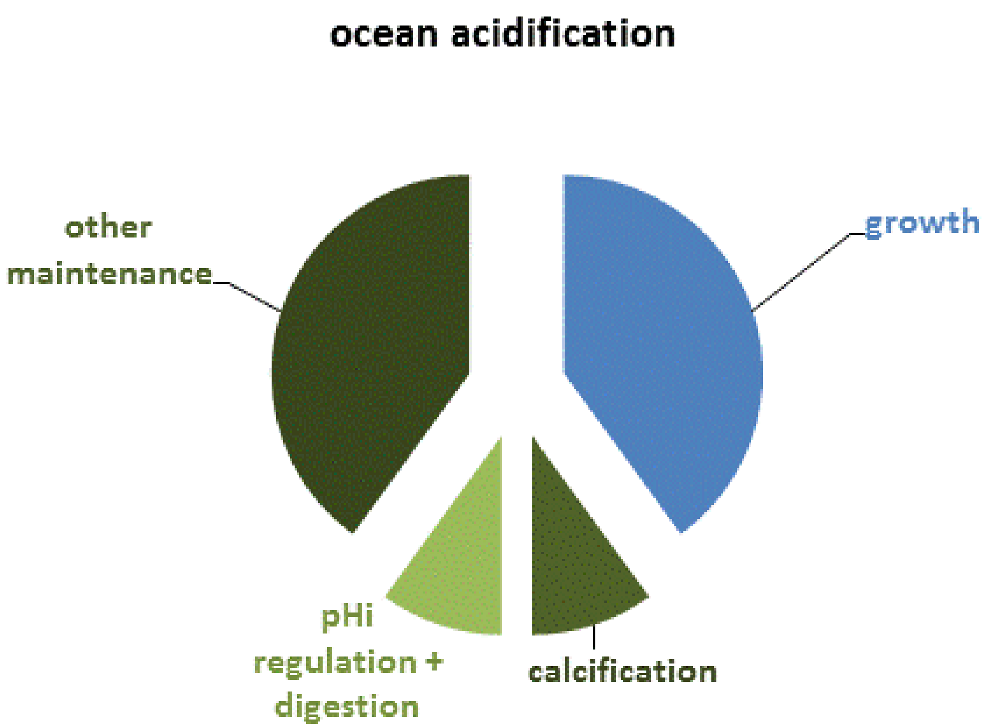
Feeding
[Stumpp et al. 2013]

Respirating
[Dorey et al. 2013]

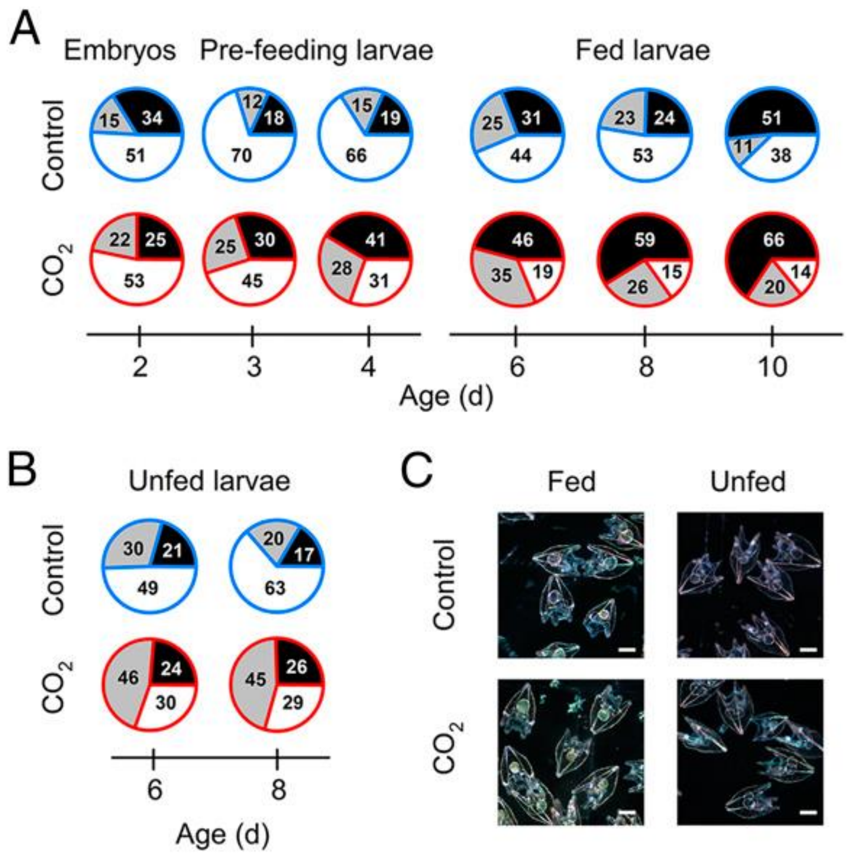
Acid-base regulation
[Stumpp et al. 2012]

Shift in energy budget





Now measured



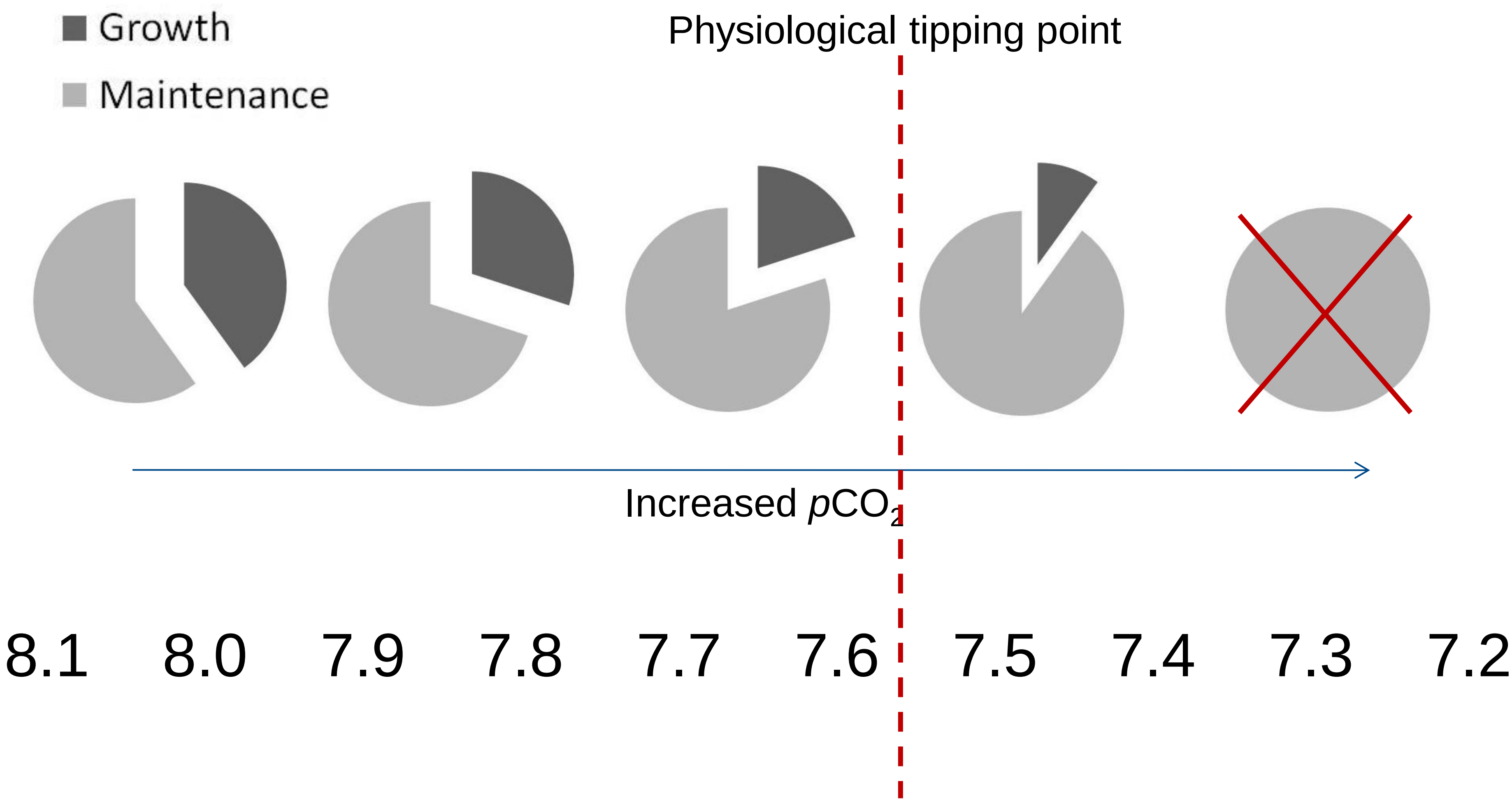
Experimental ocean acidification alters the allocation of metabolic energy

T.-C. Francis Pan[1], Scott L. Applebaum[1], and Donal T. Manahan[2]

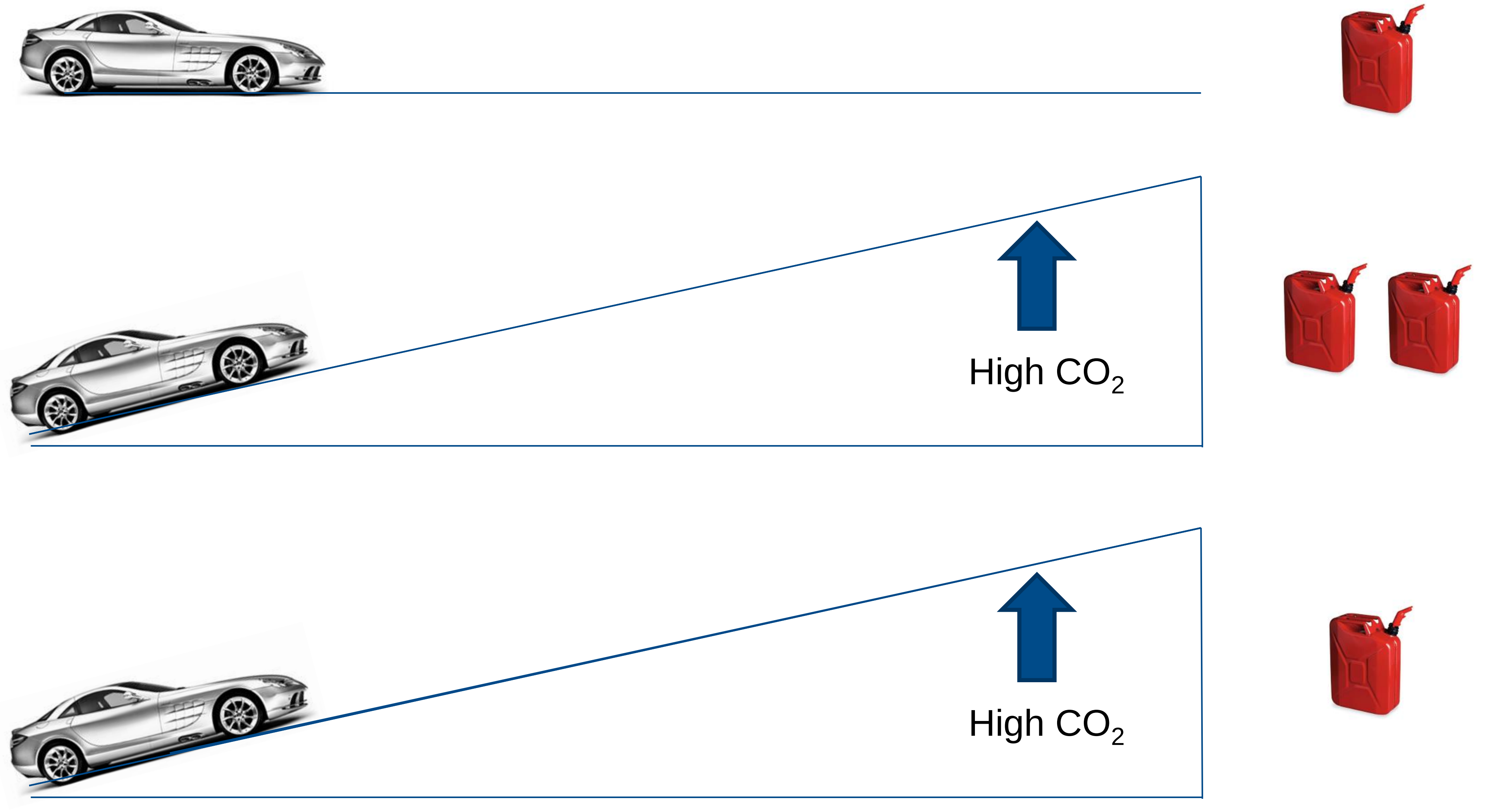
Department of Biological Sciences, University of Southern California, Los Angeles, CA 90089

Edited by George N. Somero, Stanford University, Pacific Grove, CA, and approved March 4, 2015 (received for review September 2, 2014)

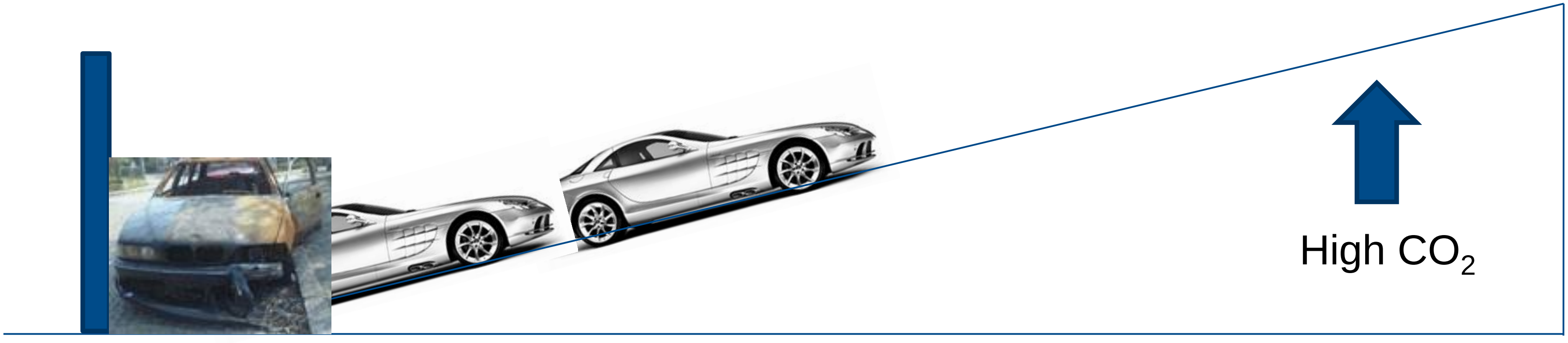
Shift in energy budget



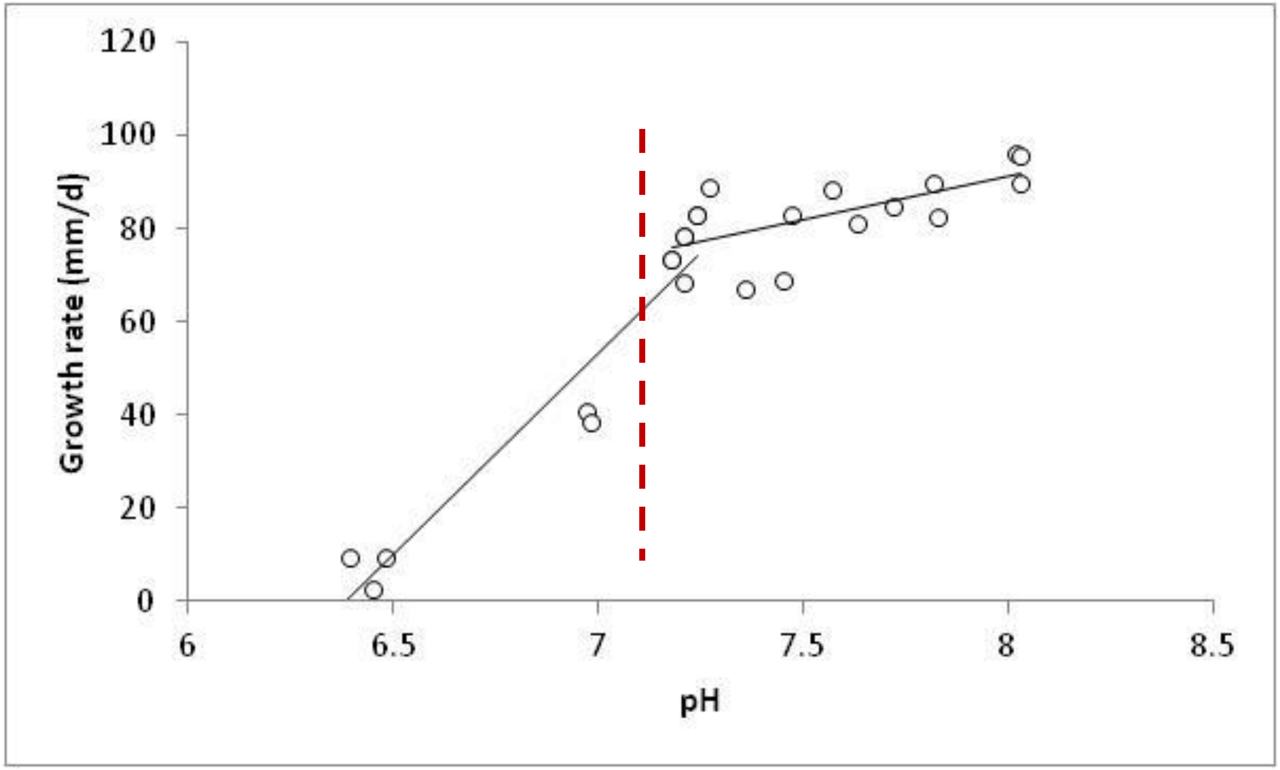
It’s all about energy

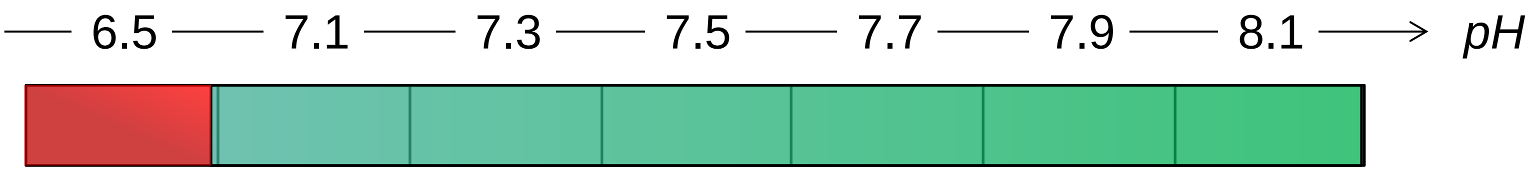


It’s all about energy



Testing the hypothesis





Grow slower at low pH (tip point pH<7.3)

Dorey et al. (2013) Glob. Change Biol.

E.g. organism response to multiple drivers

1. Good data on local variability / future scenarios
2. Good understanding of biological response for each driver [mode of action]
3. Build models

Mix all the ingredients & test using scenarios [field, laboratory]

DEB modeling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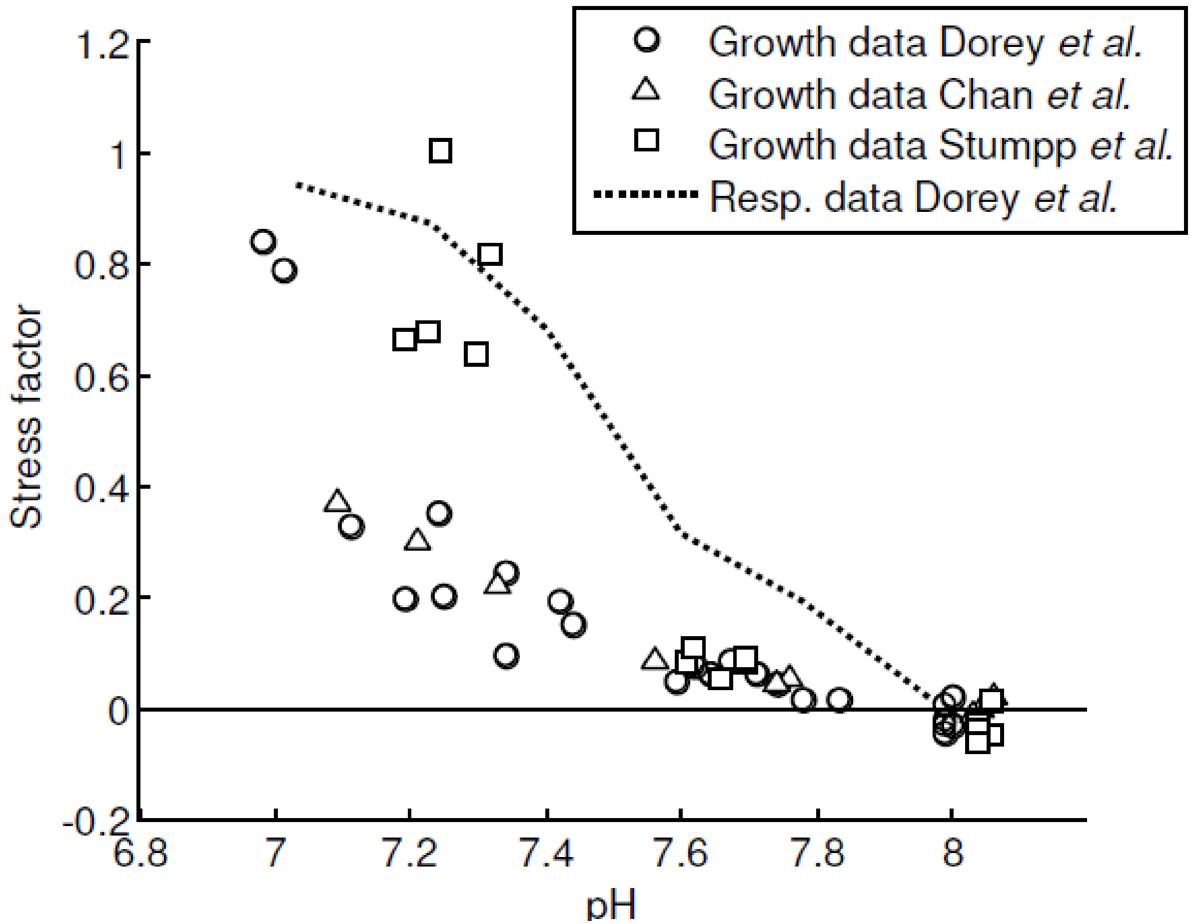
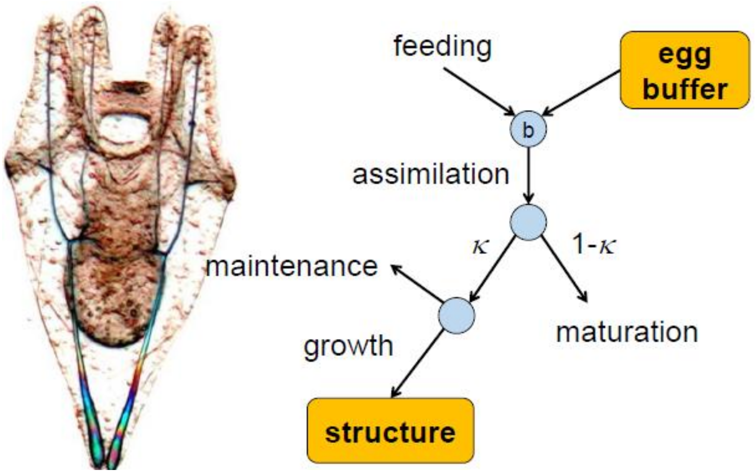
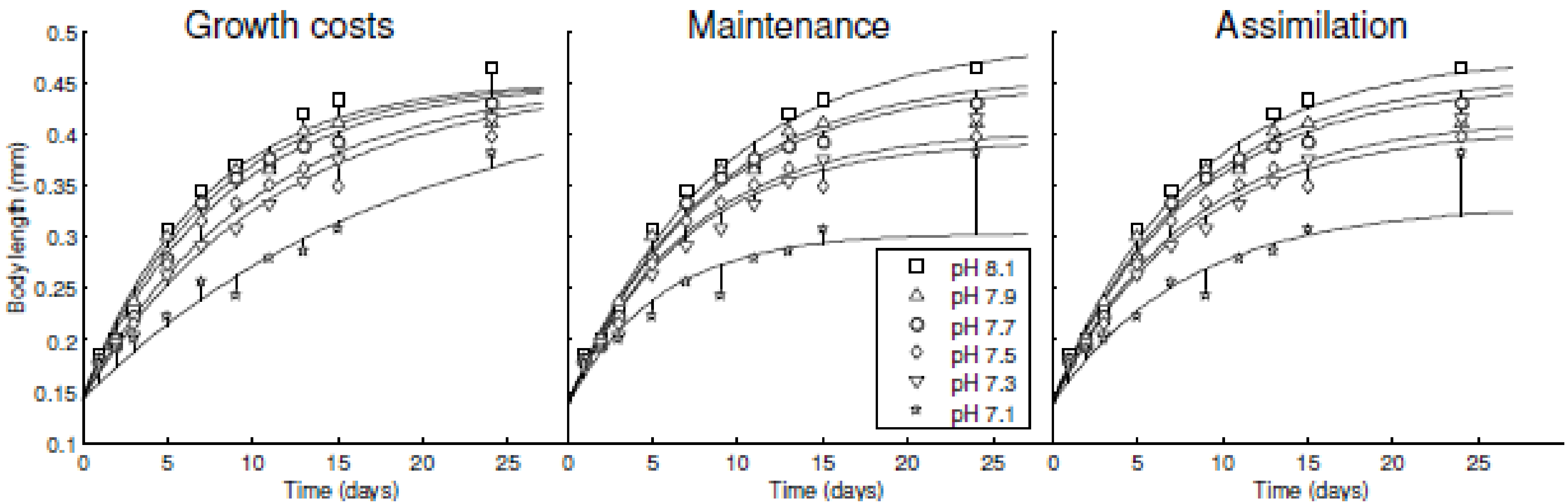
Near-future ocean acidification impacts maintenance costs in sea-urchin larvae: Identification of stress factors and tipping points using a DEB modelling approach

Tjalling Jager [a,*], Elisa Ravagnan [b], Sam Dupont [c]

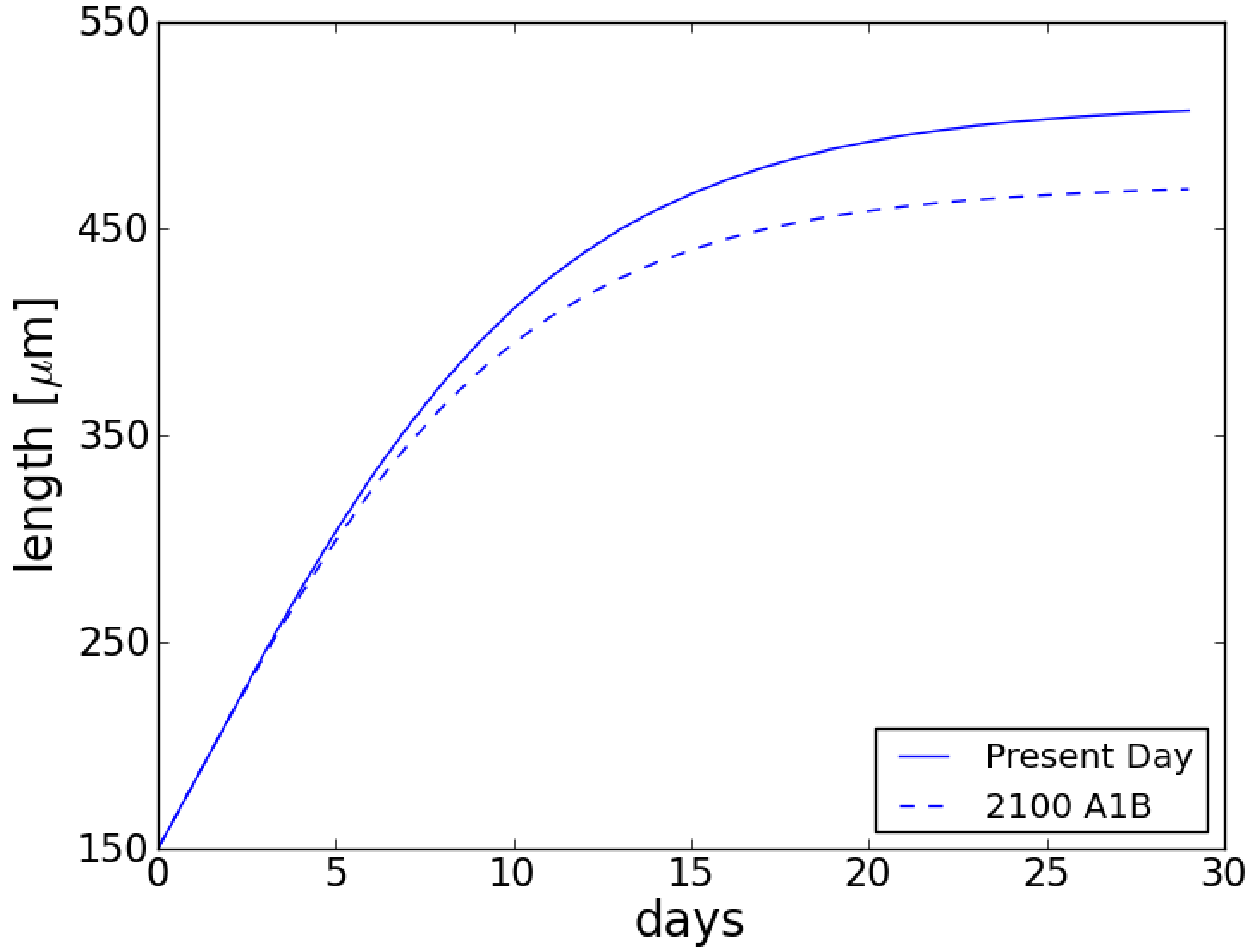


DEB modeling

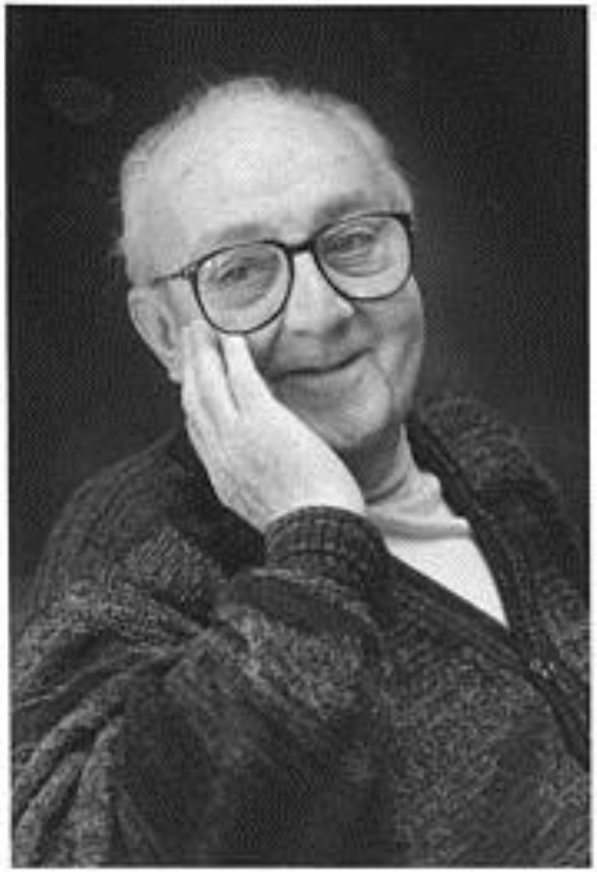




Good models



Model + physiology = prediction on field impacts [also allow to add ecological interactions]



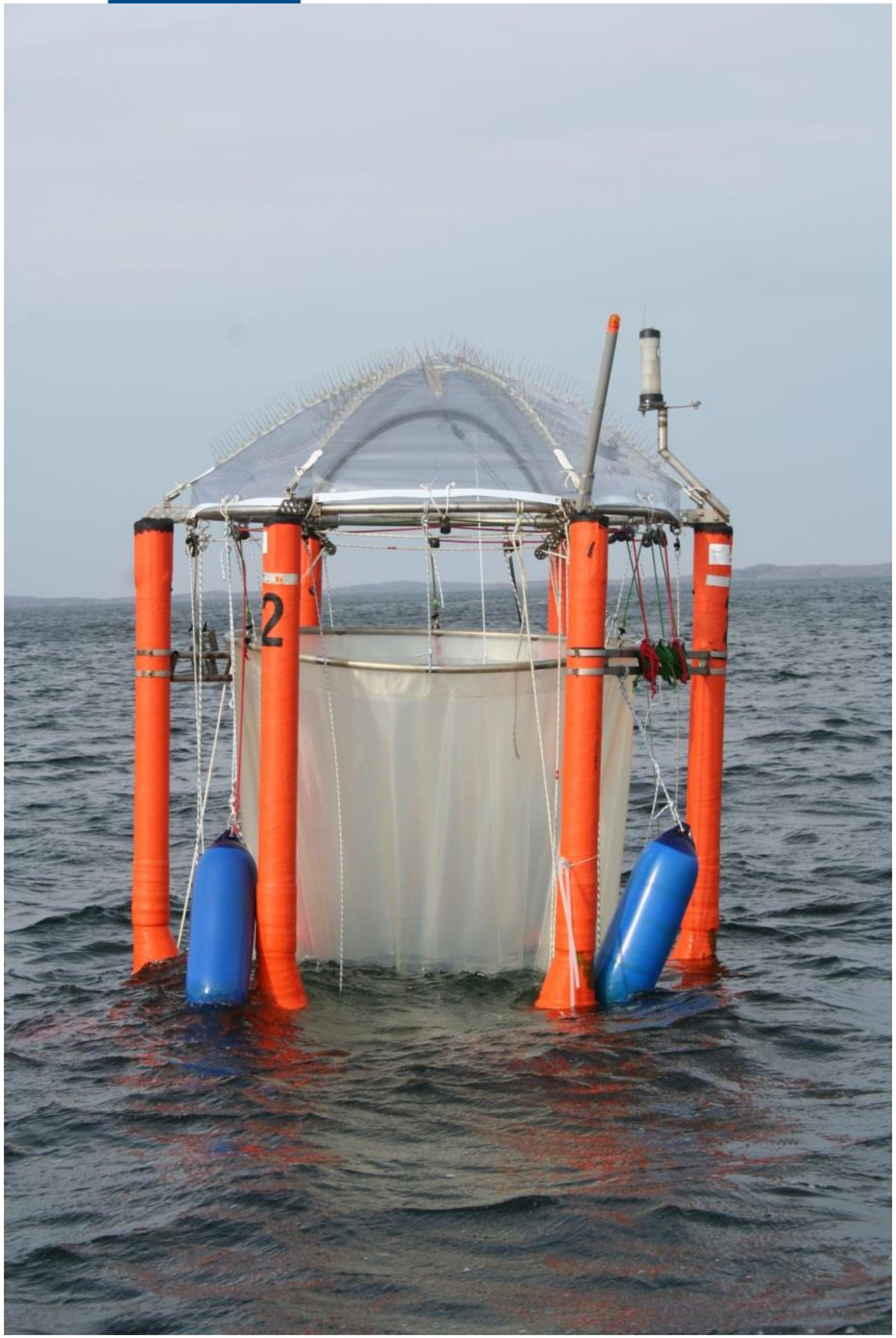
George E. P. Box

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

E.g. organism response to multiple drivers

1. Good data on local variability / future scenarios
2. Good understanding of biological response for each driver [mode of action]
3. Build models

Mix all the ingredients & test using scenarios [field, laboratory]



KOSMOS rocks

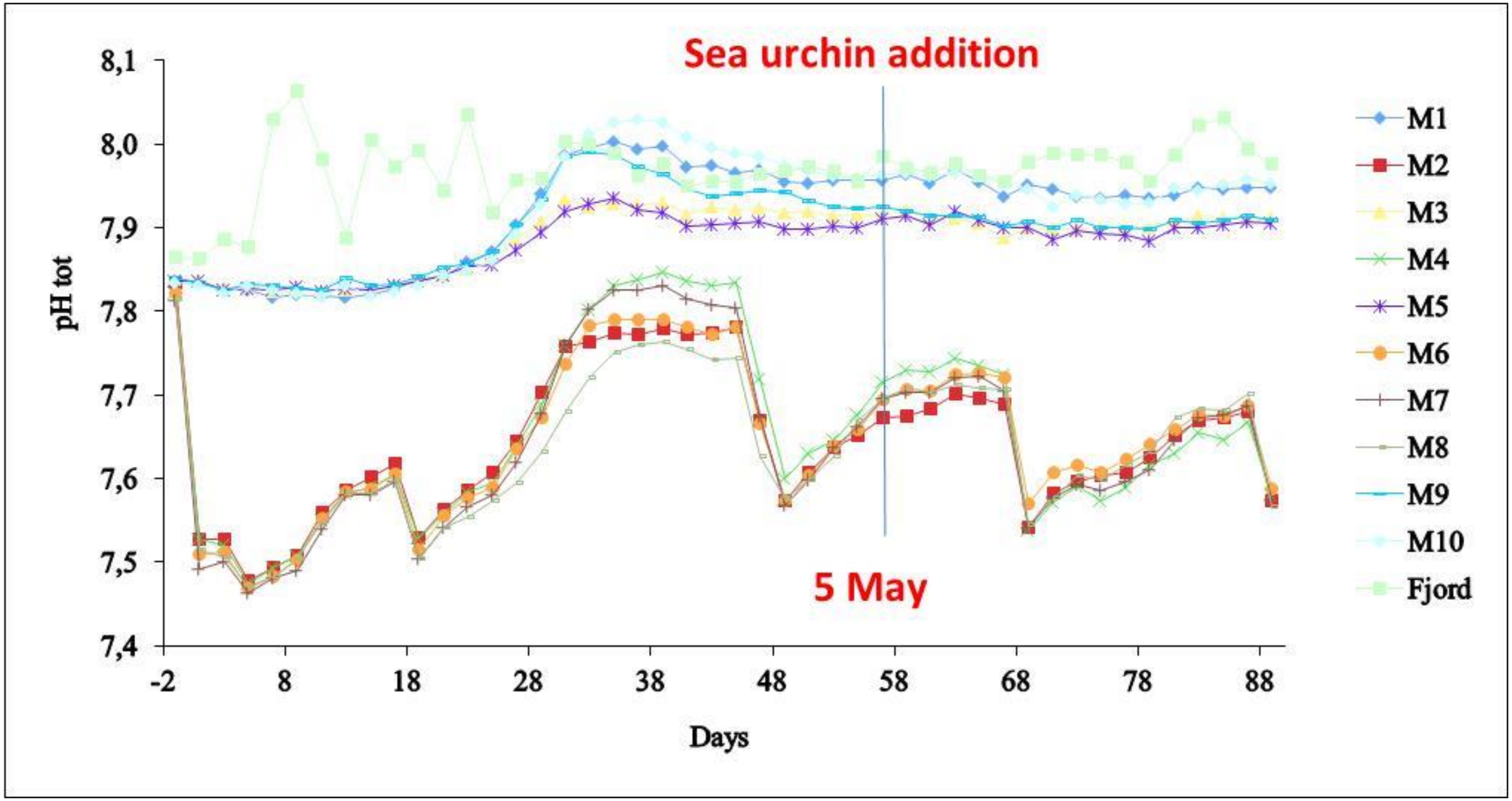
BIOACID

6 months / 50 researchers

10 x $55m^3$

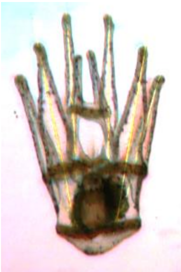
2 treatments: ctl vs low pH

Into the wild



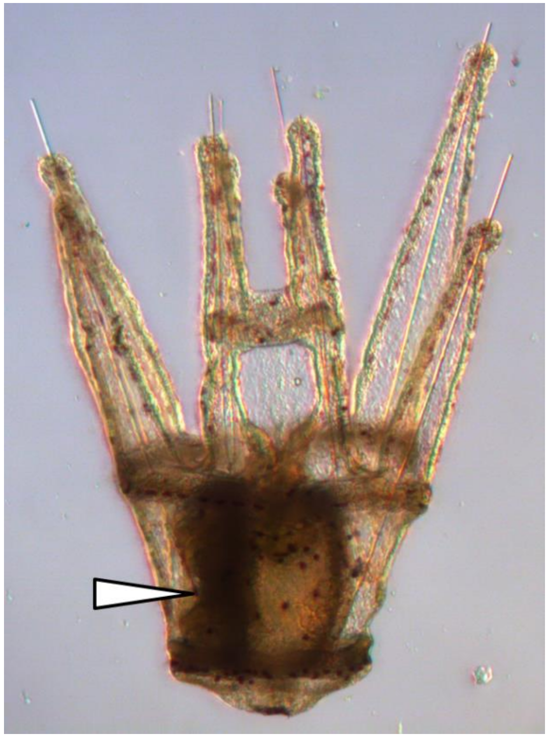
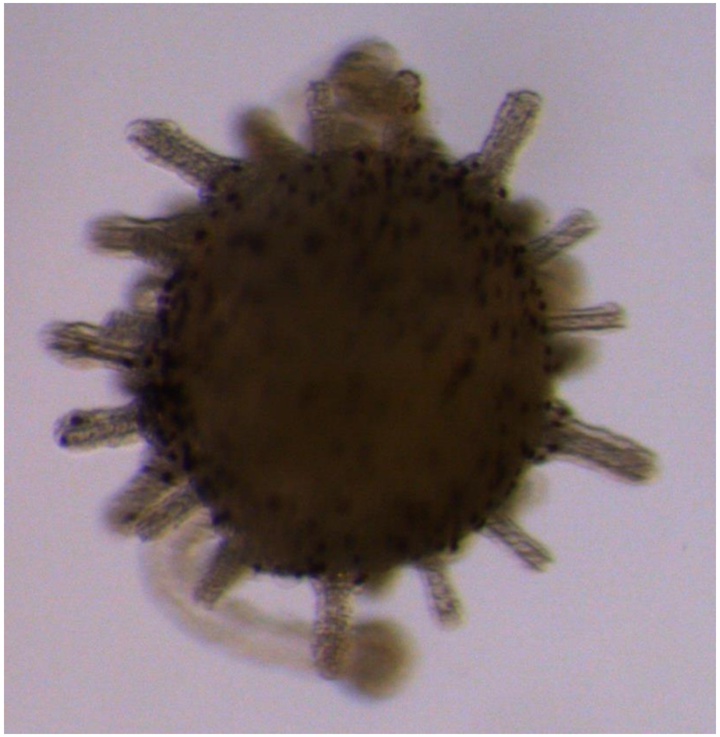
Into the wild

d1 d9 d17 d25

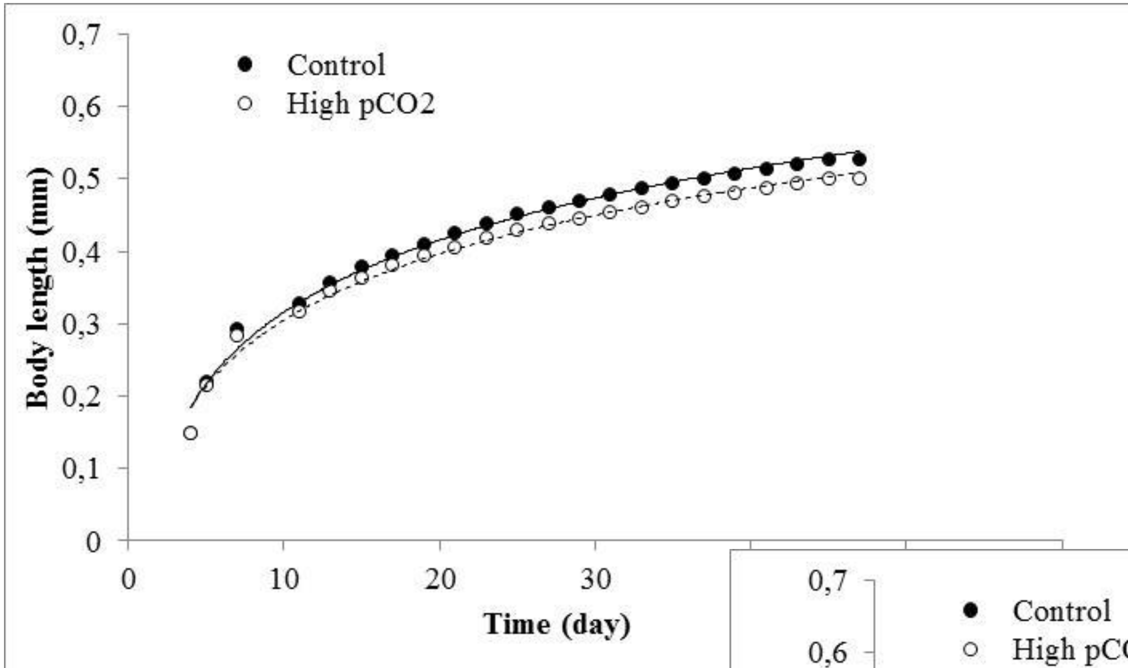
   

- **Same mortality**
- **Delay in development**
- **“Desperate” larvae**

d32

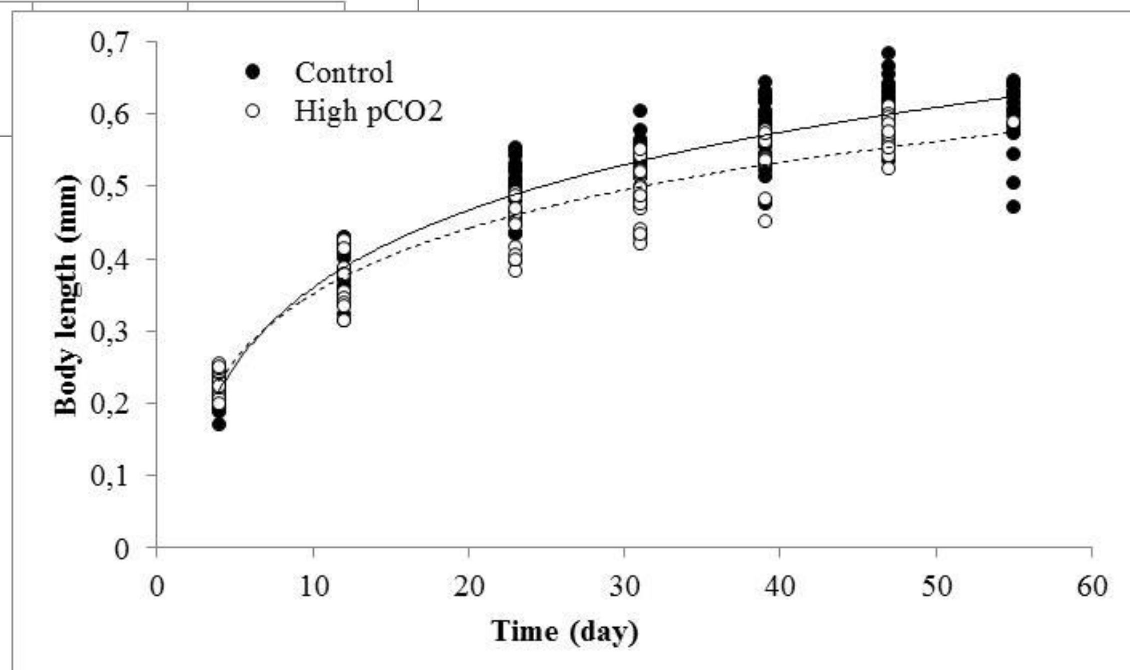
Field validation



Predicted

[mesocosm chemistry + Dorey et al. 2013]

Observed



Into the wild

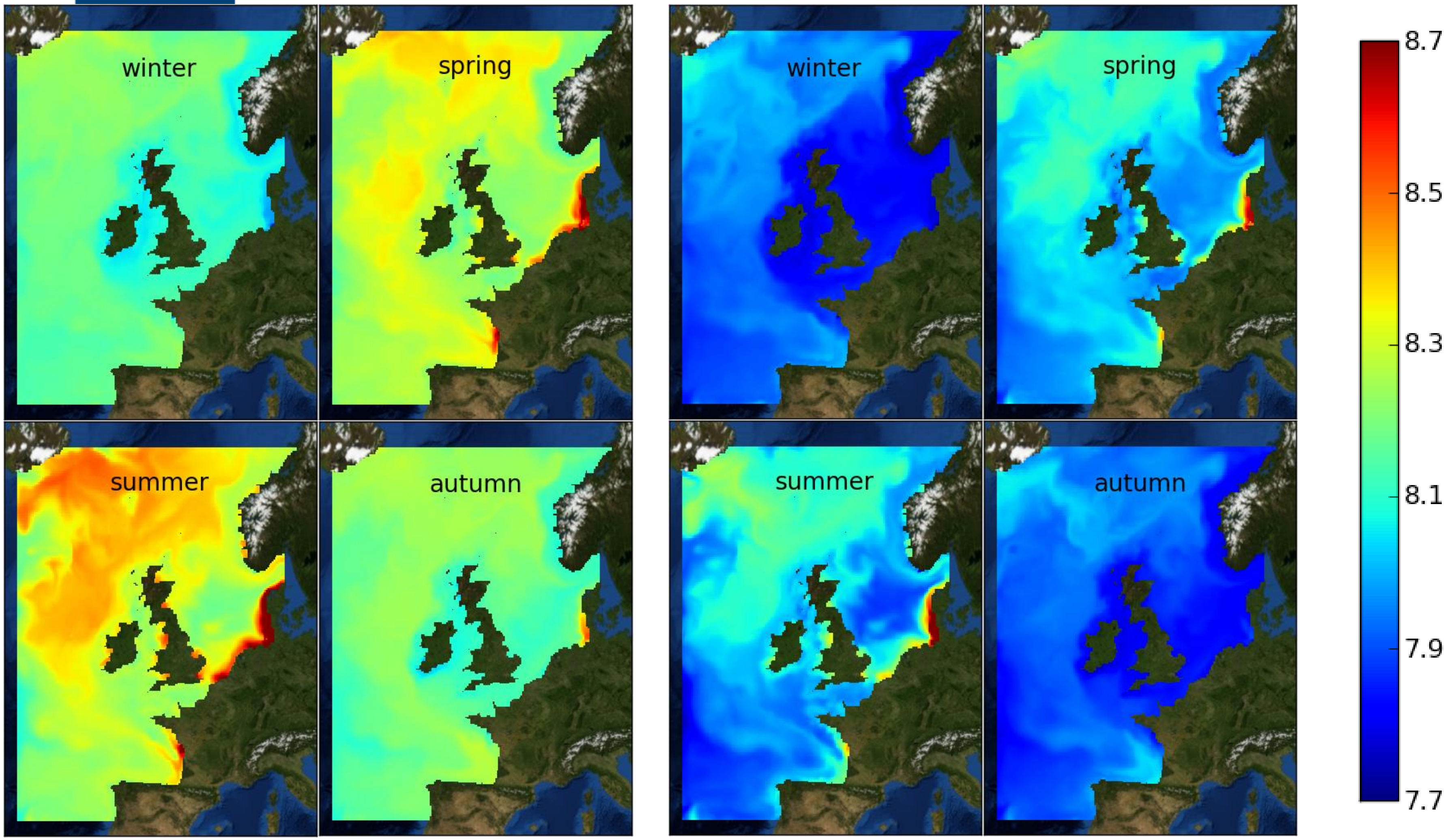
1. Good data on local variability / future scenarios
2. Good understanding of biological response for each driver [mechanisms – ecology, evolution, physiology]
3. Build models

Mix all the ingredients & test using scenarios [field, laboratory]

It works !!!

Good models

POLCOMS-ERSEM



Surface pH 1981-2000

Surface pH 2080-2099
(A1B scenario)

(Holt et al. 2012 BG; Artioli et al. 2012 JMS)

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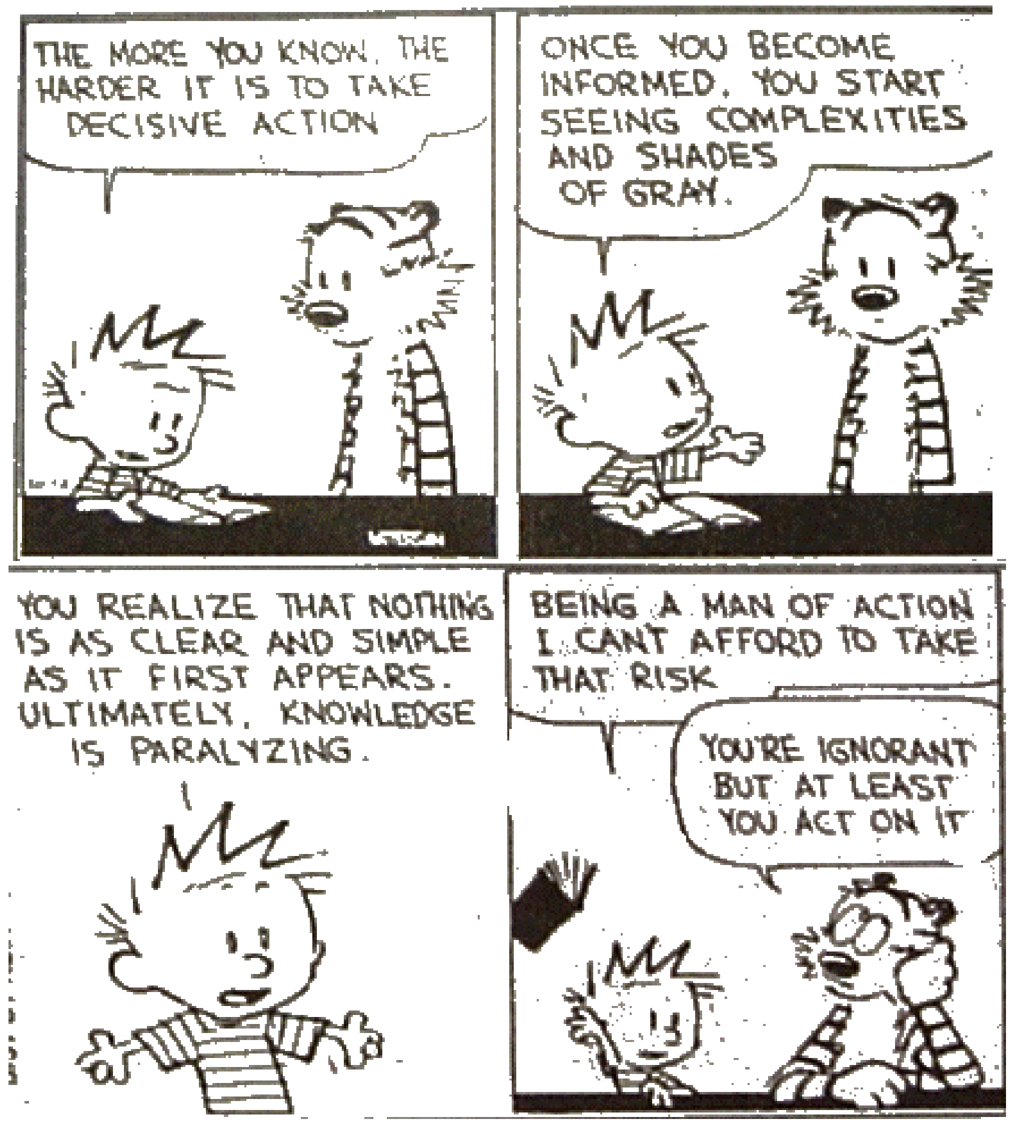
And act !



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?