Using functional genomics to explore the impacts of ocean acidification on calcifying marine organisms

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Thanks to: Workshop Steering Committee
OCB &
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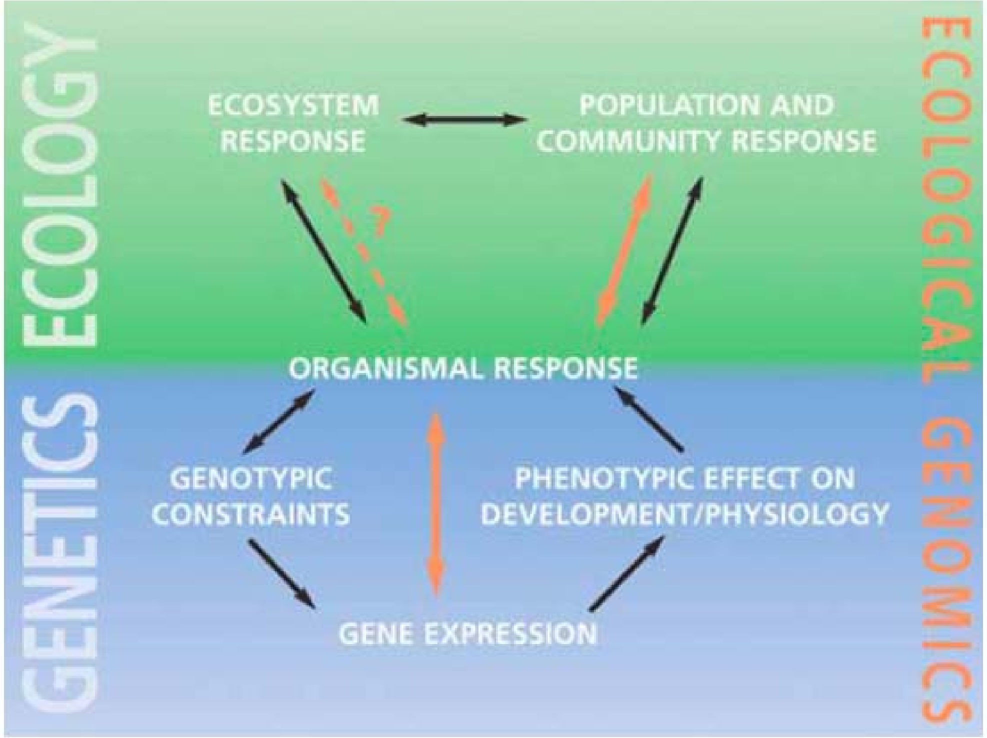
Outline of the presentation

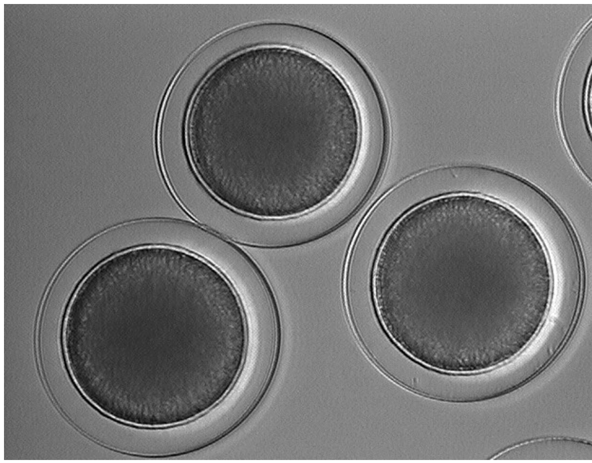
- Highlight gene expression approaches
- Sample data from sea urchin studies
  - *Organismal response* larval skeleton development
  - *Compensation/plasticity/ adaptation*
  - *Synergistic effect of temperature and CO_2*
- Strategies for future research



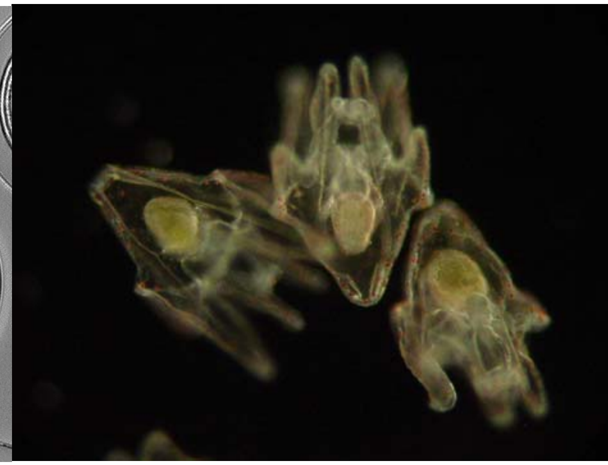
From: Ungerer et al. (2007) *Heredity,* 1-6

#1 Organismal response: skeleton formation in sea urchin embryos and larvae

Strongylocentrotus purpuratus



Embryos
with fertilization membrane

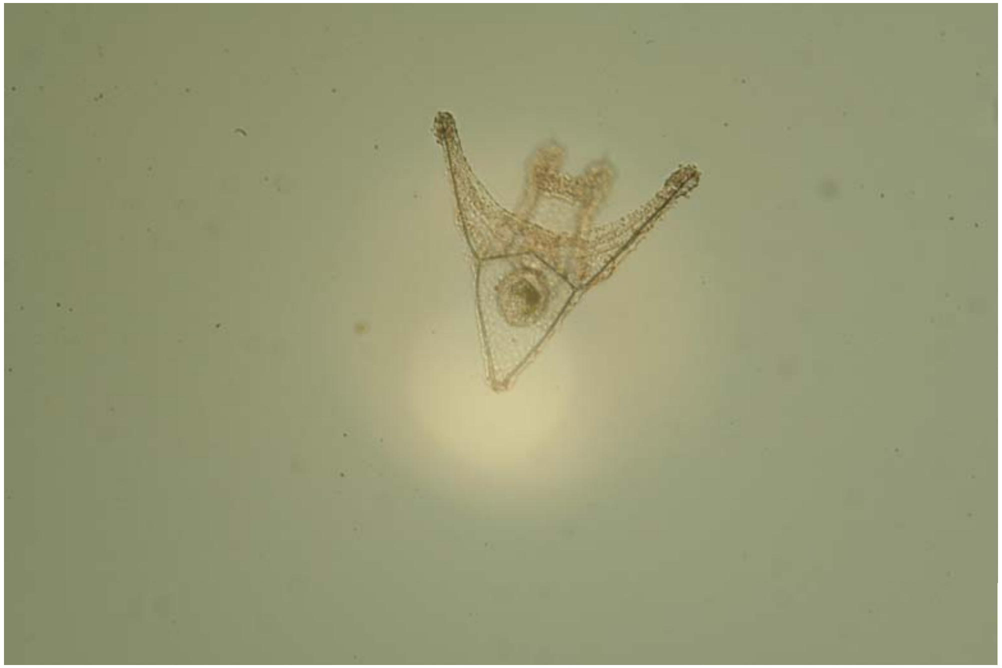


4-arm echinopluteus
larvae

Juvenile purple sea urchin

1) Utility of early life history stages
2) Sea urchin: Developmental model

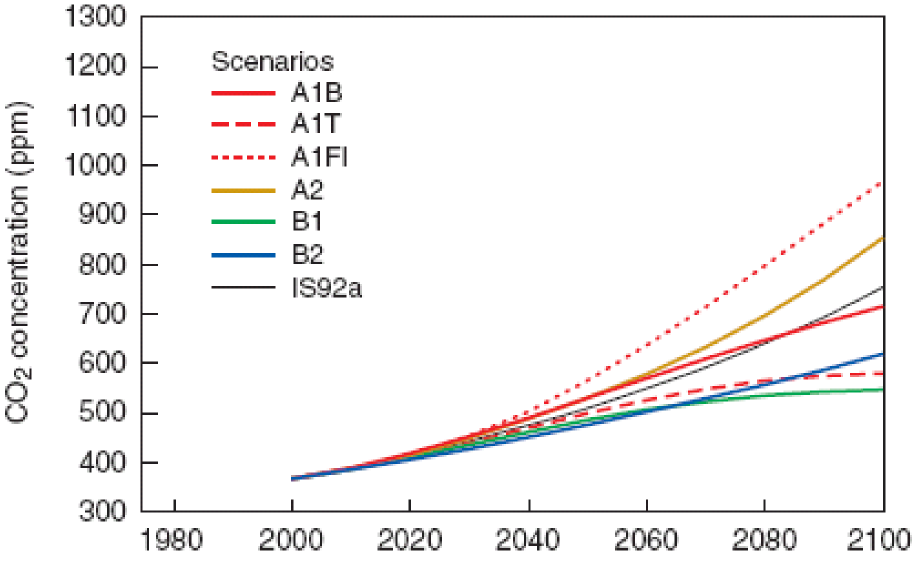
Our multidisciplinary approach



4-arm stage
Lytechinus pictus

- **Morphometrics**
  - Measure the elements of the larval skeleton
- **Gene expression analysis**
  - Ask the embryo to tell you the answer.
  - Generating “physiological fingerprints”

First-cut experimental approach



- IPCC gives predictions
- We use B1 and A1F1

Gas Mix

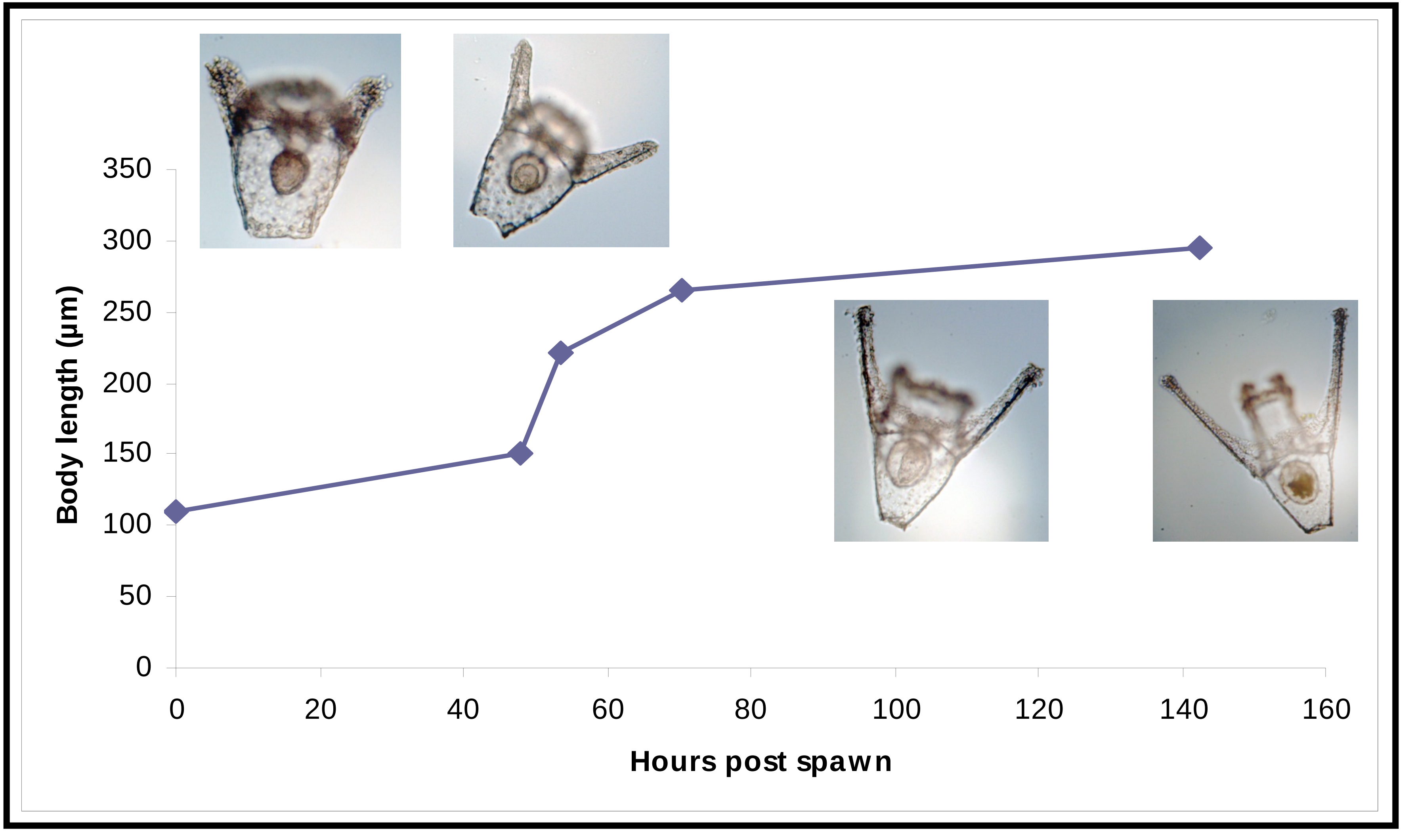
Experimental approach?
Use IPCC scenarios for future CO_2 levels

- Used IPCC Scenarios (by 2100)
  - **Present** 380 ppm CO_2
  - **B1** 540 ppm CO_2
  - **A1F1** 970 ppm CO_2

Postdoctoral Fellow
Dr. Michael 'Moose' O'Donnell

- Raise larval cultures at 15 °C

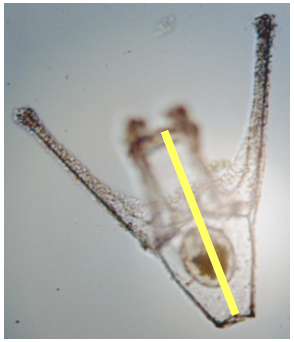
Body Length vs. Development in *Lythechinus pictus* larvae

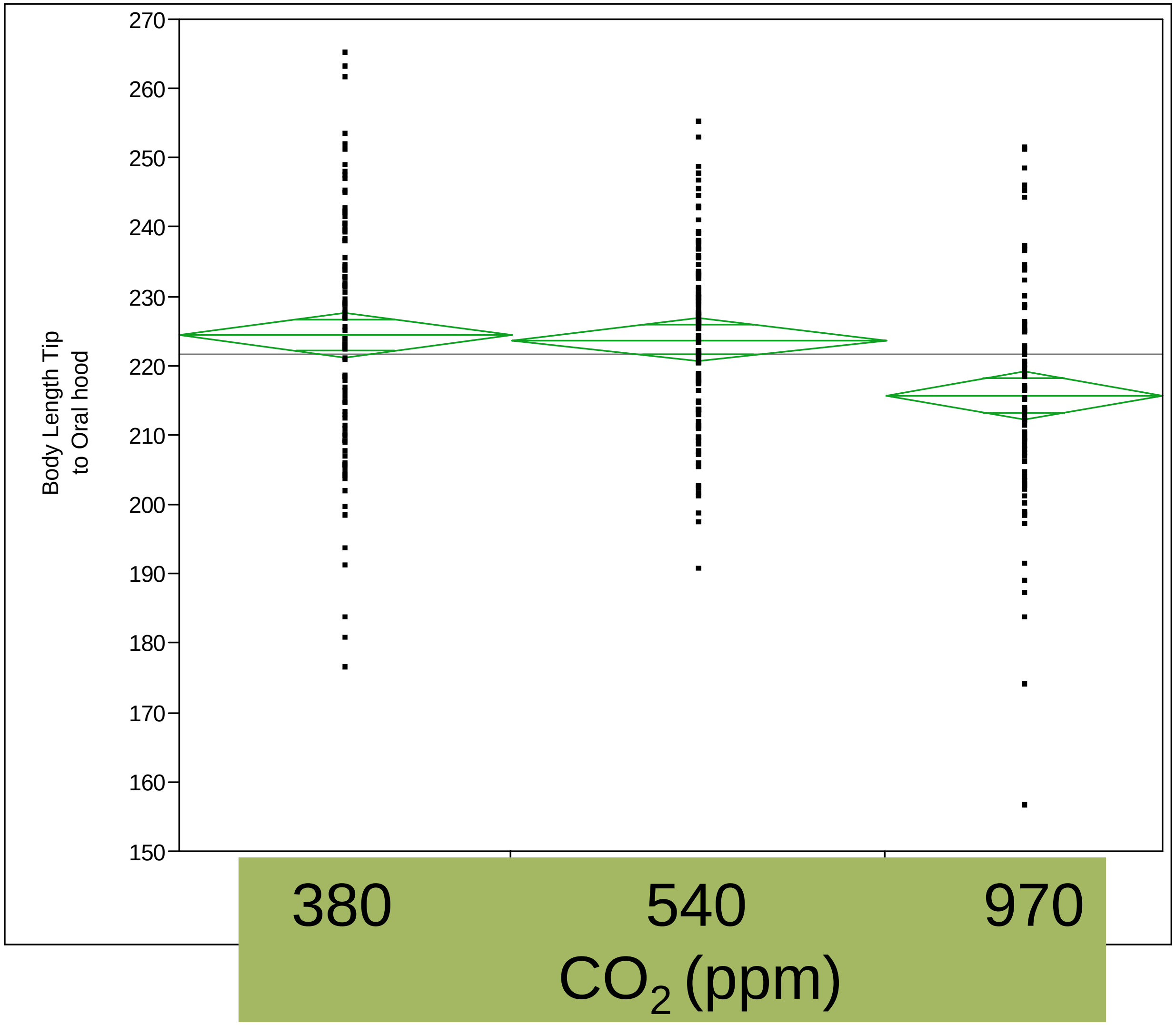


Larval Measurements

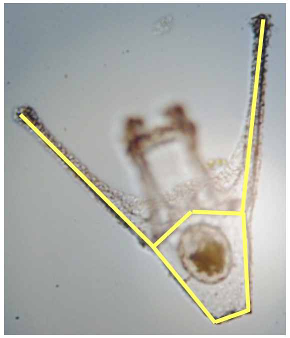
1. Body length to tip of oral hood
2. BL to ventral arch
3. Left body rod
4. Left post-oral arm
5. Right body rod
6. Right post-oral arm
7. Left transverse rod
8. Right transverse rod
9. Body width
10. Width at tip

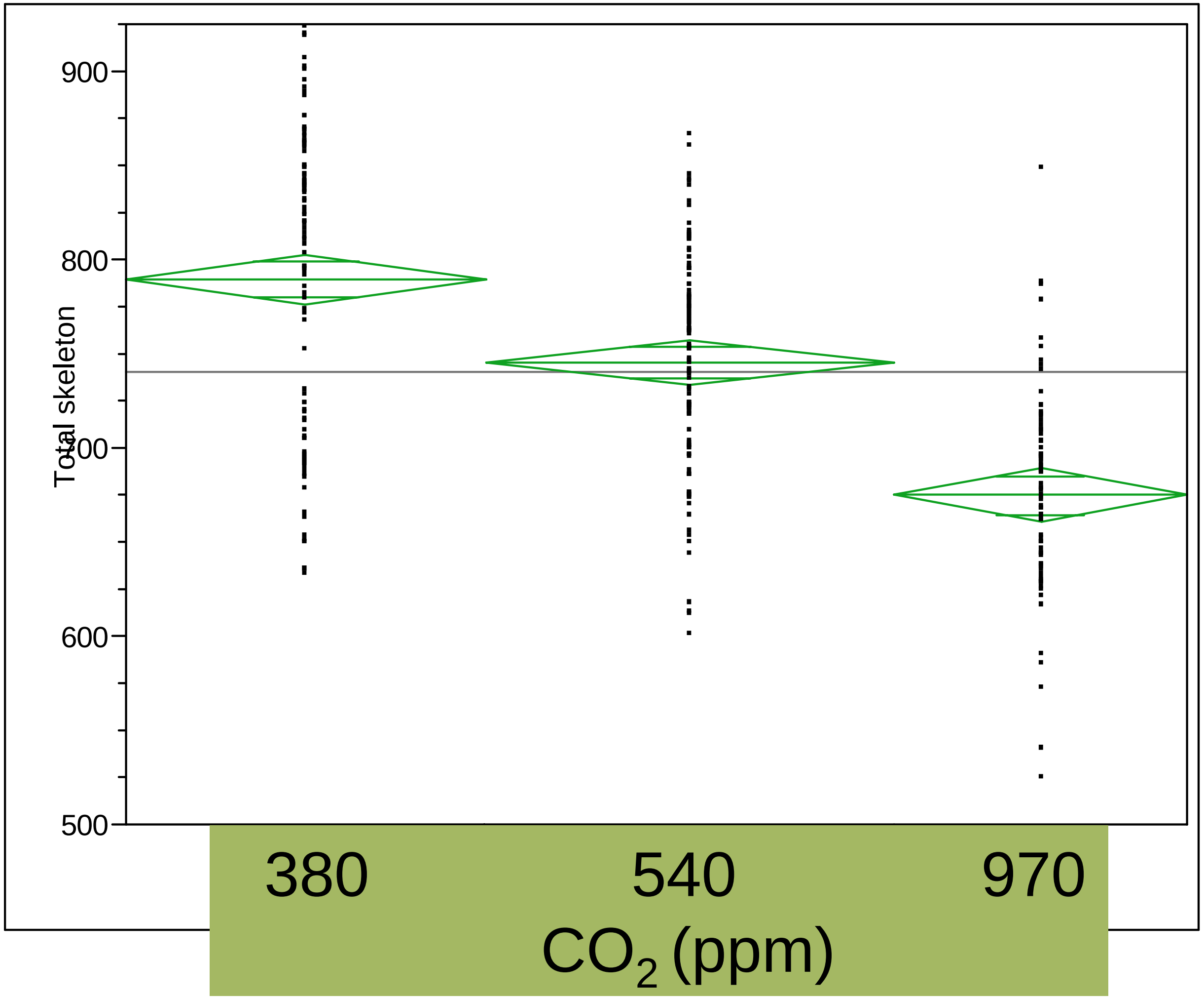
Body Length: 48 h



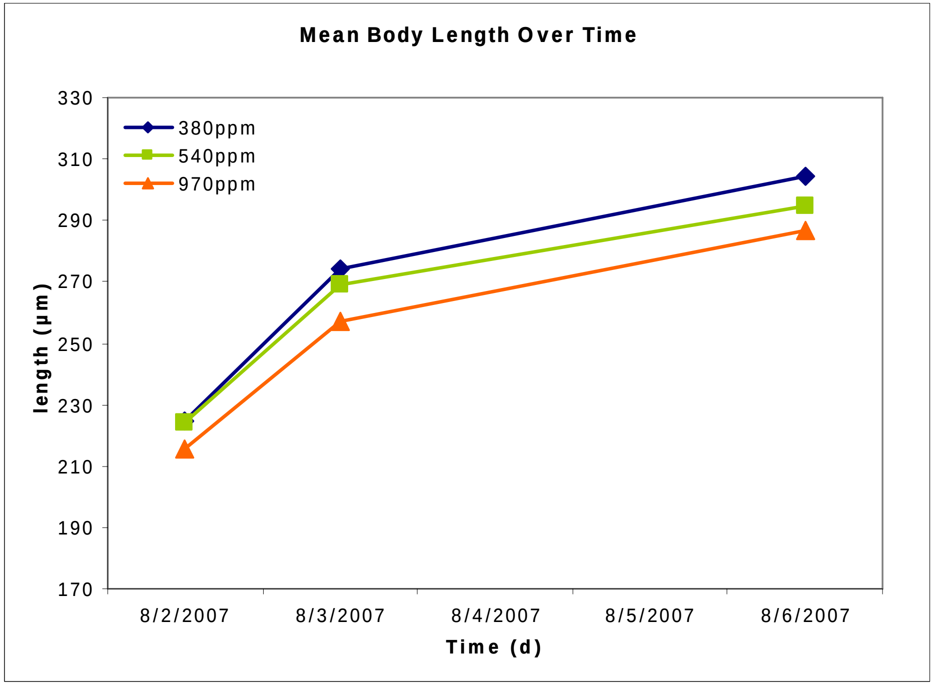


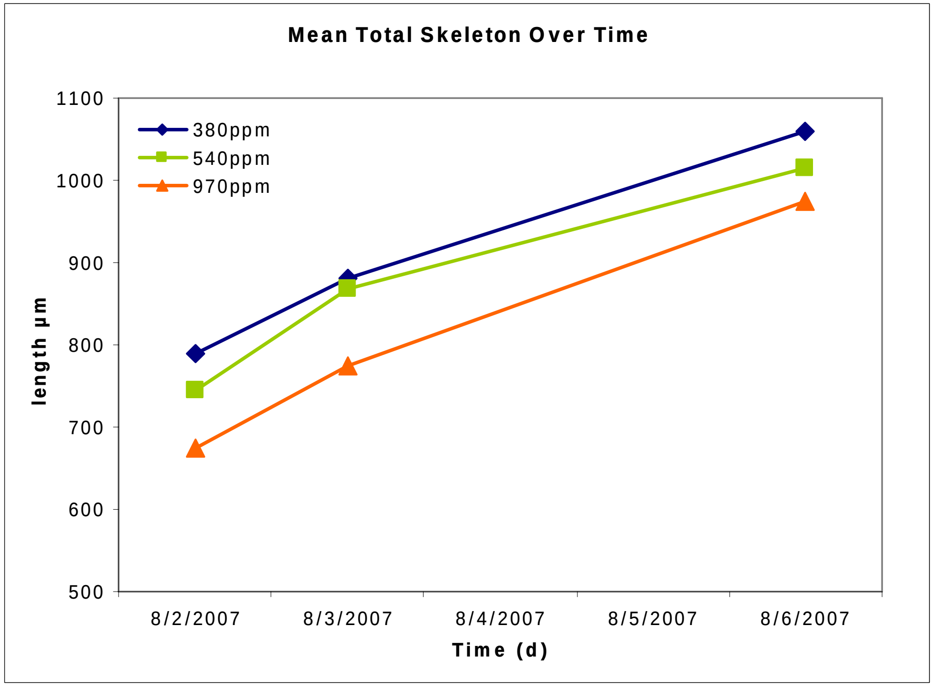
Total Skeleton: 48 h



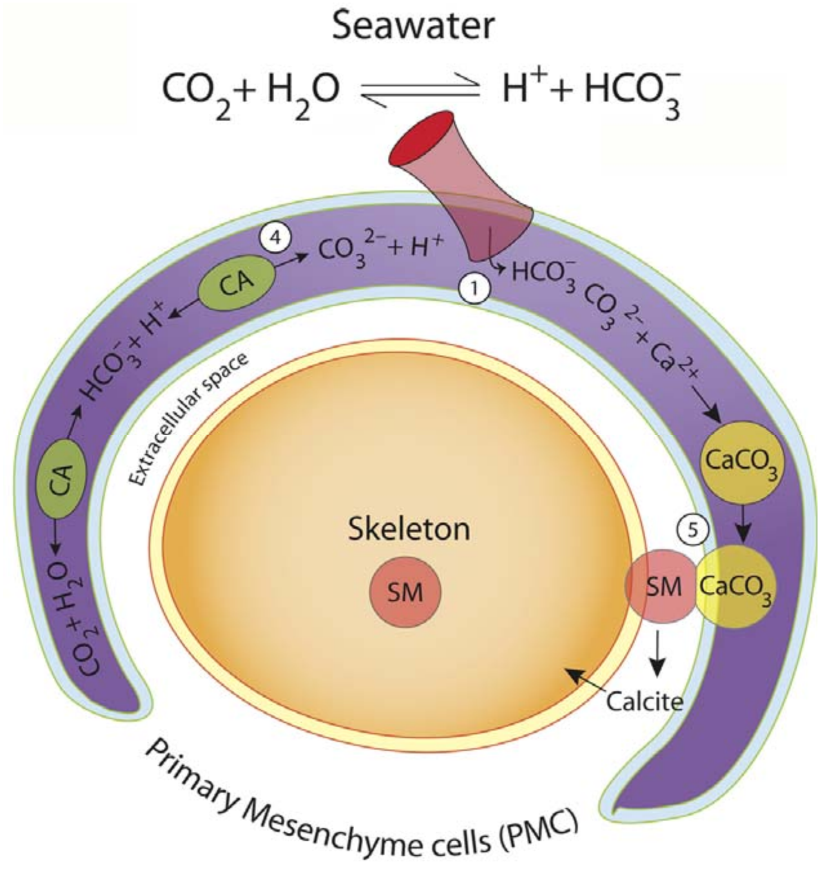


Mean Body Length and Total Skeleton vs. Time





Making Hard Parts
What genes to choose?

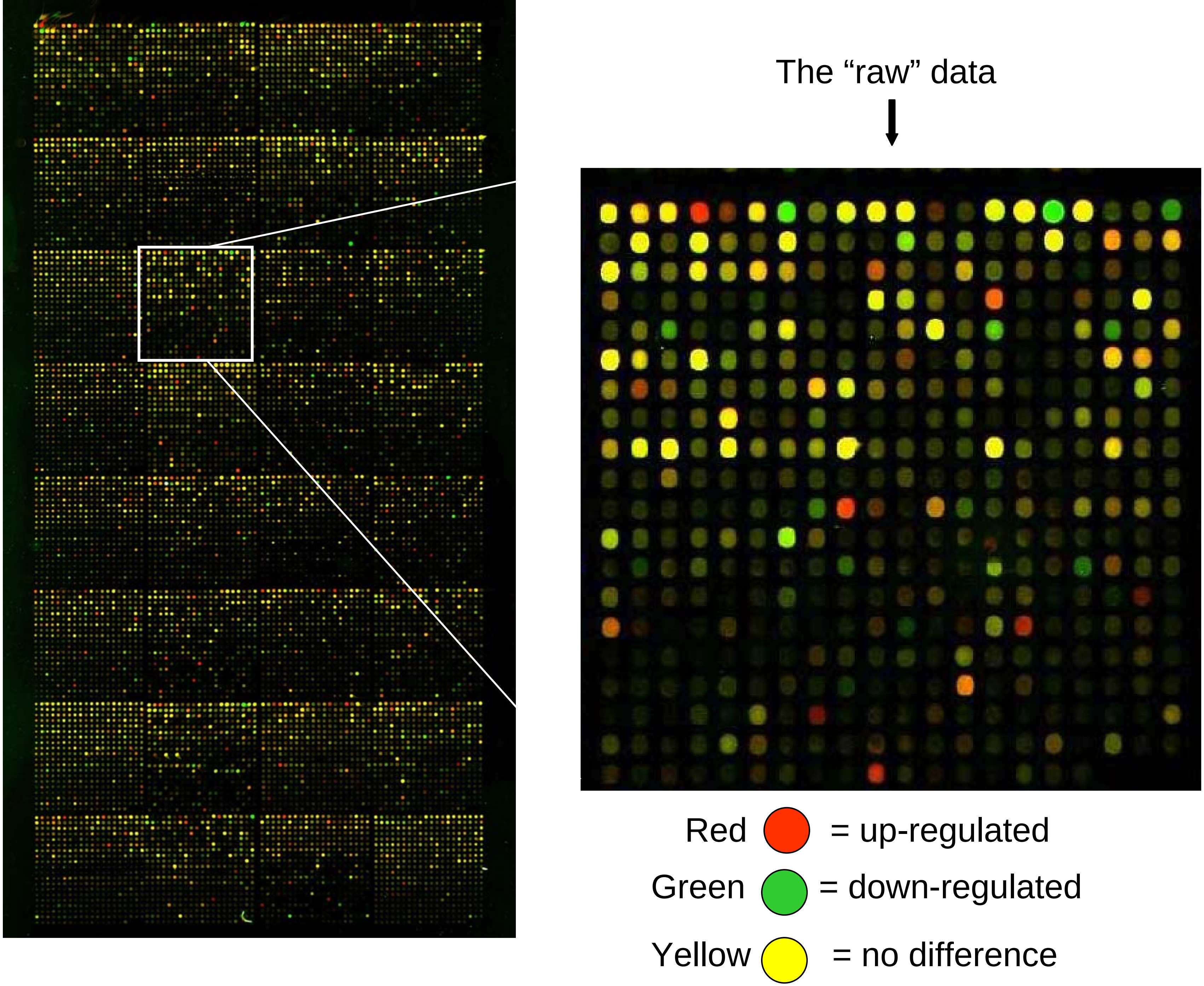


From: Hofmann and O'Donnell (in prep) *TS section on OA*

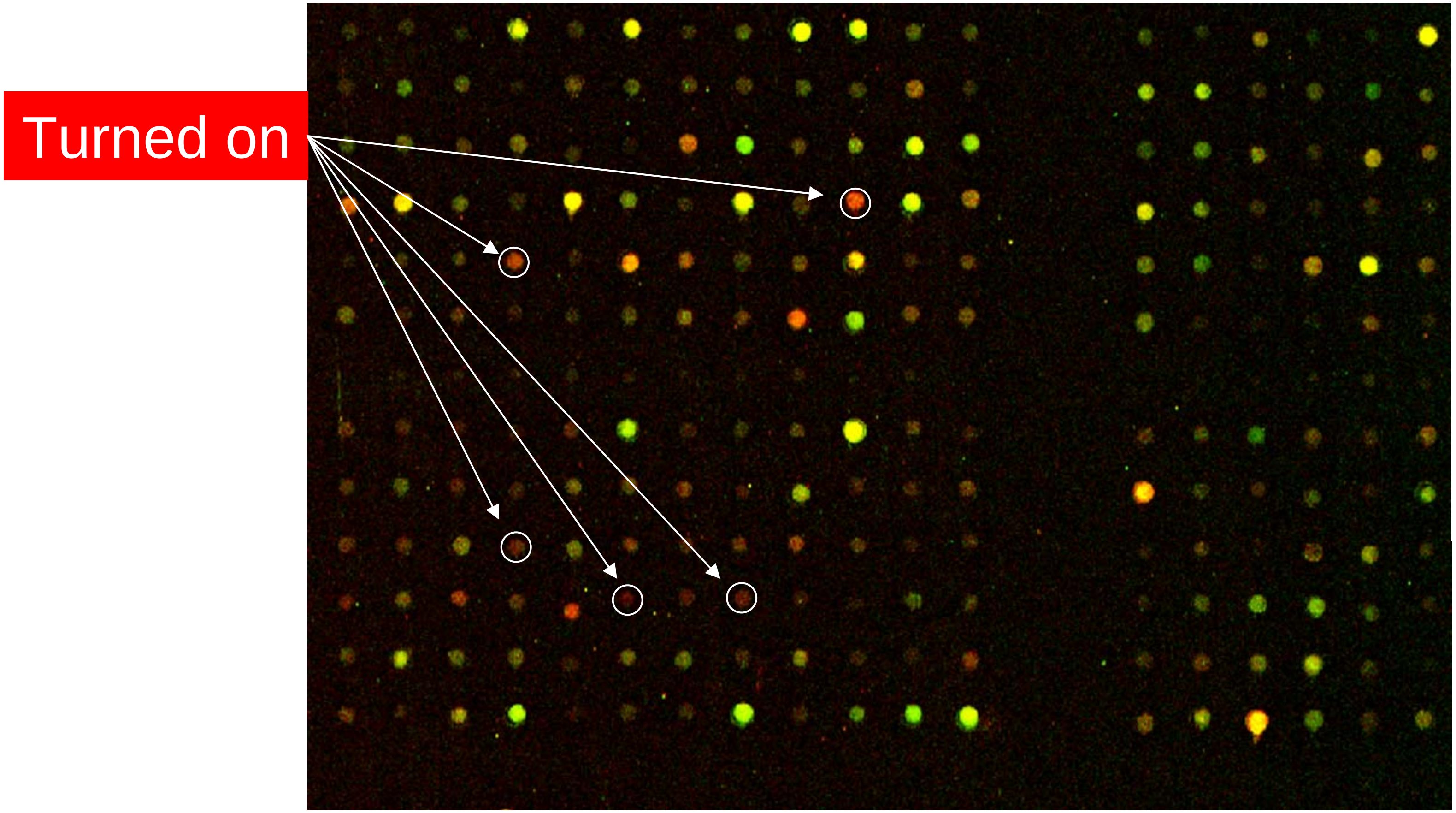
#2: Linking plasticity & physiology with gene expression

1. Biomineralization chip in prep.
2. E.g., lots of spicule matrix proteins
3. Oligo microarray from Agilent
   ~1,500 genes
   60-mers
   $170/array

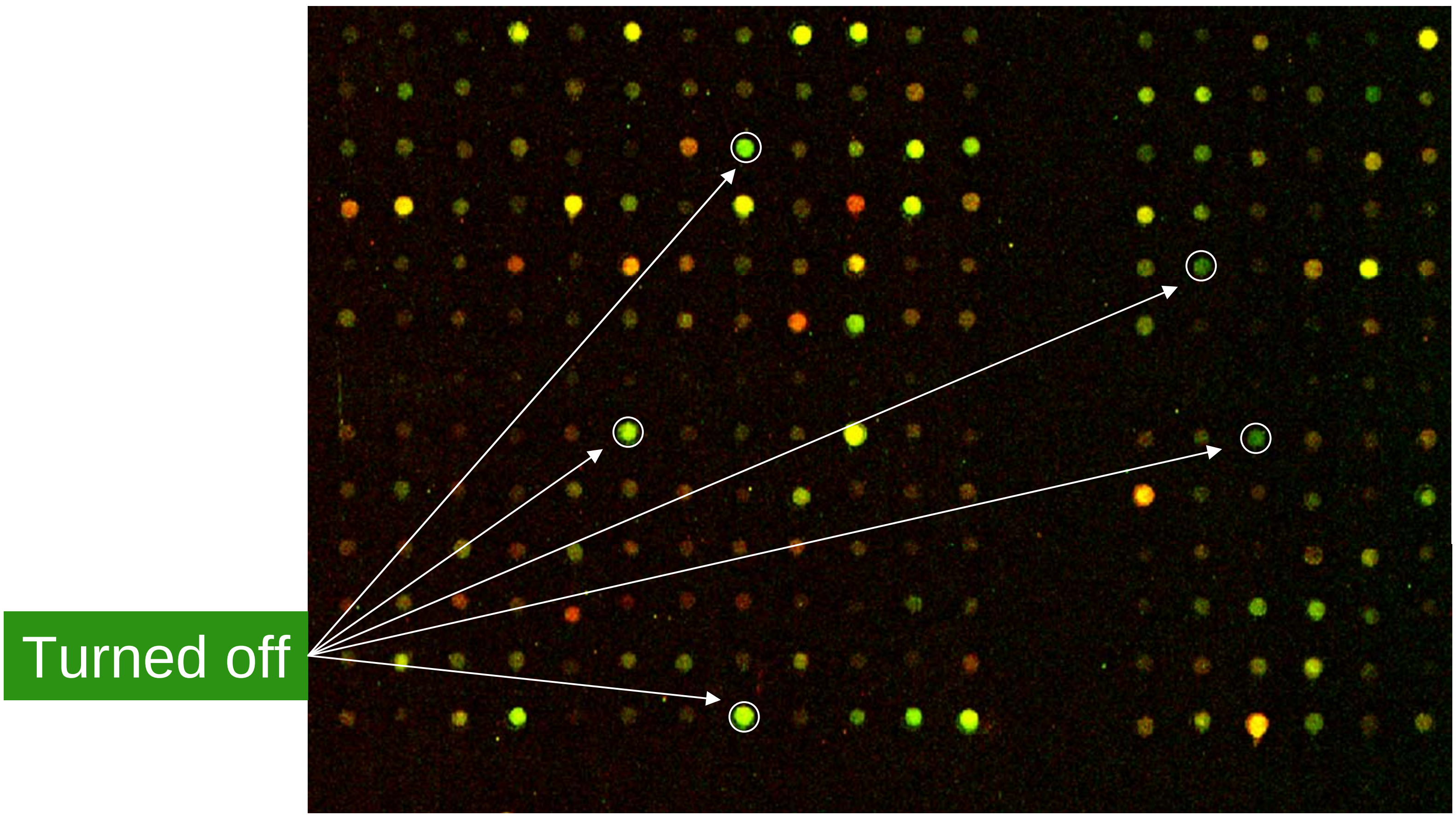
Spicule Matrix Proteins	***Gene ID***	**Exp. Notes**
SMP30-A	*SPU_000825*	High in prism
SMP30-D	*SPU_000828*	Low in prism


The “raw” data
Red
= up-regulated
Green
= down-regulated
Yellow
= no difference

Zoom in

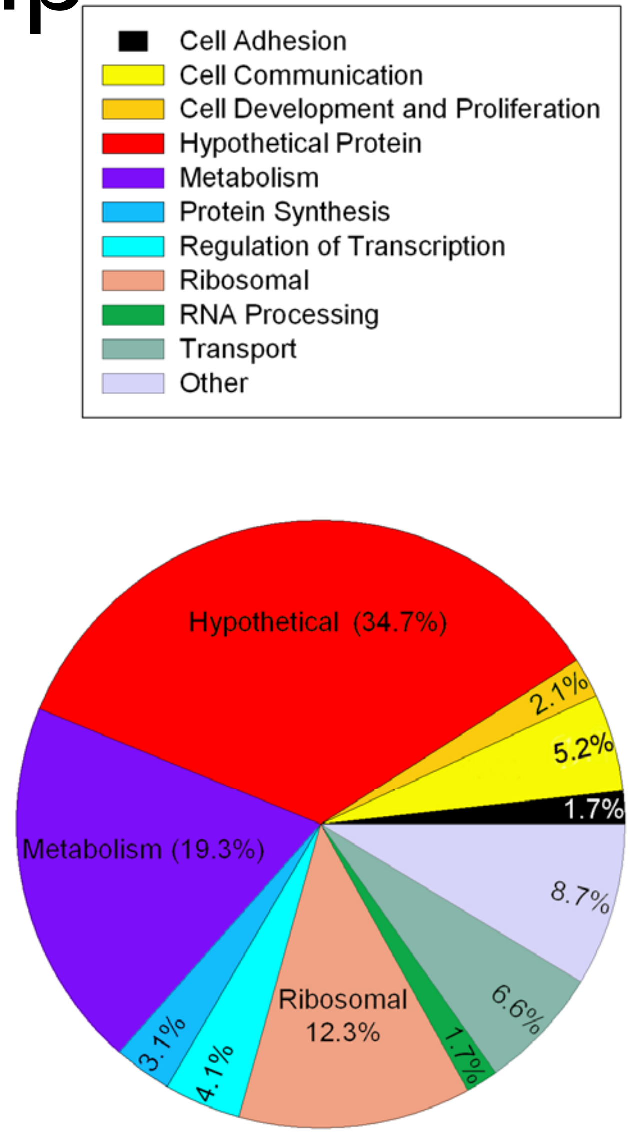


Zoom in

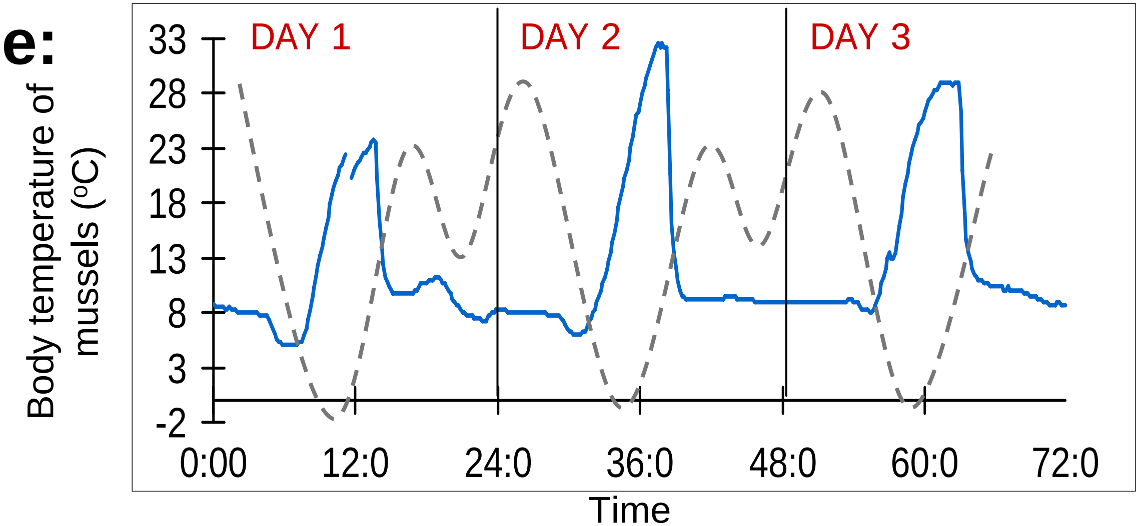


Another way to look at the data on the chip

- Spots are genes we know
- For example, gene chip for mussels
  - ~3,000 genes of interest
  - Classified by function



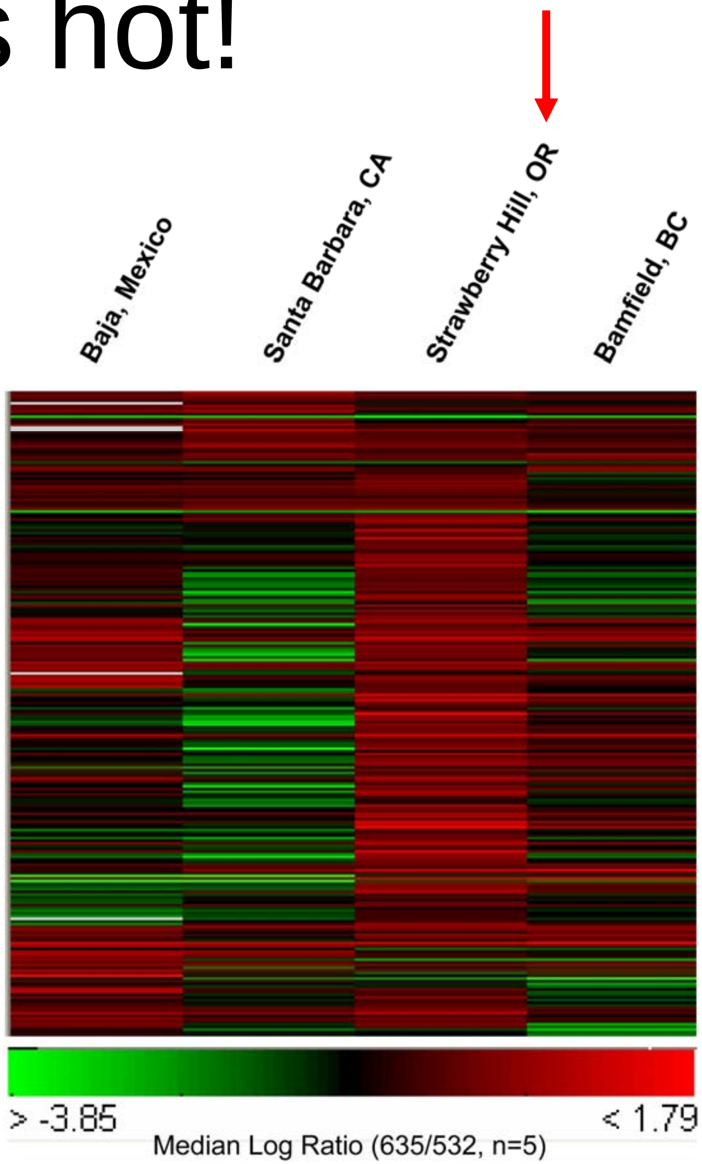
An ecological example:
Thermal stress in
Intertidal mussels



Helmuth et al. 2002 *Science* 298: 1015-1017

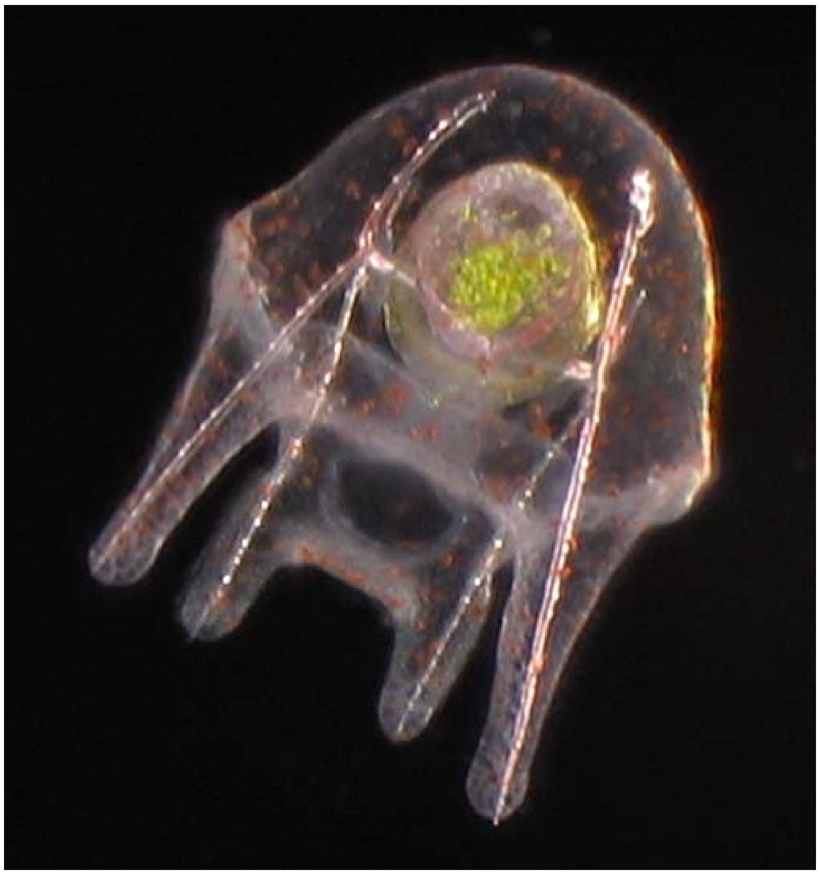
Physiological fingerprints says: Oregon is hot!

- Mussels at Oregon site have strong up-regulation of “stress” genes in **summer**



Place, Hofmann (2007) *MEPS* (in review)

#3: Synergistic effects

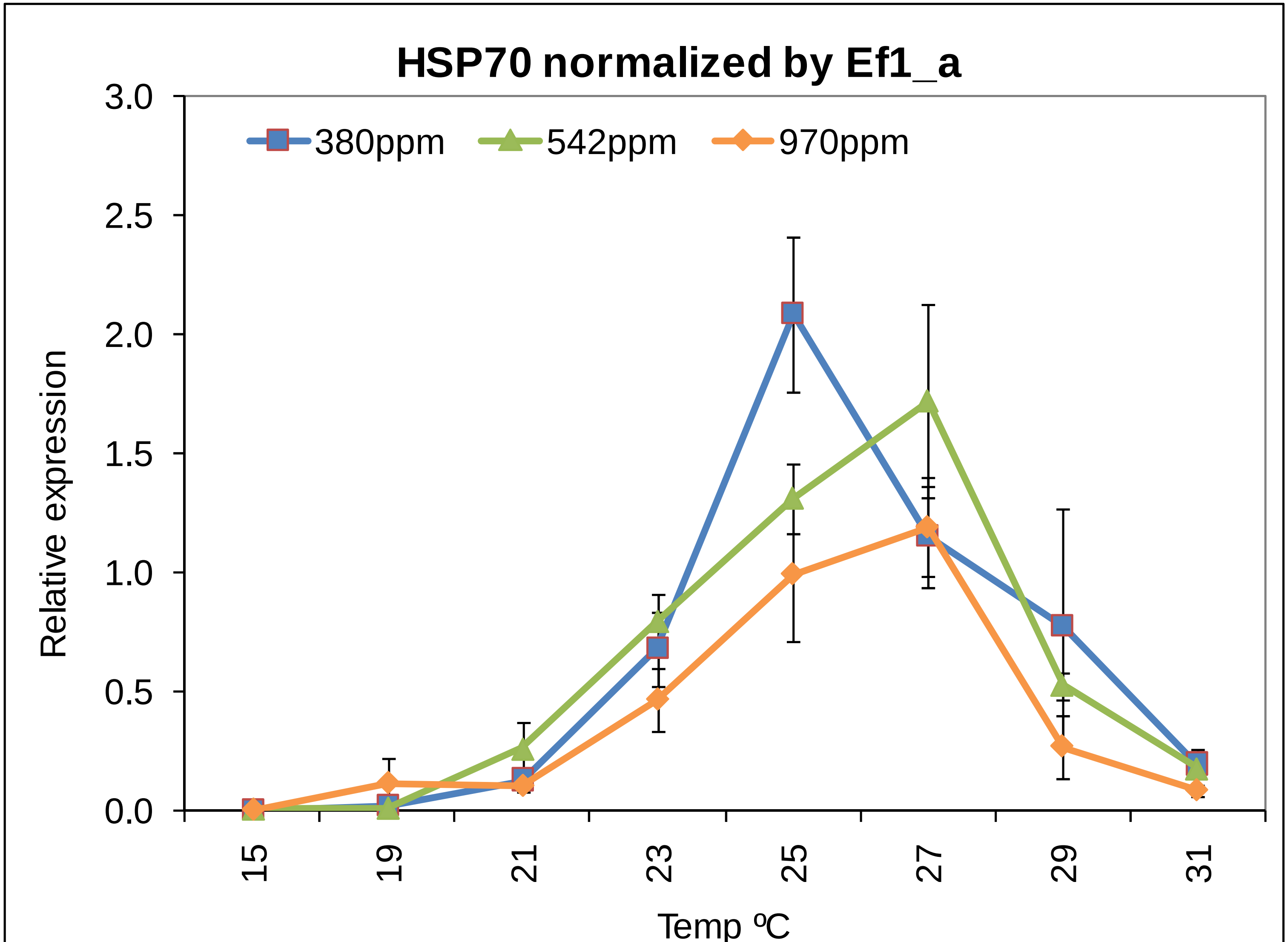


The red sea urchin: *S. franciscanus*

- All cultures raised at 15 ºC
- Three levels of CO_2
- **What happens to physiological plasticity?**

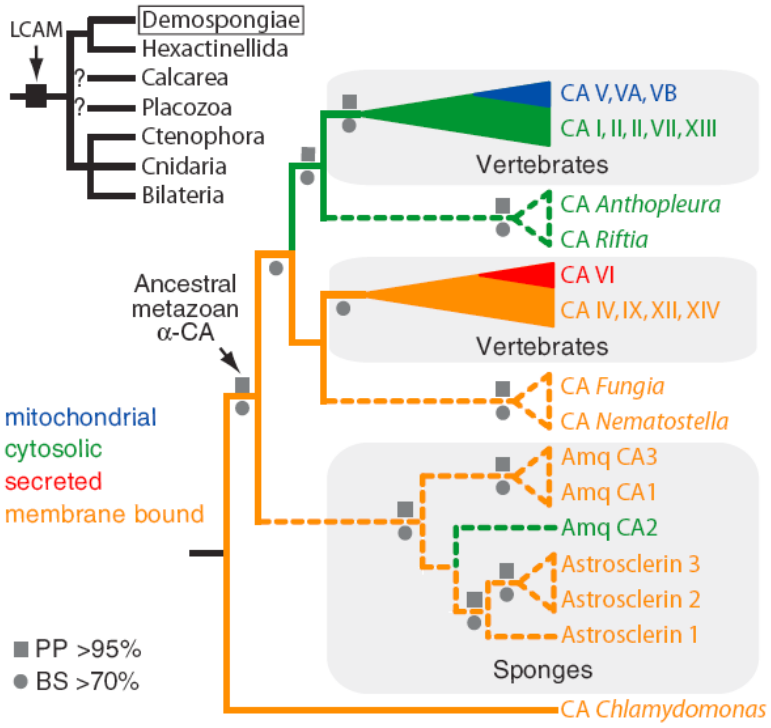
(Not just biomineralization)

Changes in thermal phenotype



Strategies or what to do while we wait for the genome…

- Study highly **conserved** genes
- Work on **targeted species** with good genomic resources
- Vegas baby! **Heterologous hybridizations**

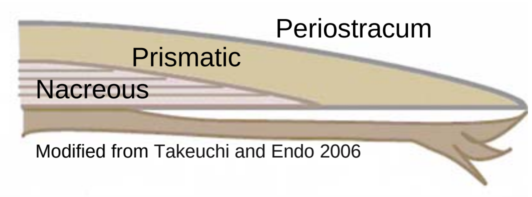


Jackson et al. (2007) *Science*

Cross-species primers

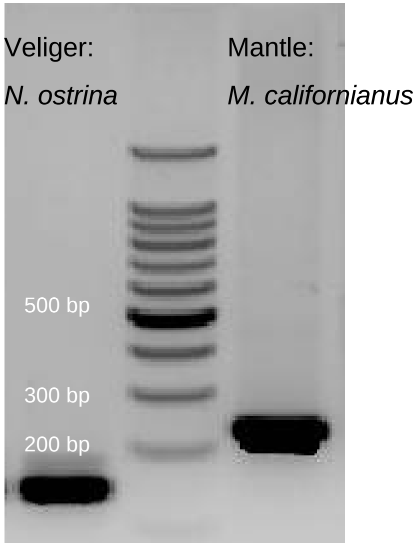
Veliger stage
Nucella ostrina

- Genes responsible for biomineralization in the pearl oyster



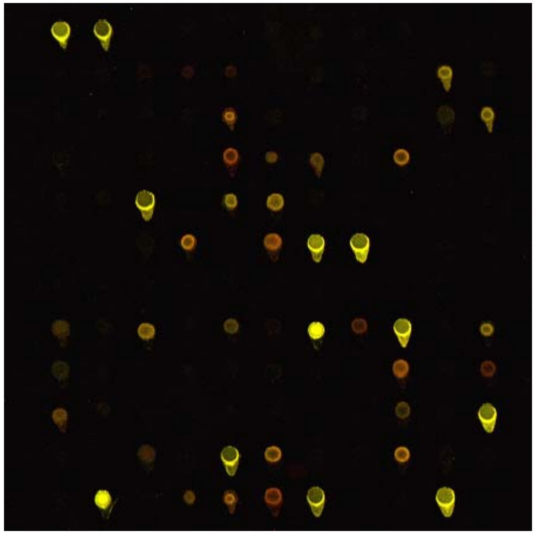
- **Nacrein** RT-primers from the pearl oyster used with other molluscs
  (Takeuchi and Endo 2006)
- PCR product size ~150-250bp
- Some success with other genes (msi60 and aspein)
- In process of sequencing the product

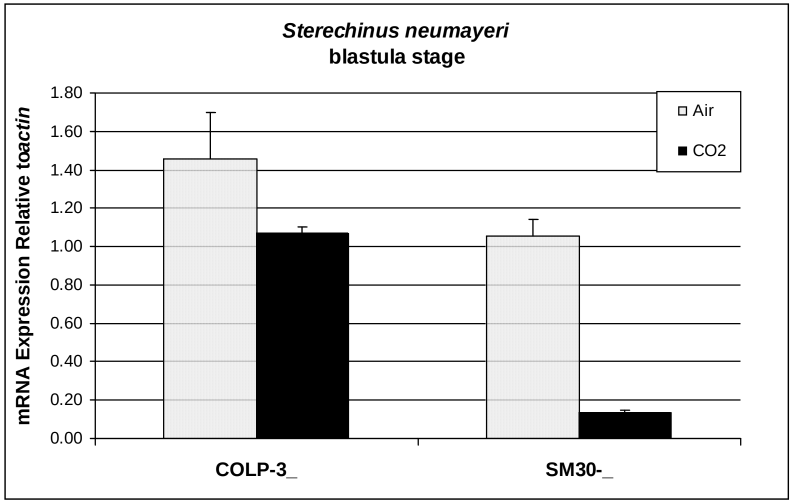
Nacrein



Antarctic sea urchin

Sterechinus neumayeri





qPCR data

□Cross hybridization to purp cDNA chip

Suggested strategies & priorities for OA studies

- These approaches can be applied to many organisms
- **Significant areas**
  - Compensation?
  - Synergistic effects
  - Species interactions
  - Metagenomics
  - Genome project proposals to JGI
  - Training gap and collaborations

QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

Metazoan examples

Acknowledgements

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- **Mackenzie Zippay**