

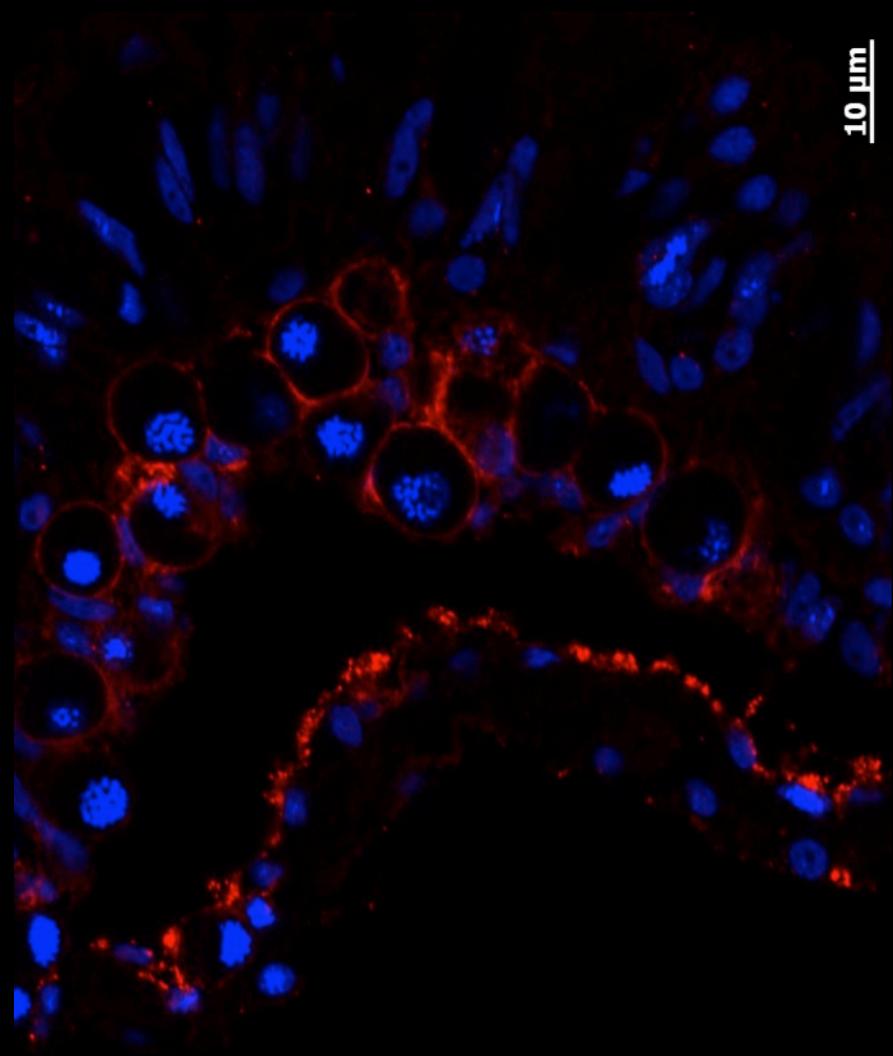
Molecular and cellular mechanisms for pH sensing, ion transport and regulation of metabolism in corals

Martin Tresguerres



The Alfred P. Sloan
Foundation

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Dr. Dimitri Deheyn
Ms. Megan Barron
Ms. Lauren Linsmayer
Ms. Sidney Pérez
Ms. Jenny Tu
Dr. Alex Venn and Dr. Sylvie Tambutteé
(Monaco Scientific Center)



10 μm

THE IMPORTANCE OF KNOWING BASIC BIOLOGY AND MECHANISMS

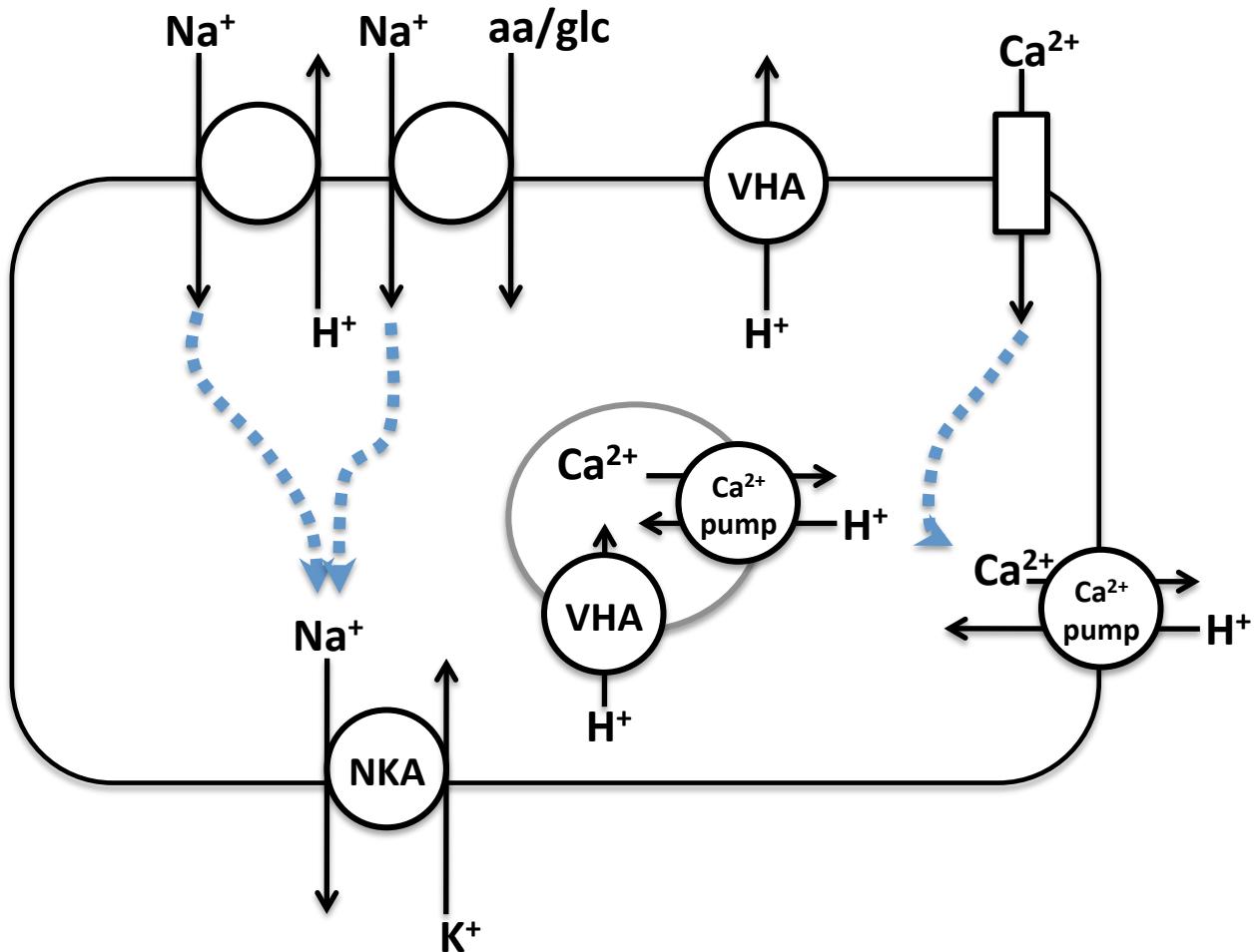
- 1) Proton pump (VHA): NOVEL PHYSIOLOGY AND MULTIPLE LOCALIZATION WITHIN CORAL CELL TYPES
- 2) NKA AND PMCA ATPases: *SPECIES SPECIFIC DIFFERENCES*
- 3) *Potential applications for field studies*

*Implications for how we study coral responses to environmental stress
(or why large scale proteomics and transcriptomics is not a good
approach in corals –at least yet–)*

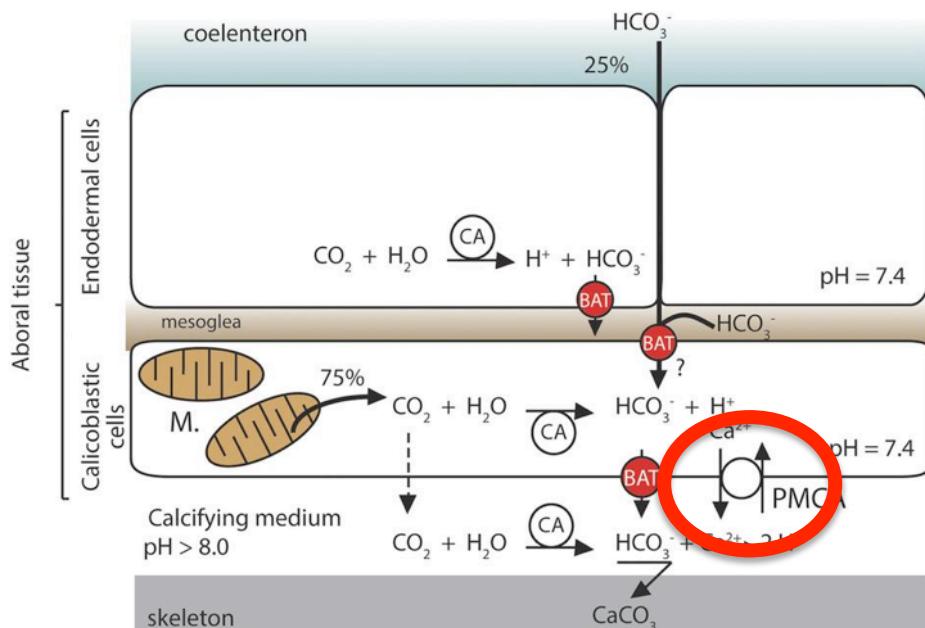
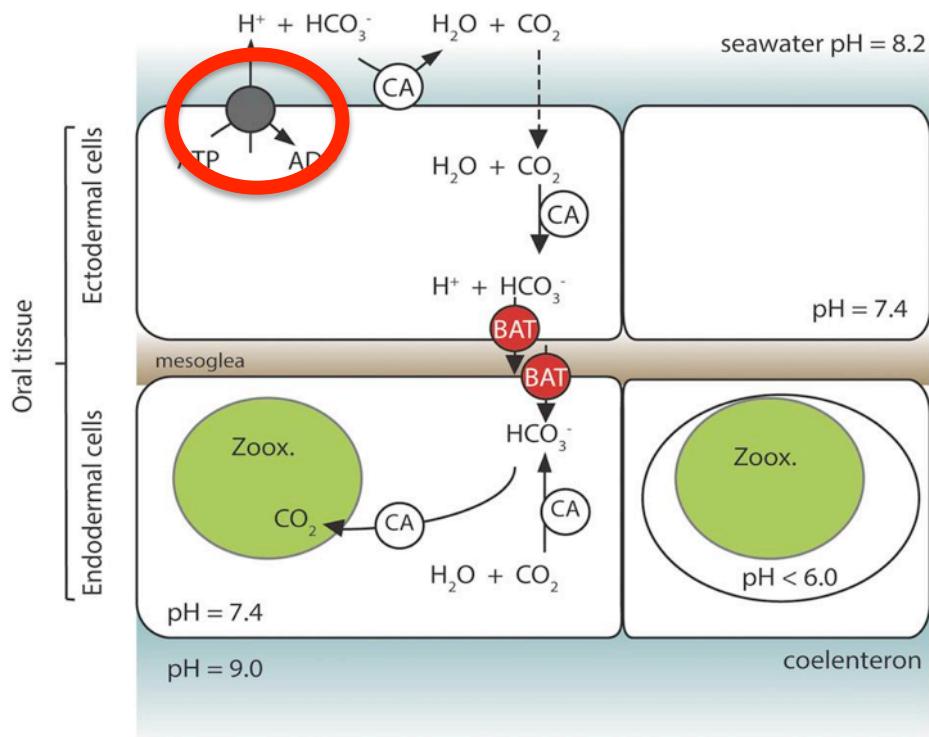
General roles of ATPases

Use ATP to move cations against the concentration gradient

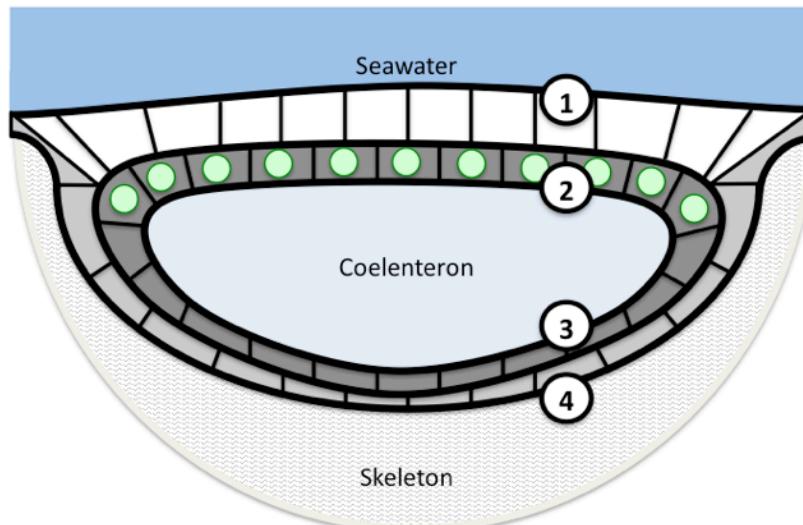
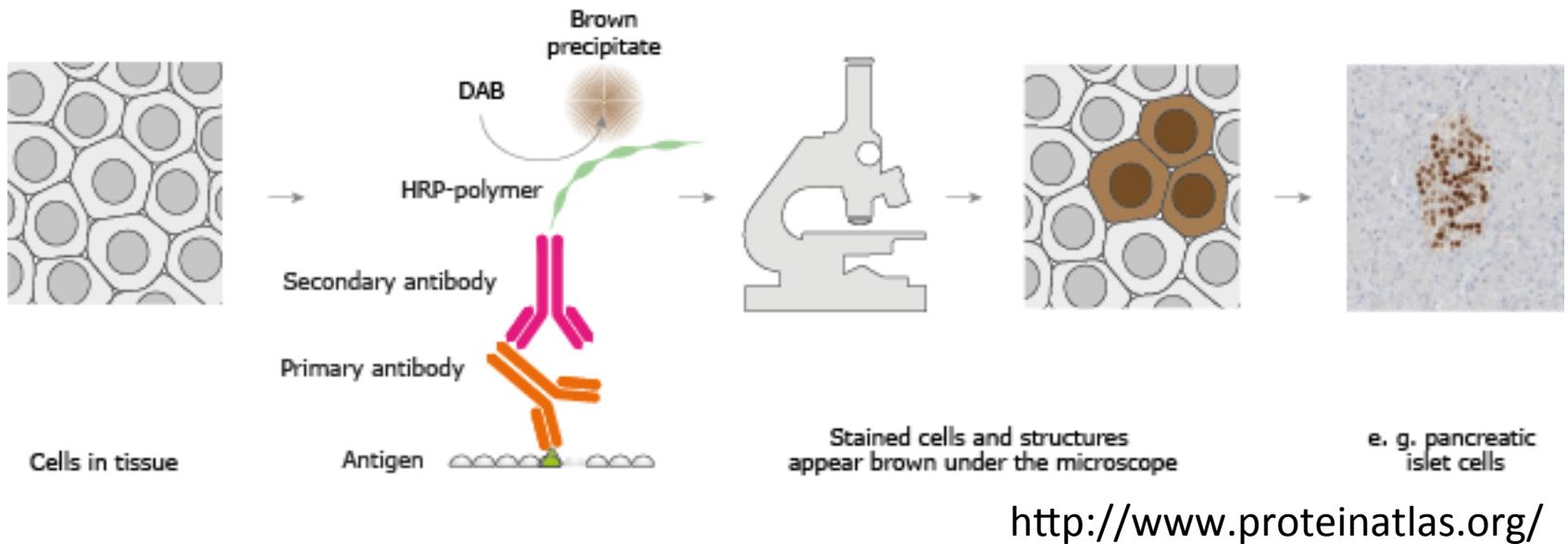
- active transport
- secondary active transport
- membrane potential



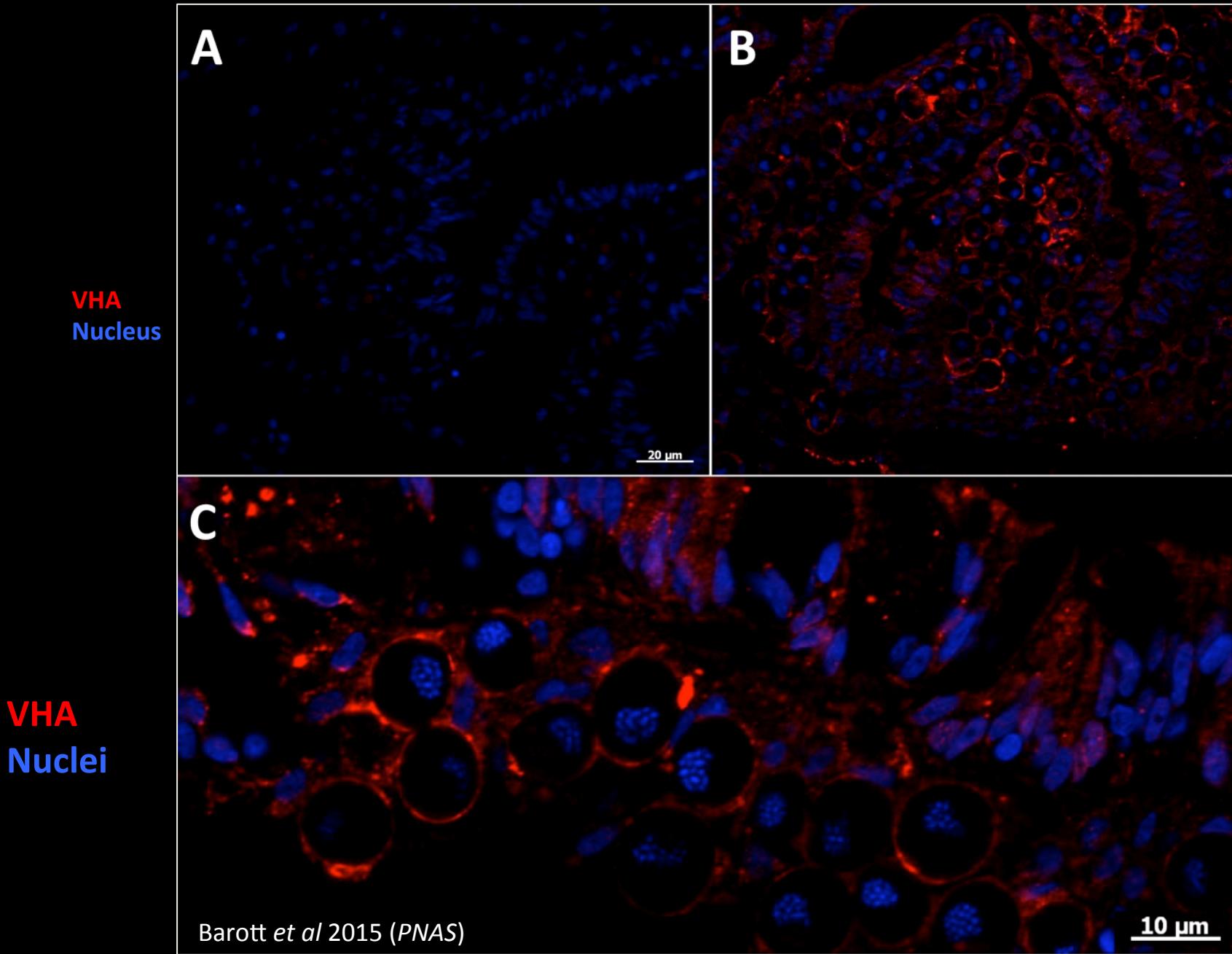
Proposed localization (and function) in corals



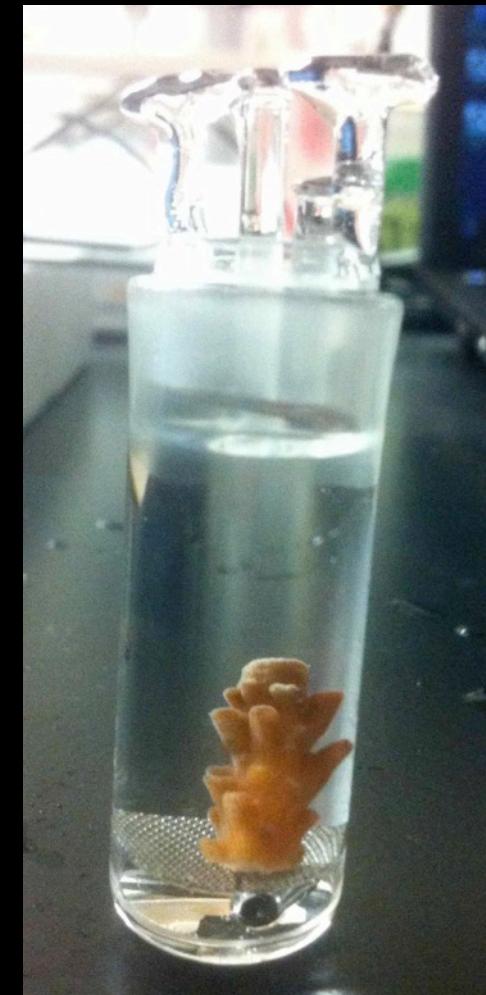
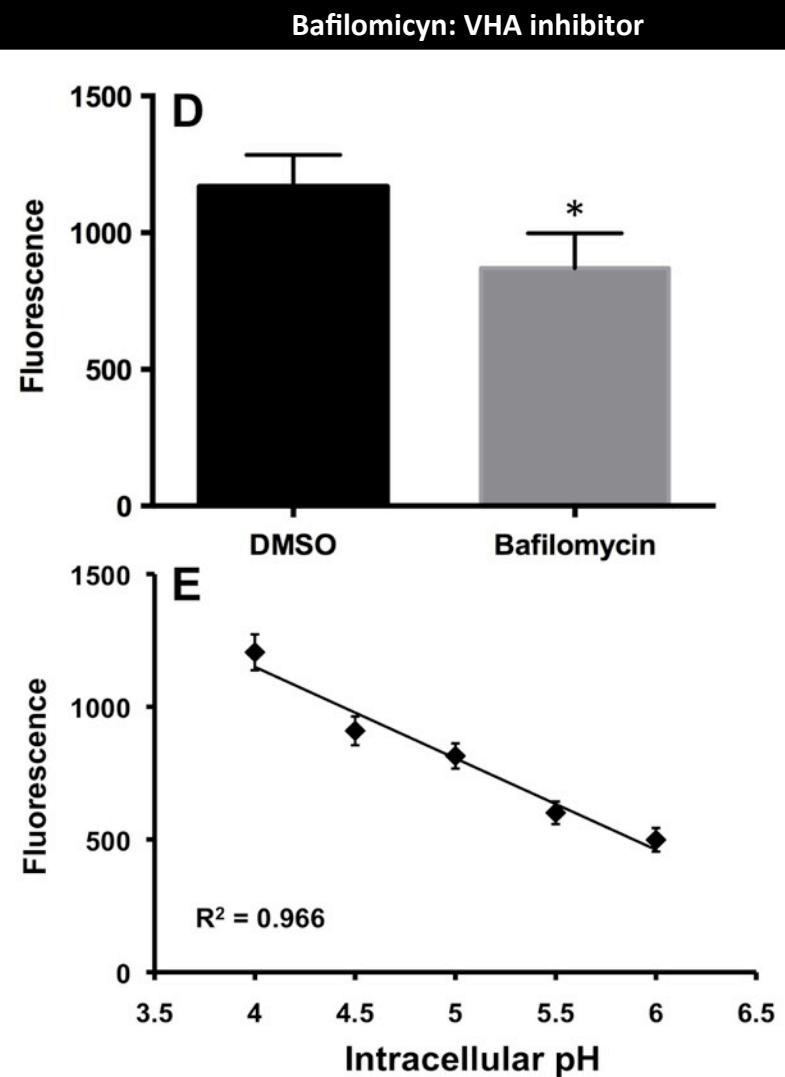
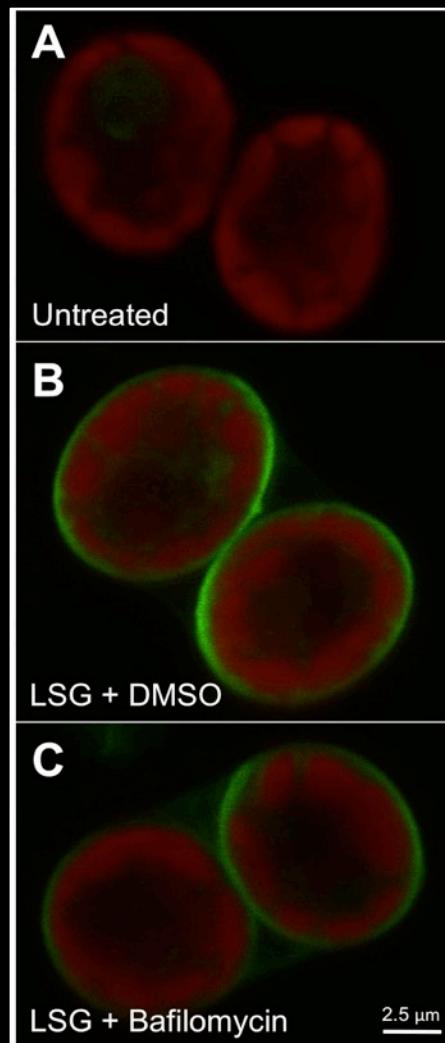
Immunohistochemistry



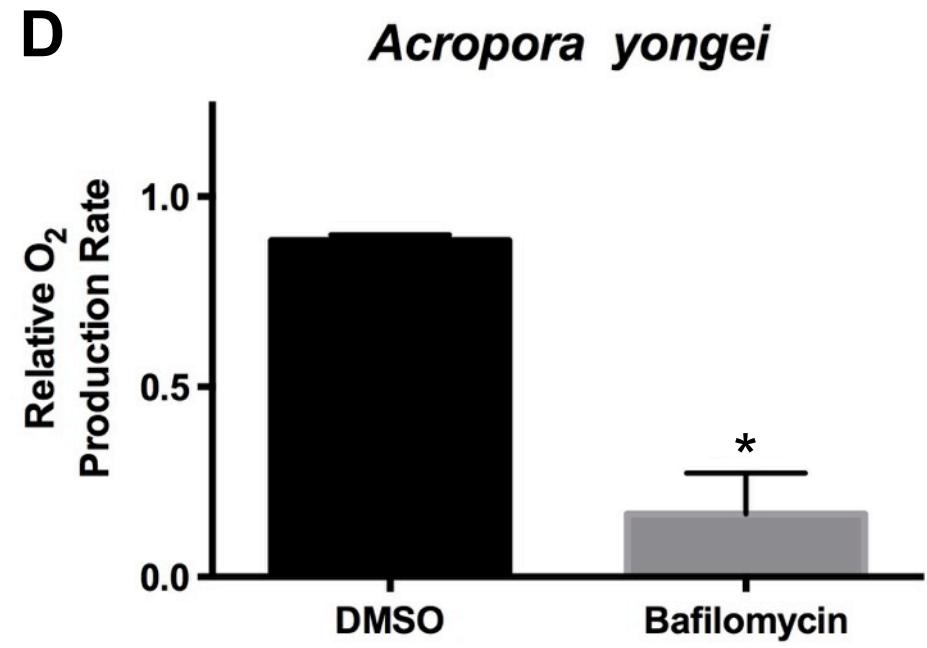
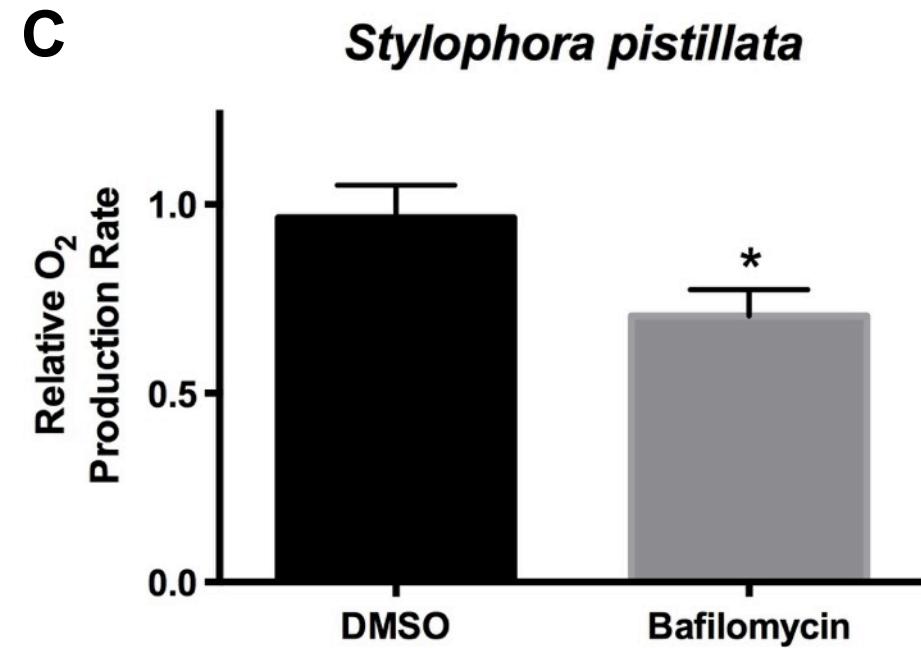
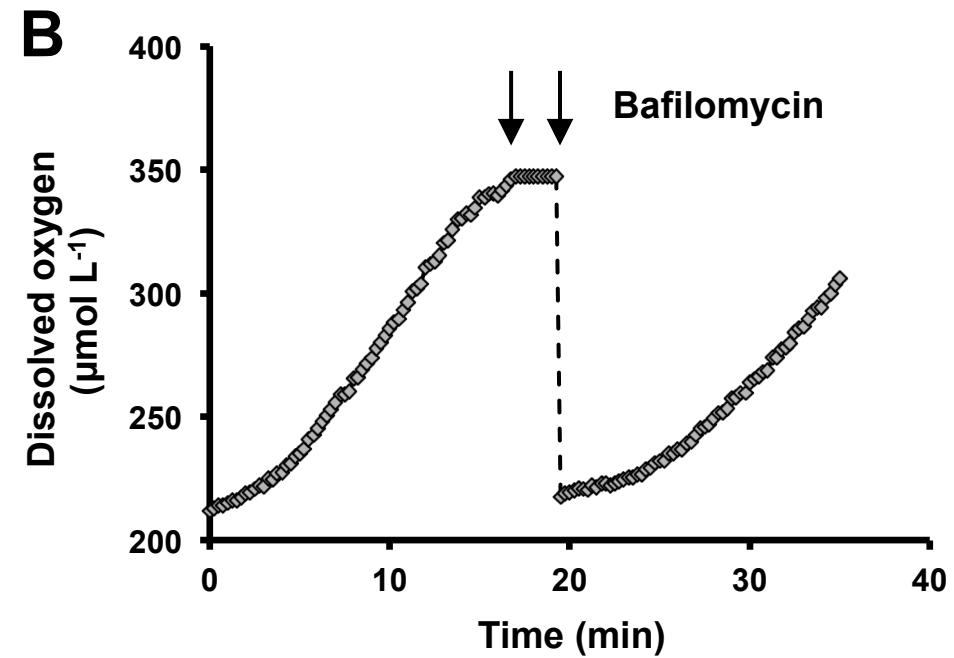
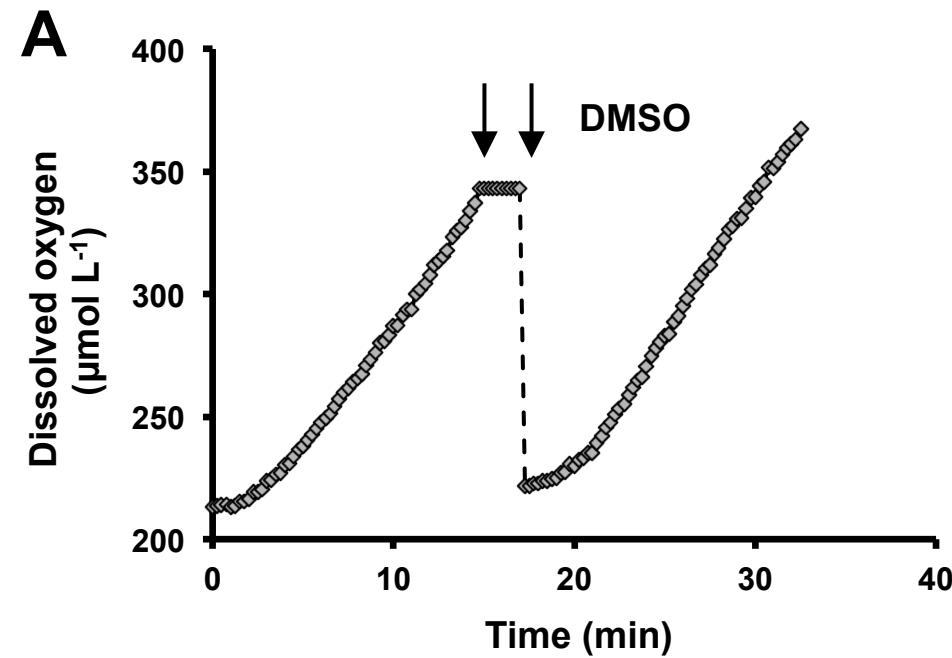
VHA is abundantly expressed in the symbiosome membrane

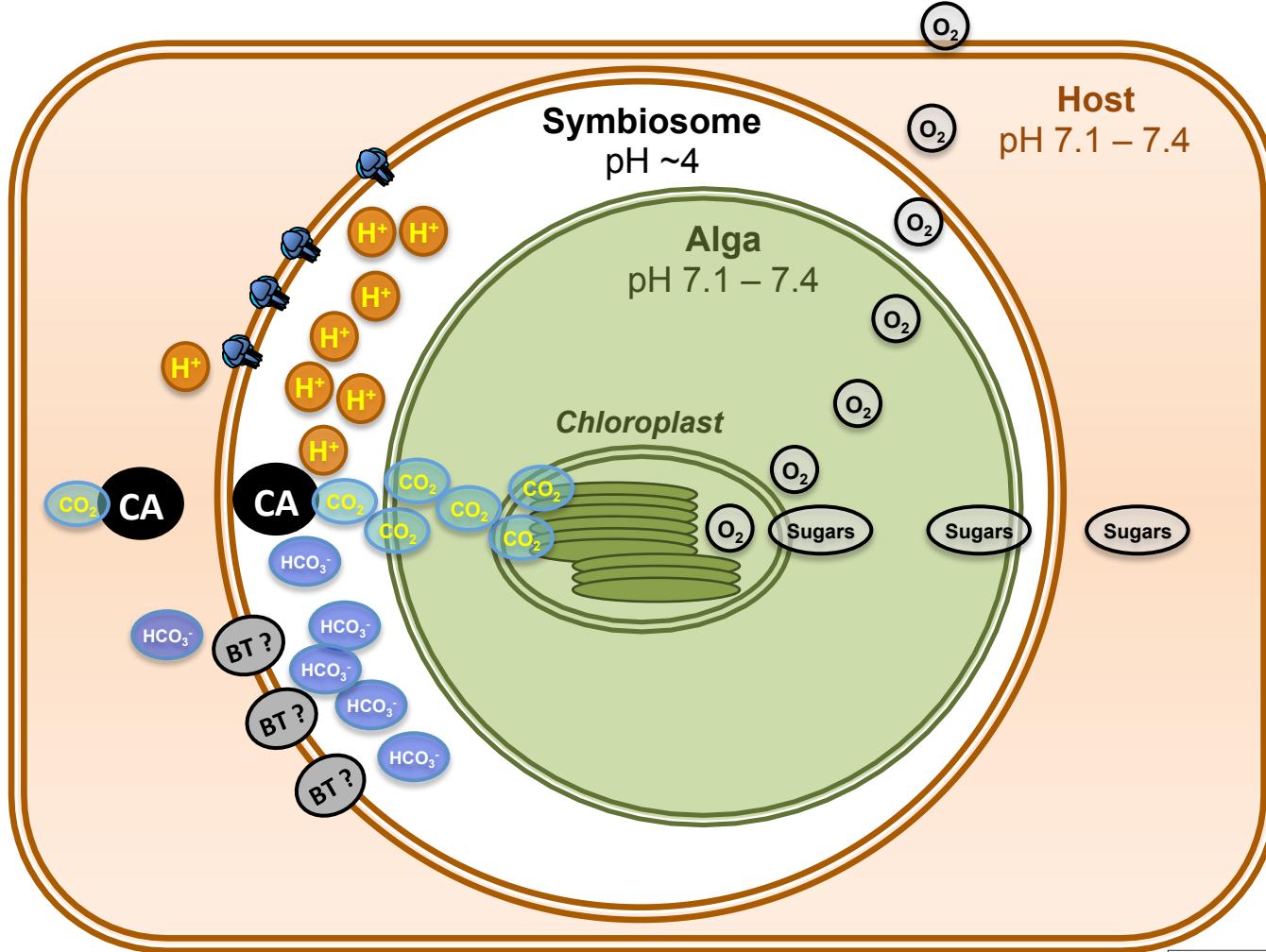


Host VHA acidifies the symbiosome and promotes photosynthesis



Lysosensor green: accumulates in acidic compartments



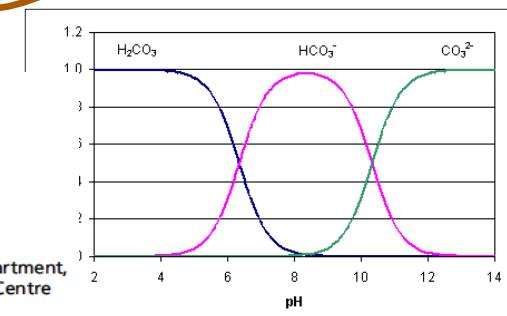


Coral host cells acidify symbiotic algal microenvironment to promote photosynthesis

Katie L. Barott^a, Alexander A. Venn^{b,c}, Sidney O. Perez^a, Sylvie Tambutté^b, and Martin Tresguerres^{a,1}

^aMarine Biology Research Division, Scripps Institution of Oceanography, University of California, San Diego, La Jolla, CA 92093; ^bMarine Biology Department, Centre Scientifique de Monaco, MC-98000 Monaco, Monaco; and ^cLaboratoire Européen Associé 647 "Biosensib," Centre Scientifique de Monaco–Centre National de la Recherche Scientifique, MC-98000 Monaco, Monaco

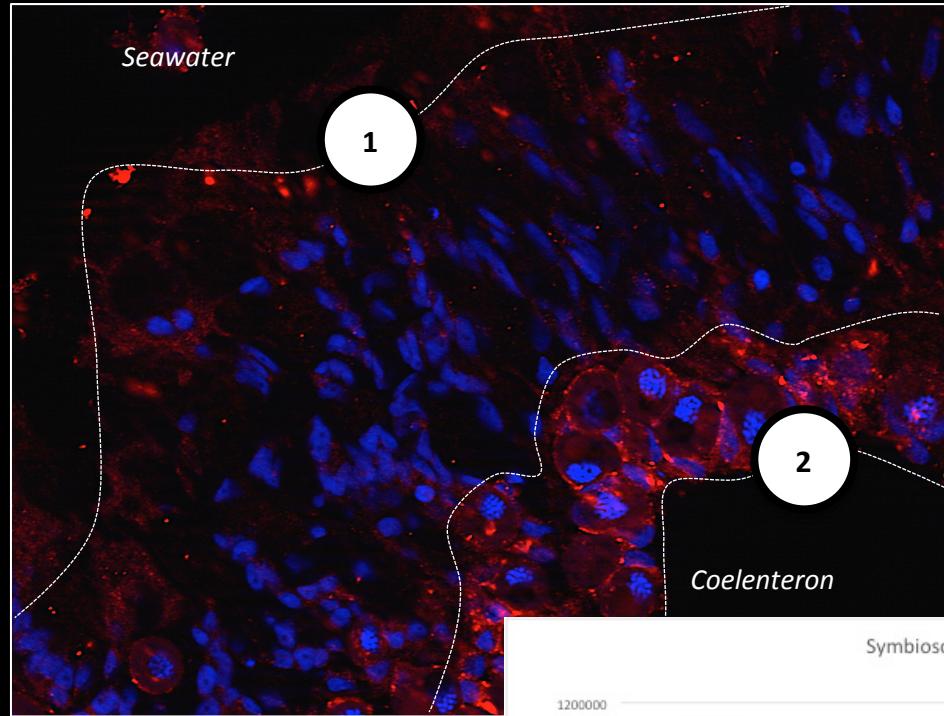
Edited by George N. Somero, Stanford University, Pacific Grove, CA, and approved November 18, 2014 (received for review July 16, 2014)



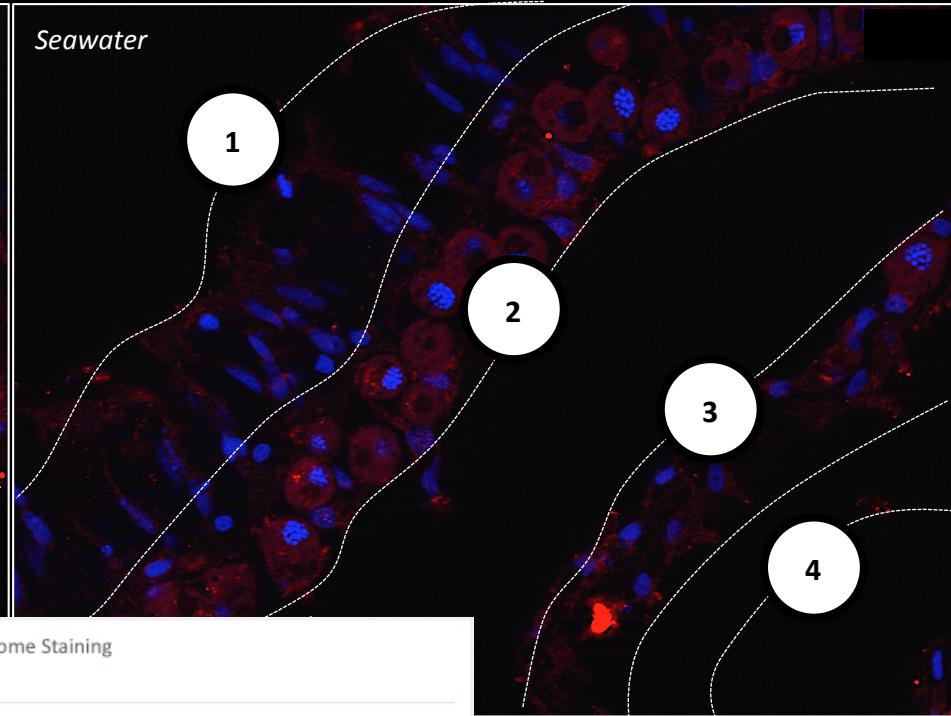


Symbiosome VHA protein abundance during “OA” (\uparrow CO_2)

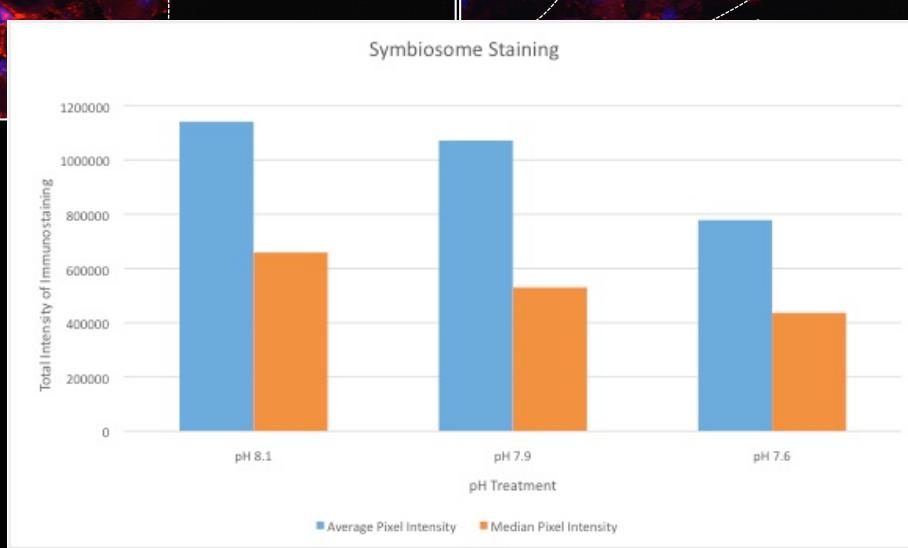
pH 8.0, PCO_2 450 μatm



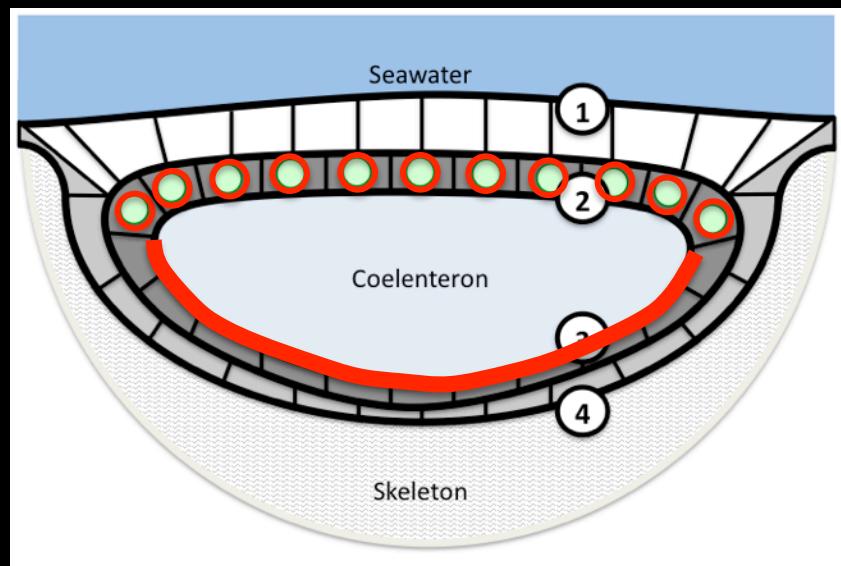
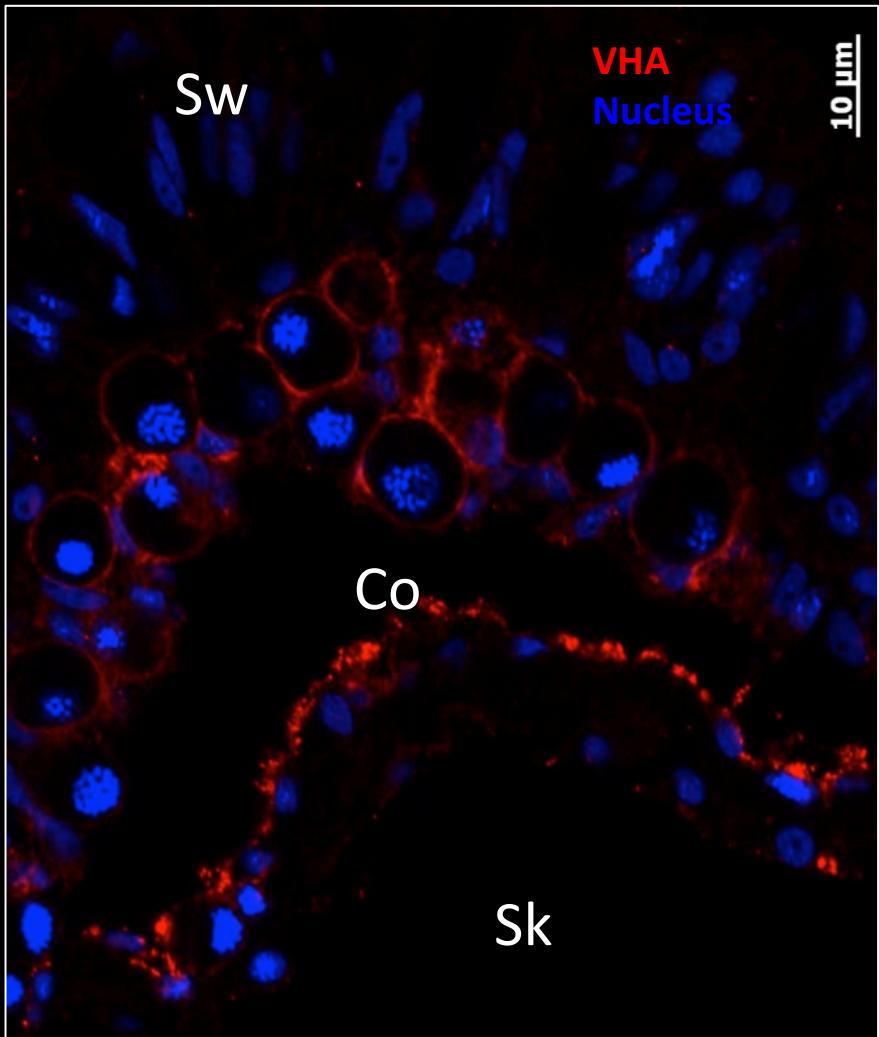
pH 7.6 PCO_2 1800 μatm



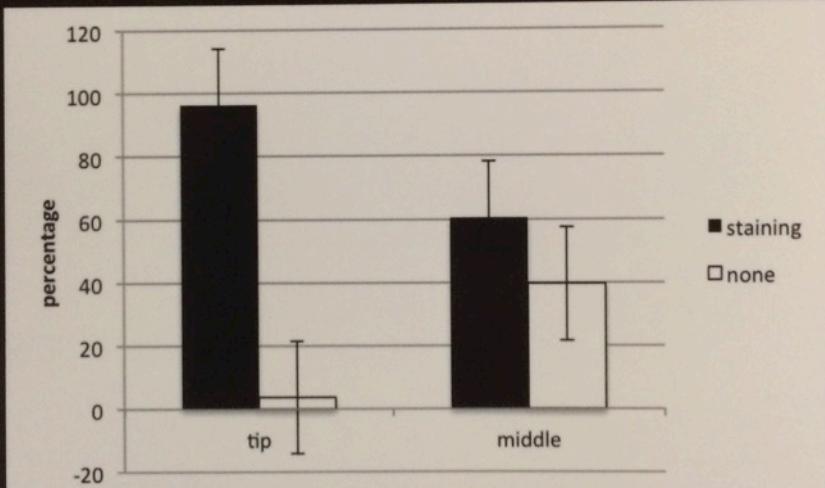
VHA
Nuclei



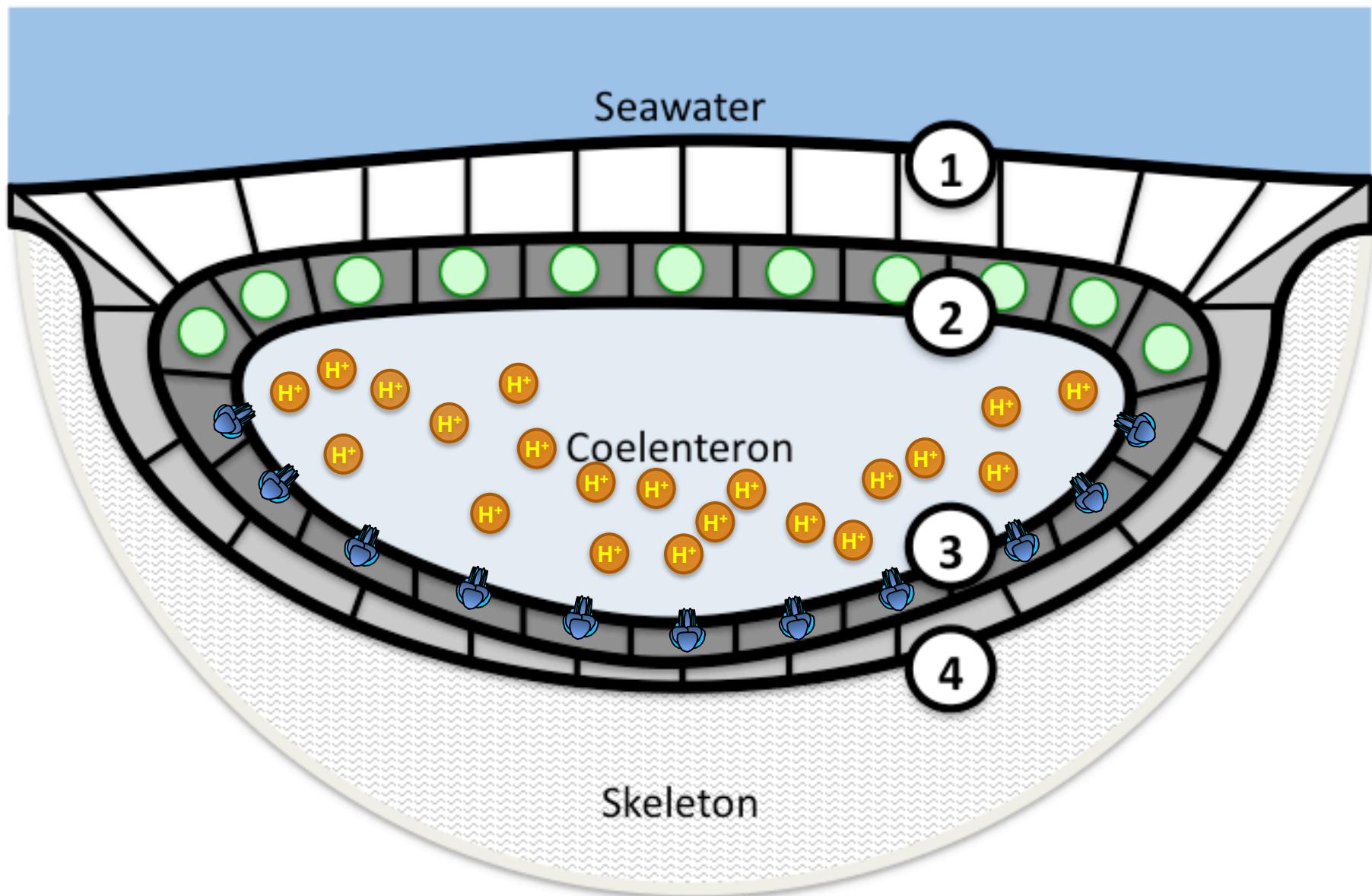
BUT VHA IS NOT ONLY IN THE
SYMBIOSOME.....CORAL TIP?



VHA staining in aboral ectoderm

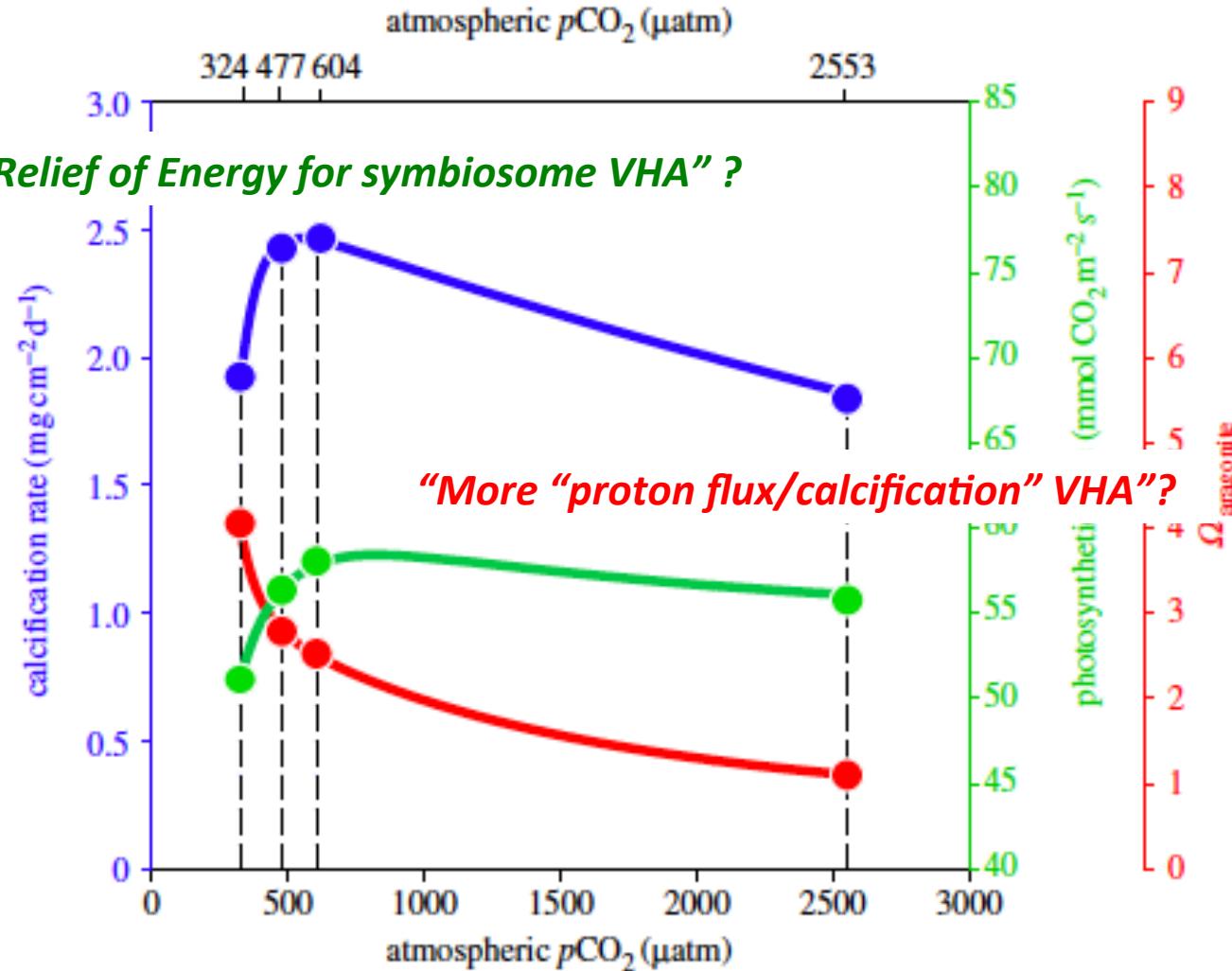


Remove H⁺ from the site of calcification?



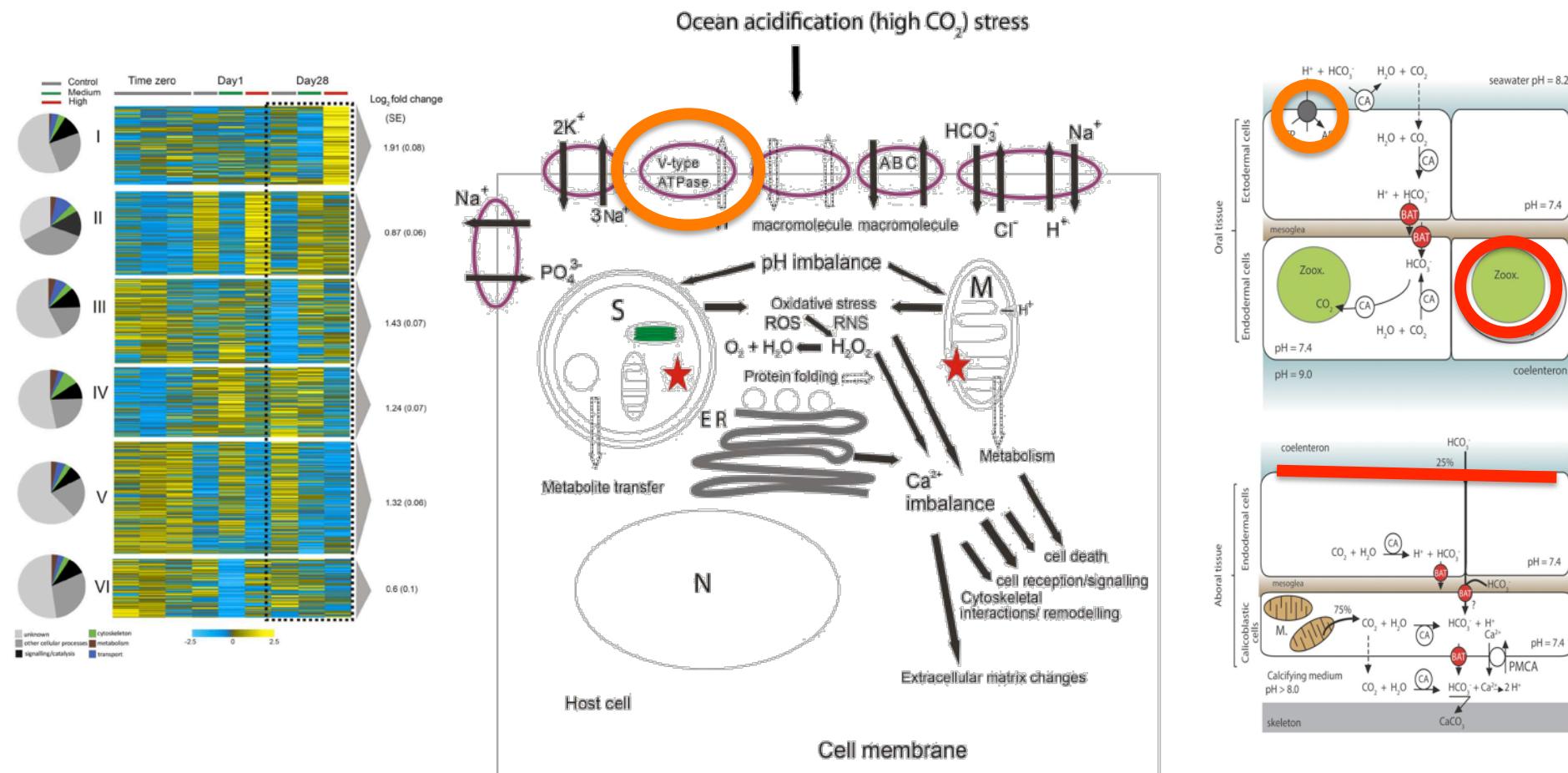
The reef-building coral *Siderastrea siderea* exhibits parabolic responses to ocean acidification and warming

Karl D. Castillo¹, Justin B. Ries^{1,2}, John F. Bruno³ and Isaac T. Westfield^{1,2}

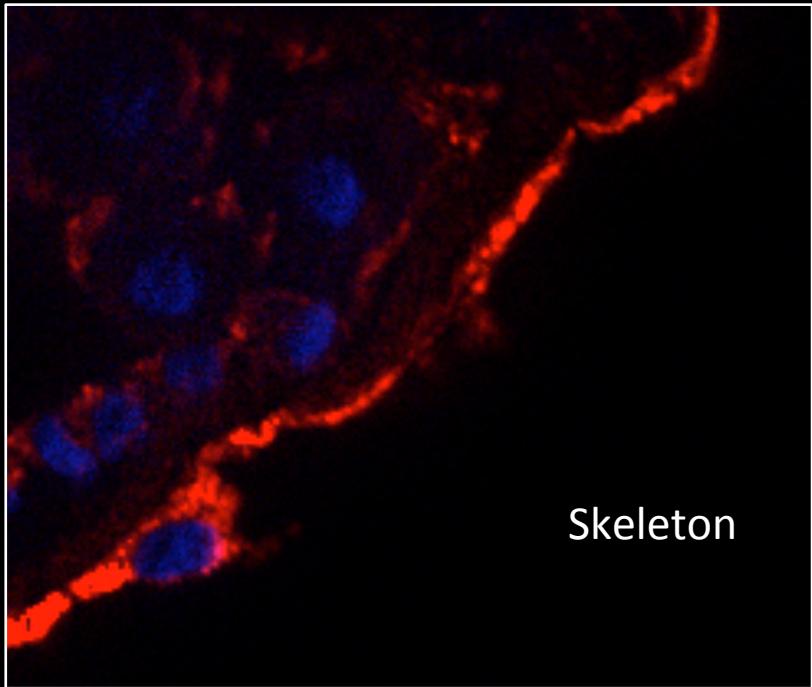


Major Cellular and Physiological Impacts of Ocean Acidification on a Reef Building Coral

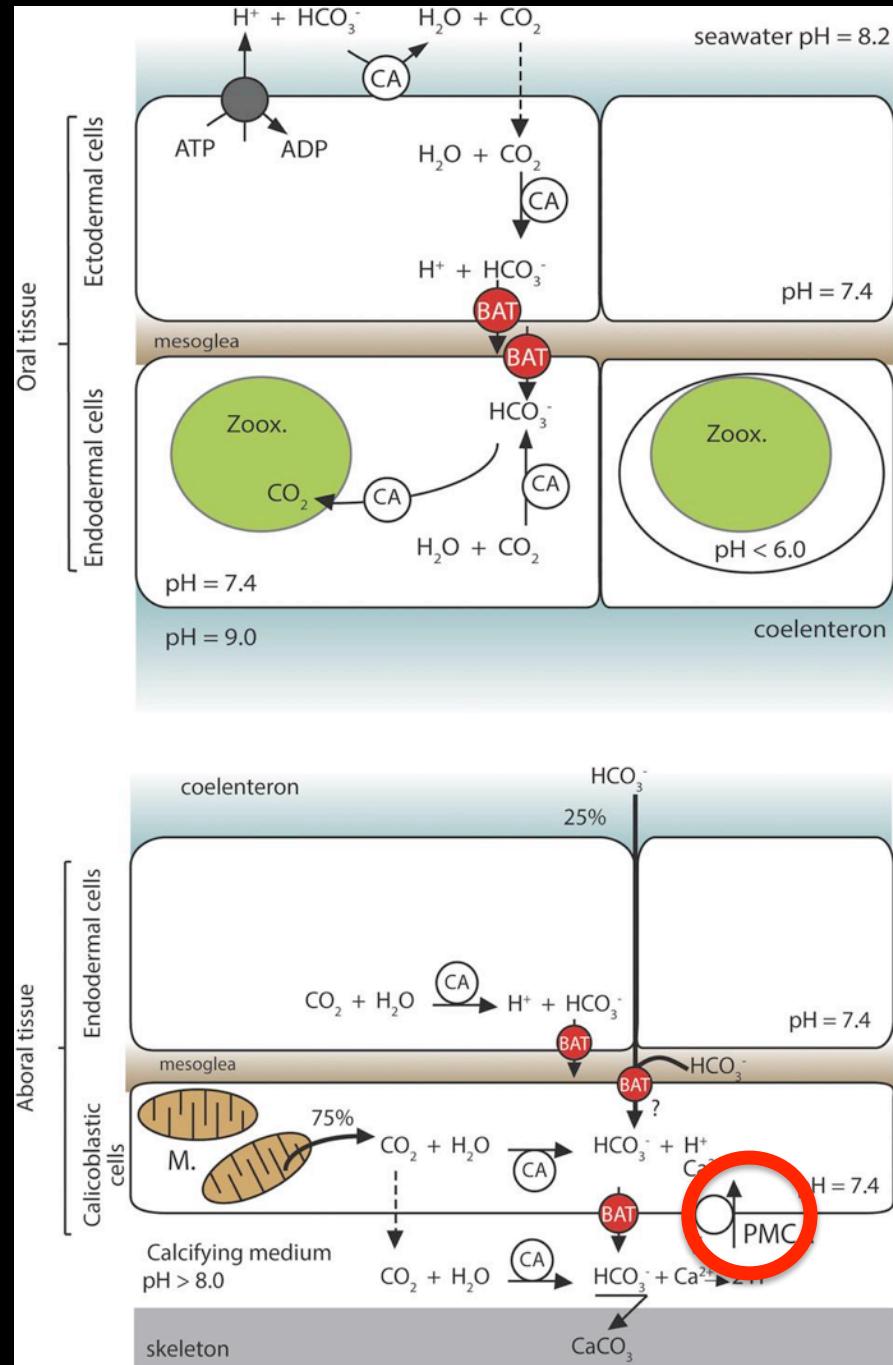
Paulina Kaniewska^{1,*^a}, Paul R. Campbell², David I. Kline¹, Mauricio Rodriguez-Lanetty³, David J. Miller⁴, Sophie Dove^{1,4^b}, Ove Hoegh-Guldberg^{4,5^b}



Revisiting other parts of the model= Calcium pump may not be in the membrane



**PMCA
Nuclei**



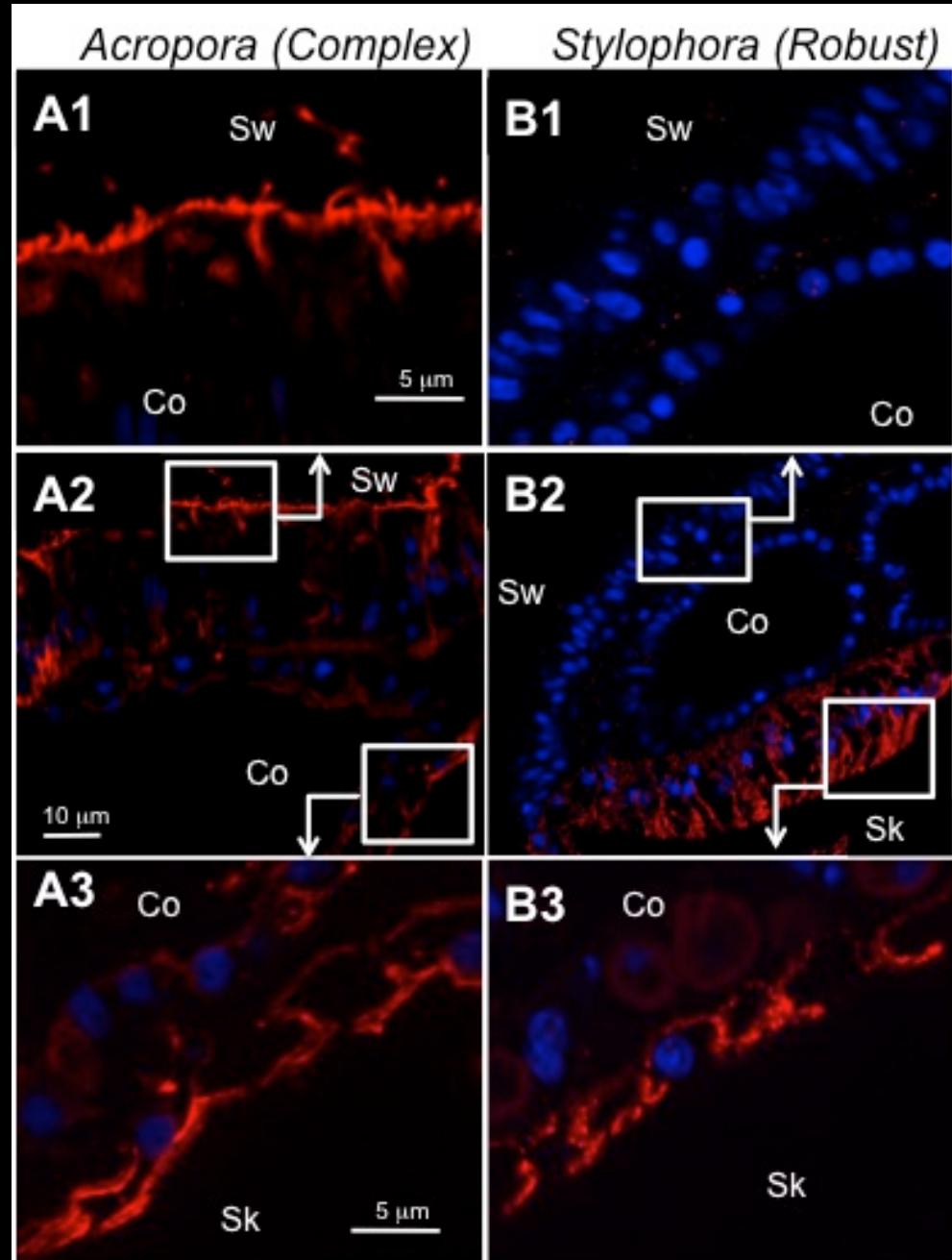
Differential tissue localization of NKA in two coral species

NKA=can consume between 20-90% of all the energy in a cell/organism

Roles?

Implications for analyzing results

NKA
Nuclei



CONCLUSIONS

- Localization and function is (obviously) key for interpreting data
- (Protein and mRNA) Responses in one cell type might mask responses in another
- Other regulation (PTMs, e.g. phosphorylation)
- Species specific differences (“a coral is not a coral is not a coral”)
- Relevance for corals in the ‘real world’?

Year 1

A) Sample 9 fragments from 2 sites and 2 depths at each site.



= 36 samples

= 36 samples

B) Long-term Reciprocal transplant of 5m corals between sites



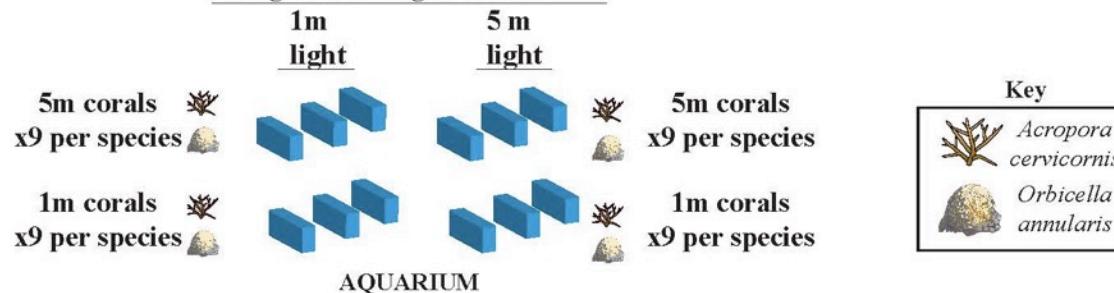
= 36 samples (9 to other site and 9 controls for each site)

= 36 samples



National Science Foundation
WHERE DISCOVERIES BEGIN

C) Aquarium study manipulating light with Lagoon corals



Year 2

D) Sample Long-term Reciprocal transplant set up in year 1 (B above)

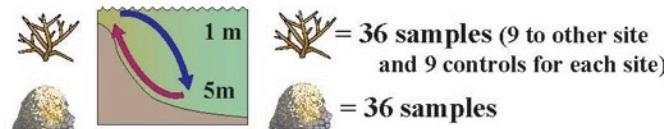
E) Short-term Reciprocal transplant of 5m corals between sites



= 36 samples

= 36 samples

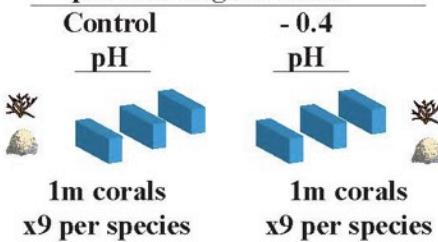
F) Short-term Reciprocal transplant of corals between 1 and 5m within Lagoon site



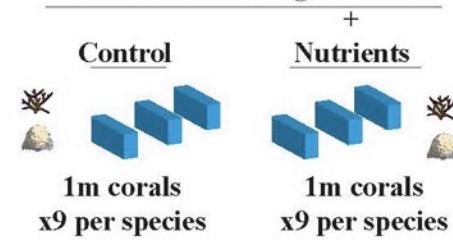
= 36 samples (9 to other site and 9 controls for each site)

= 36 samples

G) Aquarium study manipulating pH with Lagoon corals



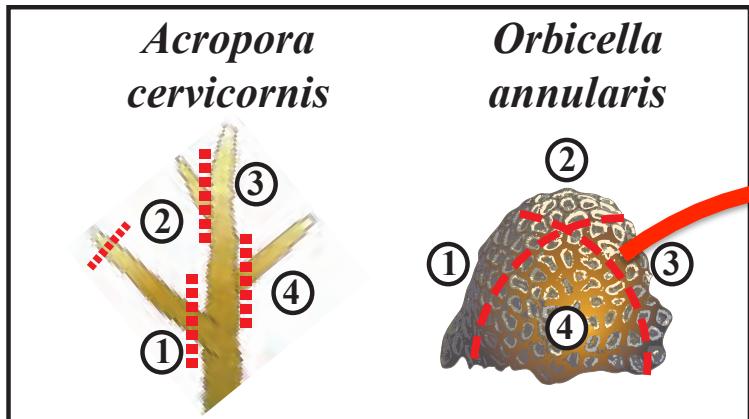
H) Aquarium study manipulating nutrients with Lagoon corals



Davey Kline

Simple sampling and processing methods

Panama



Fixative

Scripps (San Diego)



70% Ethanol
(ship)
Decalcification
Buffer (ship)

-Storage at 4 °C (archival)
-Processing (VHA, NKA,
PMCA, sAC, 4 other proteins)



Money from NOAA, NSF, Foundations

**Technician (s) microscopy facilities
Place with low overhead and taxes**



ADCY10



GENE/PROTEIN

ANTIBODY/ANTIGEN

TISSUE ATLAS

STAINING OVERVIEW

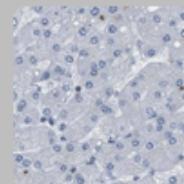
Dictionary



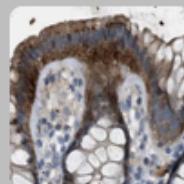
Dictionary

TISSUE ATLAS ? »

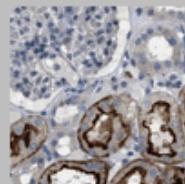
Gene description	Adenylate cyclase 10 (soluble)
RNA tissue category	Tissue enriched Ectoderm
Protein summary	Detected at High or Medium expression levels in 21 of 79 analyzed normal tissue cell types.
Protein expression	Cytoplasmic and membranous expression in selected tissues, expression in cilia.
Protein class	Cytoskeleton related proteins, Disease related genes, Enzymes, Potential drug targets, Predicted membrane proteins
Predicted localization	Membrane
Protein evidence	Evidence at protein level
Protein reliability	Uncertain based on 2 antibodies.



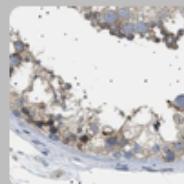
Liver



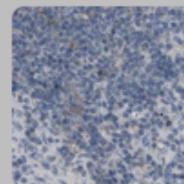
Colon



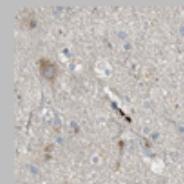
Kidney



Testis



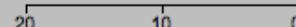
Lymph node



Cerebral cortex

RNA

Expression (FPKM)



Organ system

Liver and pancreas

Liver

Gallbladder

Pancreas

Protein

Localization (score)

