Dose-dependent impacts of ocean acidification conditions and potential resiliency in young squid

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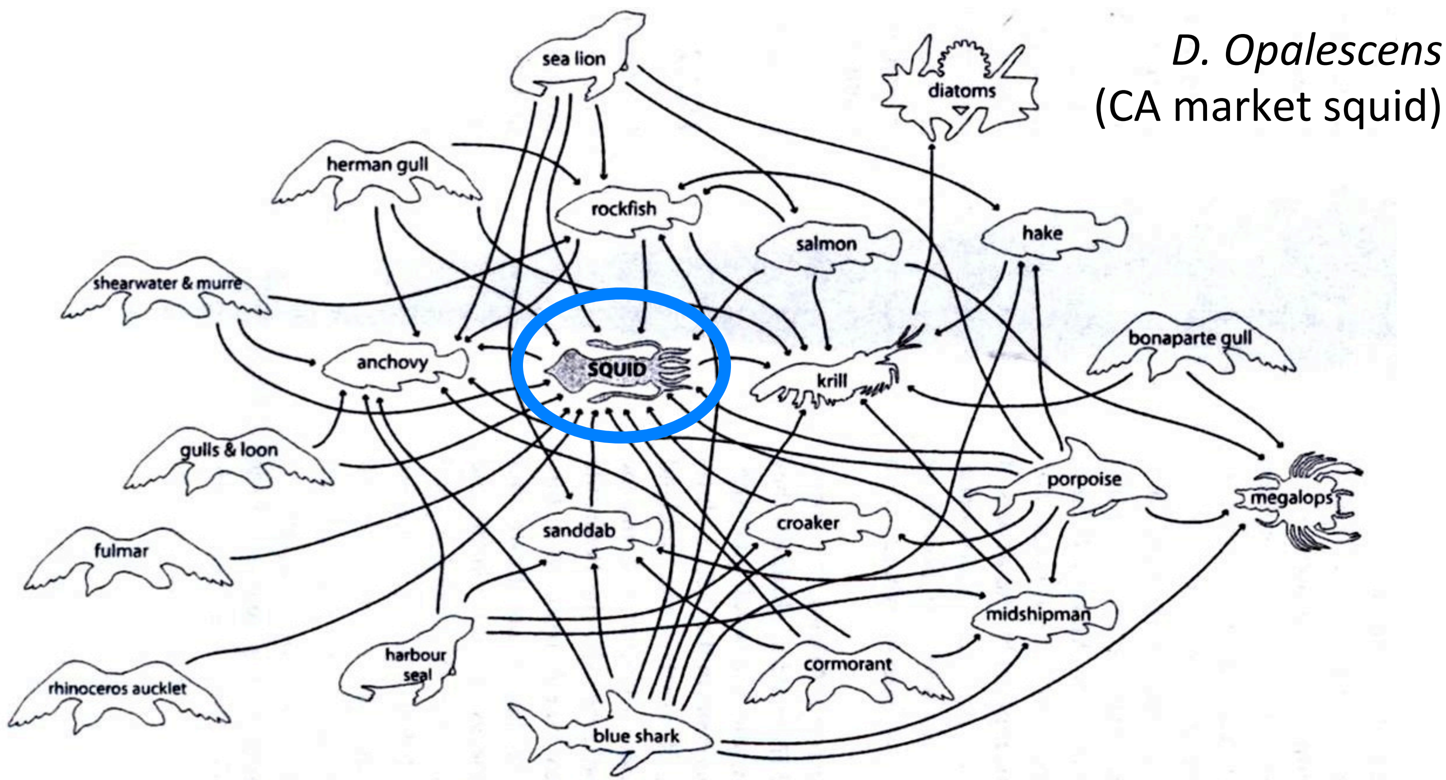
Why Cephalopods

- Play a vital (sometimes keystone) role in marine ecosystems
- Fisheries importance (20% of global landings)



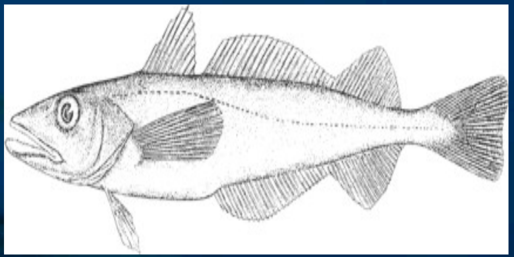
Prey of seabirds, seals, whales, fishes and other invertebrates

(Clarke 1996; Ruiz-Cooley et al. 2004; Clarke 2005; Ruiz-Cooley et al. 2006)

Ecology and Life History

- Abundant, fecund and semelparous reproduction with ~1 year lifespan
- Cephalopod physiology and populations directly tied to physical environment (temp, O_2 binding of blood)
- Squid population abundances directly affect those of other taxa

Grey-headed Albatrosses
Thalassarche chrysostoma

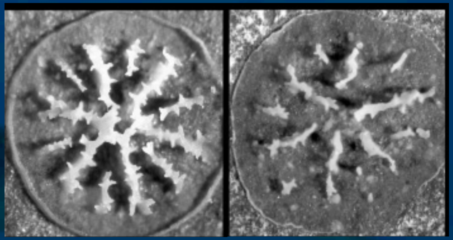


Pacific hake
Merluccius productus

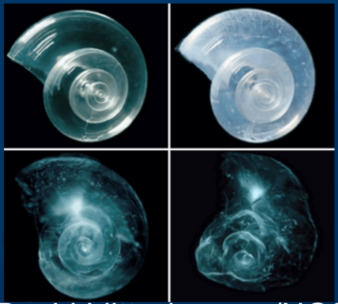
(Clark, 1996; Boyle and Rodhouse, 2005; Xavier et al 2003; Ziedberg Robinson 2007; Field et al 2007)

Early Life Sensitivities & Consequences

- Impaired development, size, and survival shown for a variety of species (Kurihara 2008, Ries et al. 2009; Baumann et al 2010)
- Impacts influence recruitment success thus could reduce populations (Munday et al. 2010)
- Studies have largely focused on marine calcifiers; Impacts to soft-bodied invertebrates is perhaps less understood
- Within cephalopods, much focus on cuttlefish



Anne Cohen, WHOI



David Liittschwager/NGS

(MBL)

(Radtke 1983, O'Dor et al. 1994, Pörtner et al. 2004; Hoegh-Guldberg et al. 2007, Gobler et al. 2014).

D. Pealeii - Coastal Squid

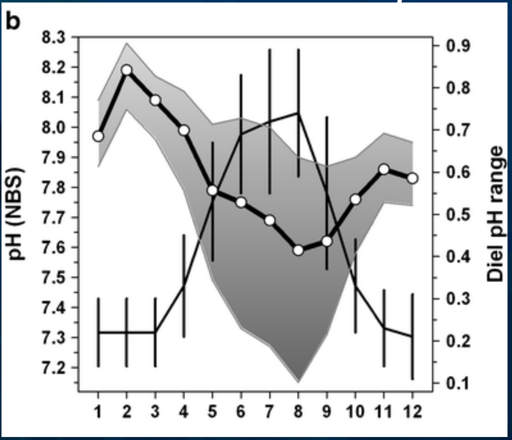
- Habitat Range: Nova Scotia to Brazil

- Adults pelagic – Benthic eggs

Breeding squid and egg mat

- Habitat: Dynamic, productive, coastal ecosystem
  - Bridge between better-addressed estuaries and open ocean

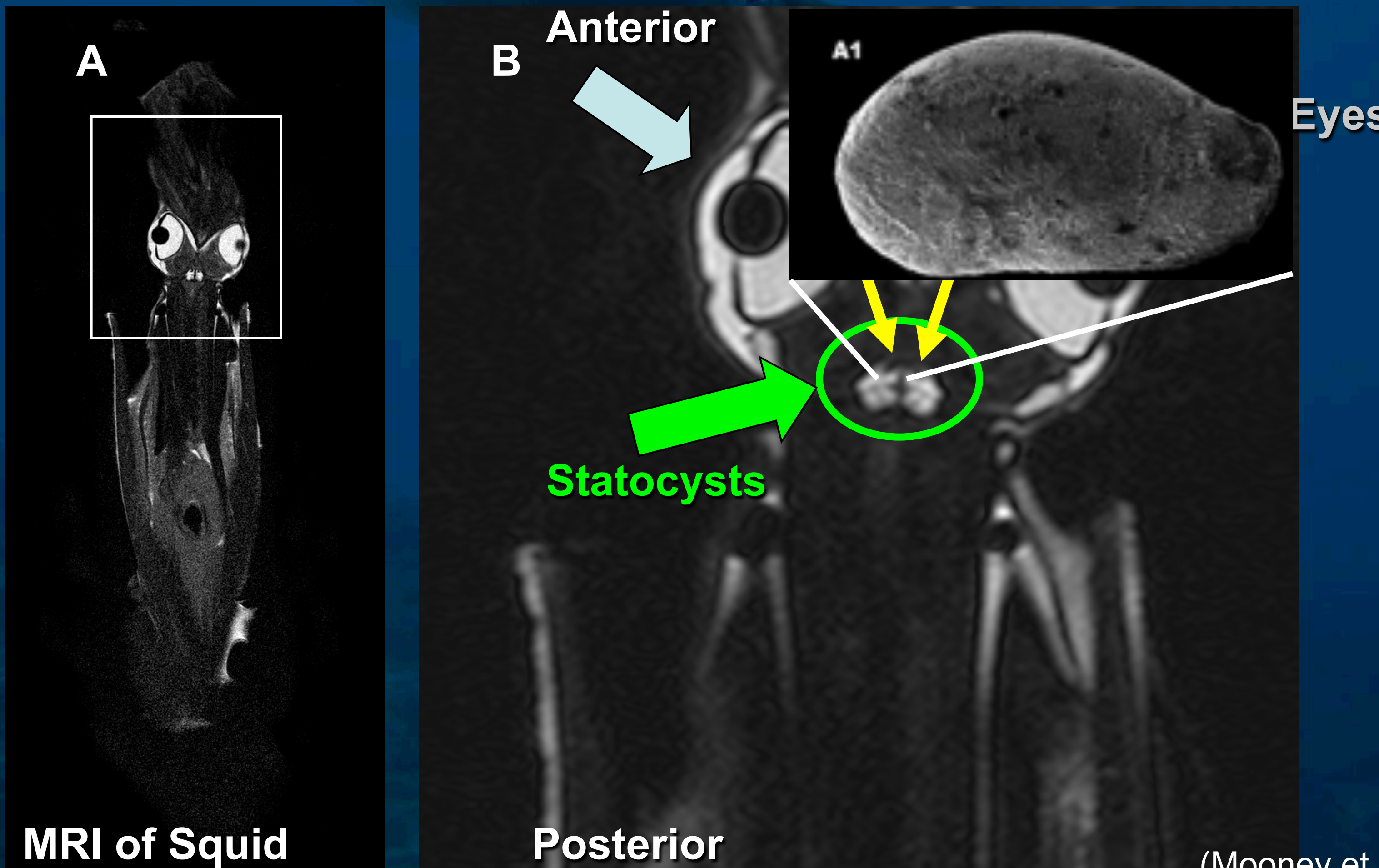
Long Island Salt Marsh pH range



(Baumann et al 2014)

Squid Statoliths

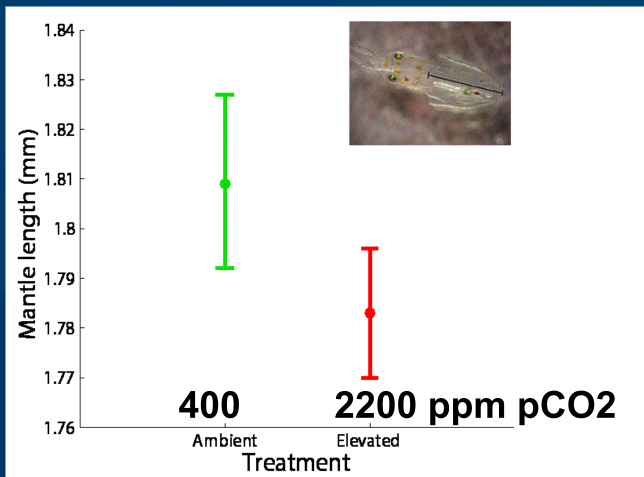
- Aragonite statoliths (inside the statocyst) = squid inner ear (vital for balance, swimming, hearing, orientation)



(Mooney et al 2010)

Our Initial Experiments

Development



($F_{1,292}$ = 9.241, p,0.003)

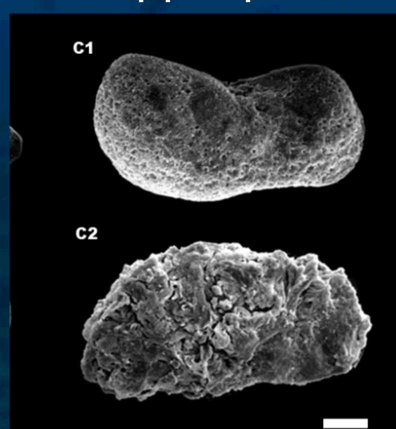
High CO_2 treated animals:

- Hatched smaller
- Hatched later

Statoliths

400 Ambient

2200 ppm pCO_2



($F_{1,55}$ = 70.722, p,0.001)

- Significantly reduced surface area
- Greater porosity

(Kaplan, Mooney, McCorkle, Cohen, 2013)

Goals of these studies

1) Quantify the dose-dependent effects of a high-CO_2 environment on squid

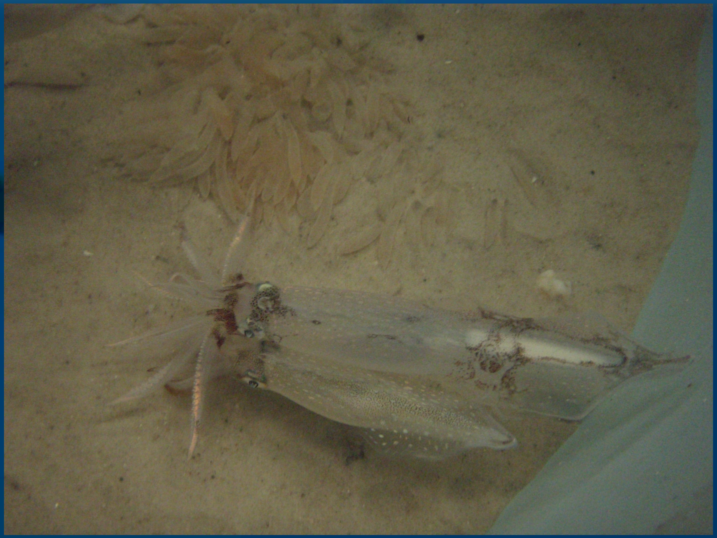
   - Where is the ‘threshold’? Seasonal (cohort) or annual differences?

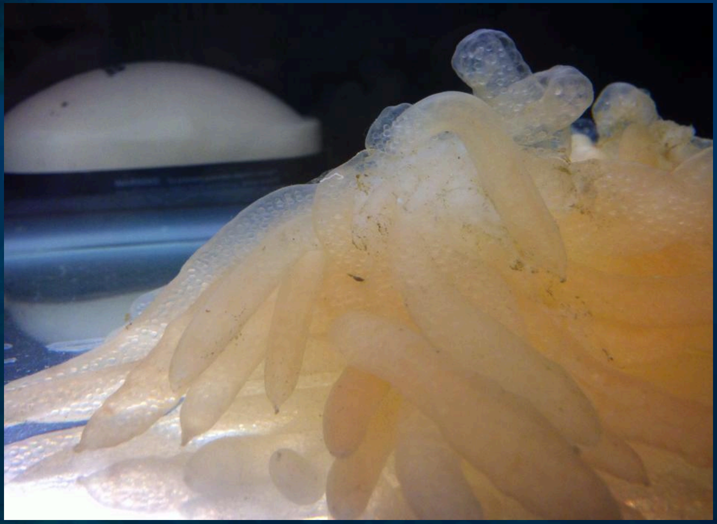
2) Address mechanisms of changes metabolism (O_2 consumption and energy reserves)

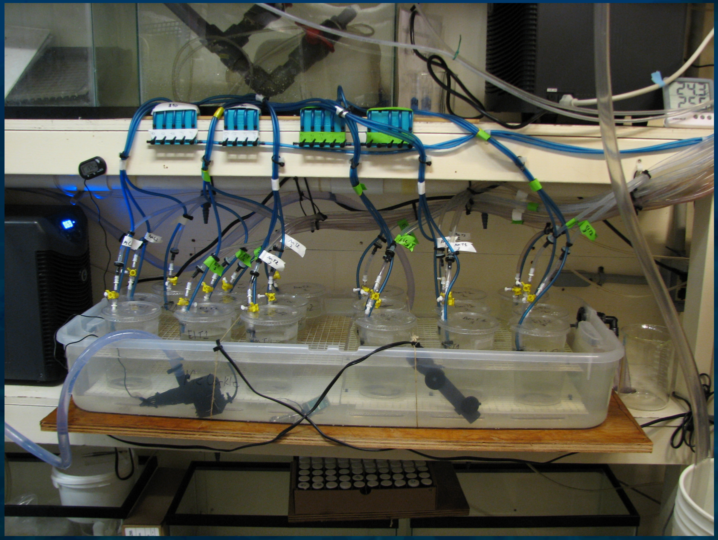
3) Examine consequences of OA impacts: survival and swimming capabilities

   - Velocity, distance, orientation…

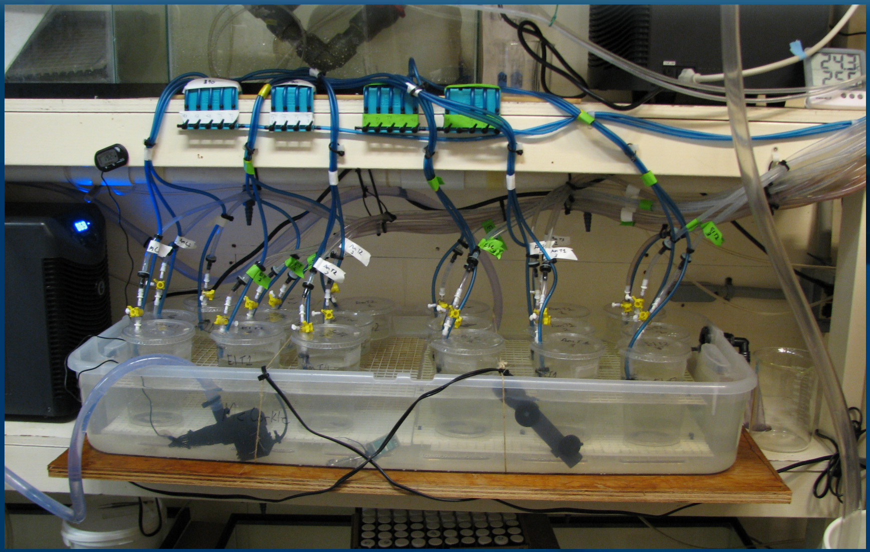
Methods

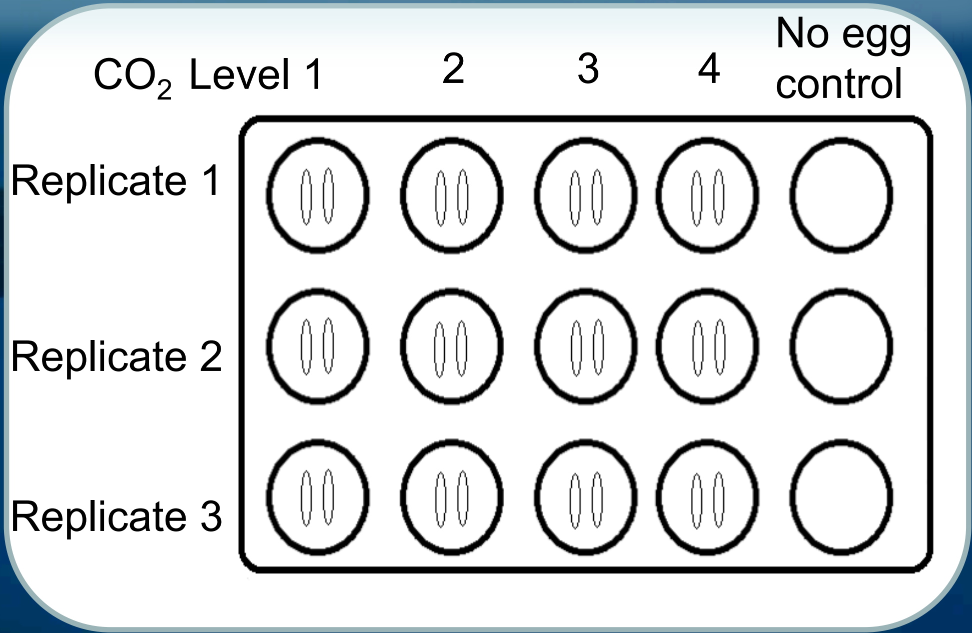






Methods

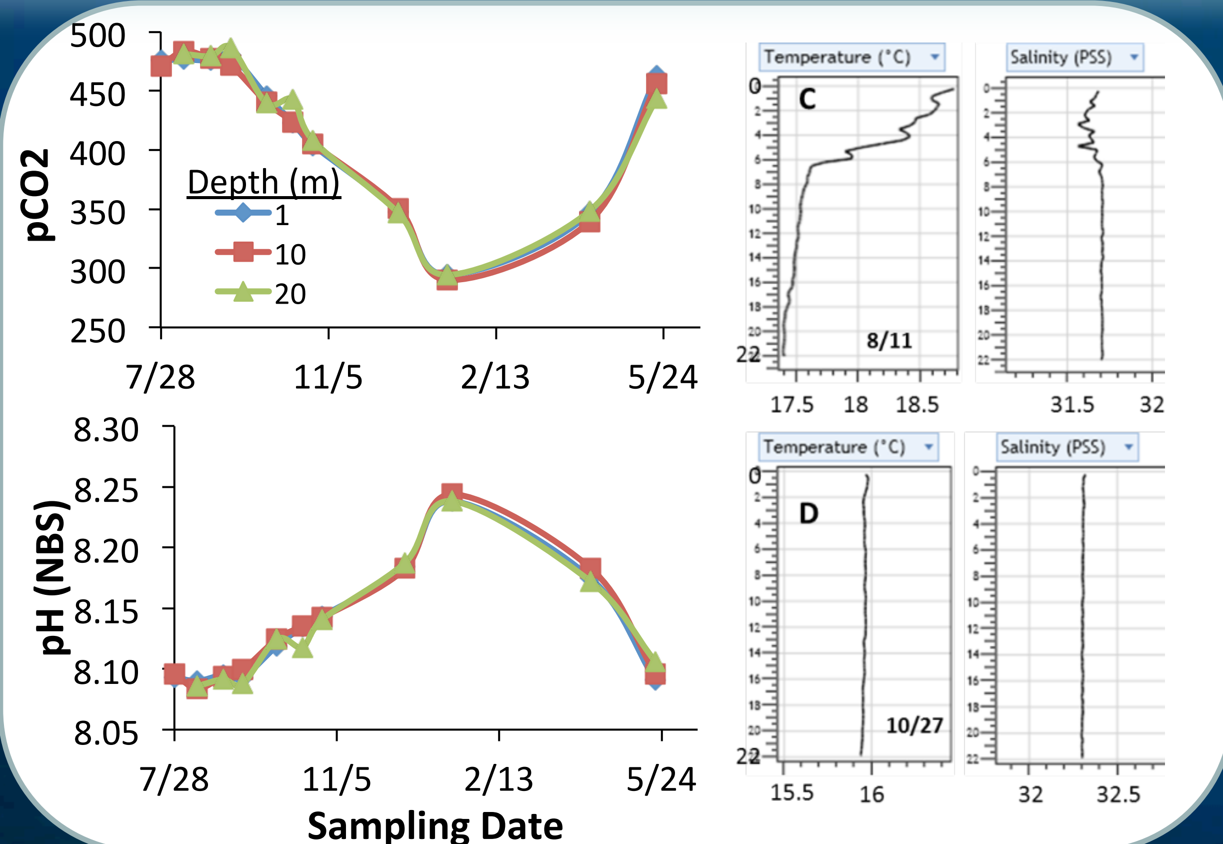


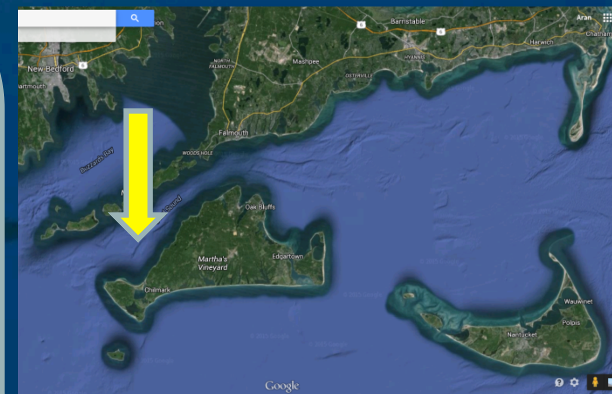


- 2+ years, 4-5 trials per season (May-Sept*)
- Each container bubbled with gas, 4 levels per trial repeating 400 and 2200 as controls
- 3772 animals process for mantle lengths alone

- CO2 conc measured weekly
- pH measured every 2nd day (meter) and weekly (spectrophotometer)
- Salinity measured every 2nd day (refractometer and weekly (autosal)
- TA measured with salinity (titration)

pH and pCO2 in squid egg sites

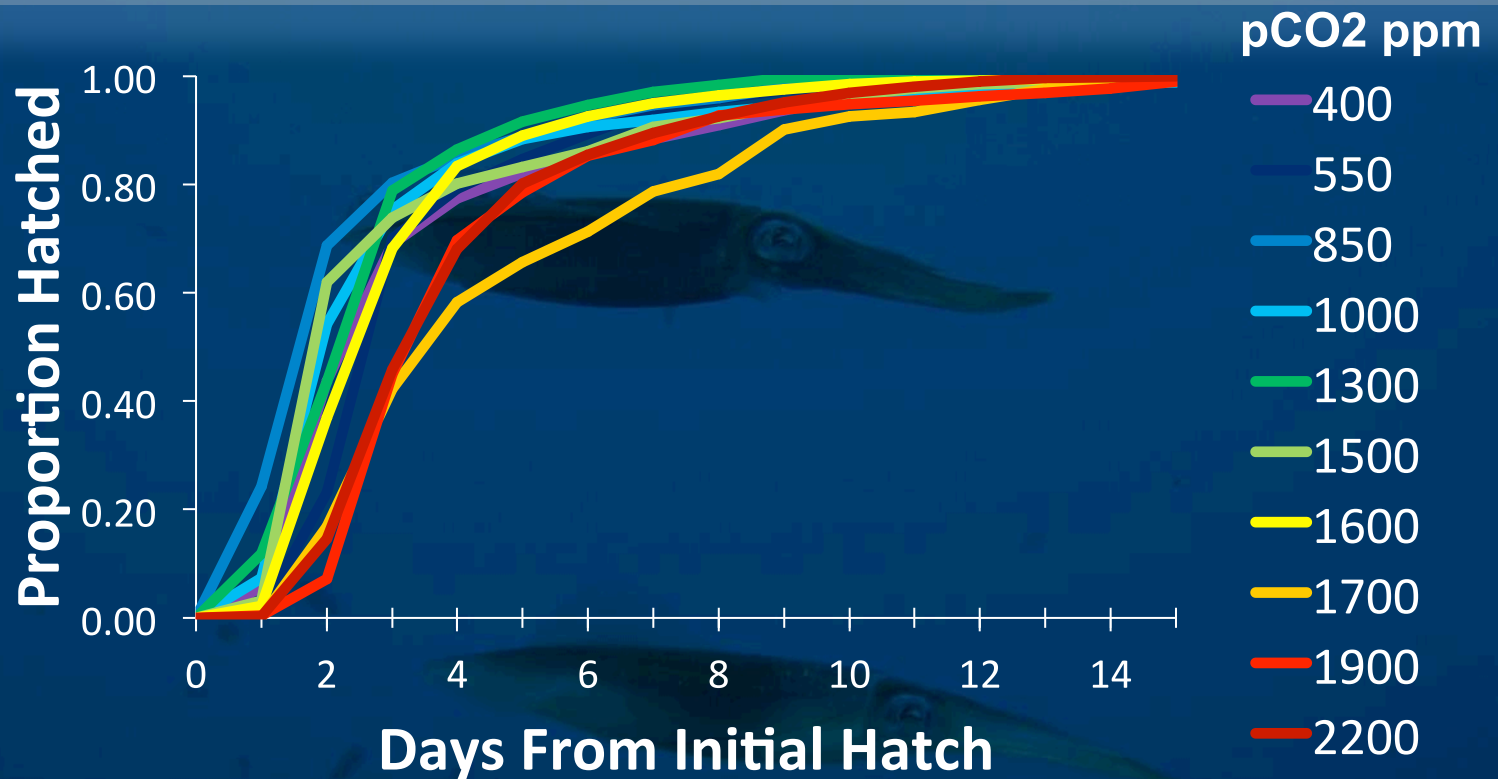




- Bottles and CTD during squid hauls
- Monthly (or more): 28 July 2014 – today
- Currently: 2 week intensive (diel) sampling

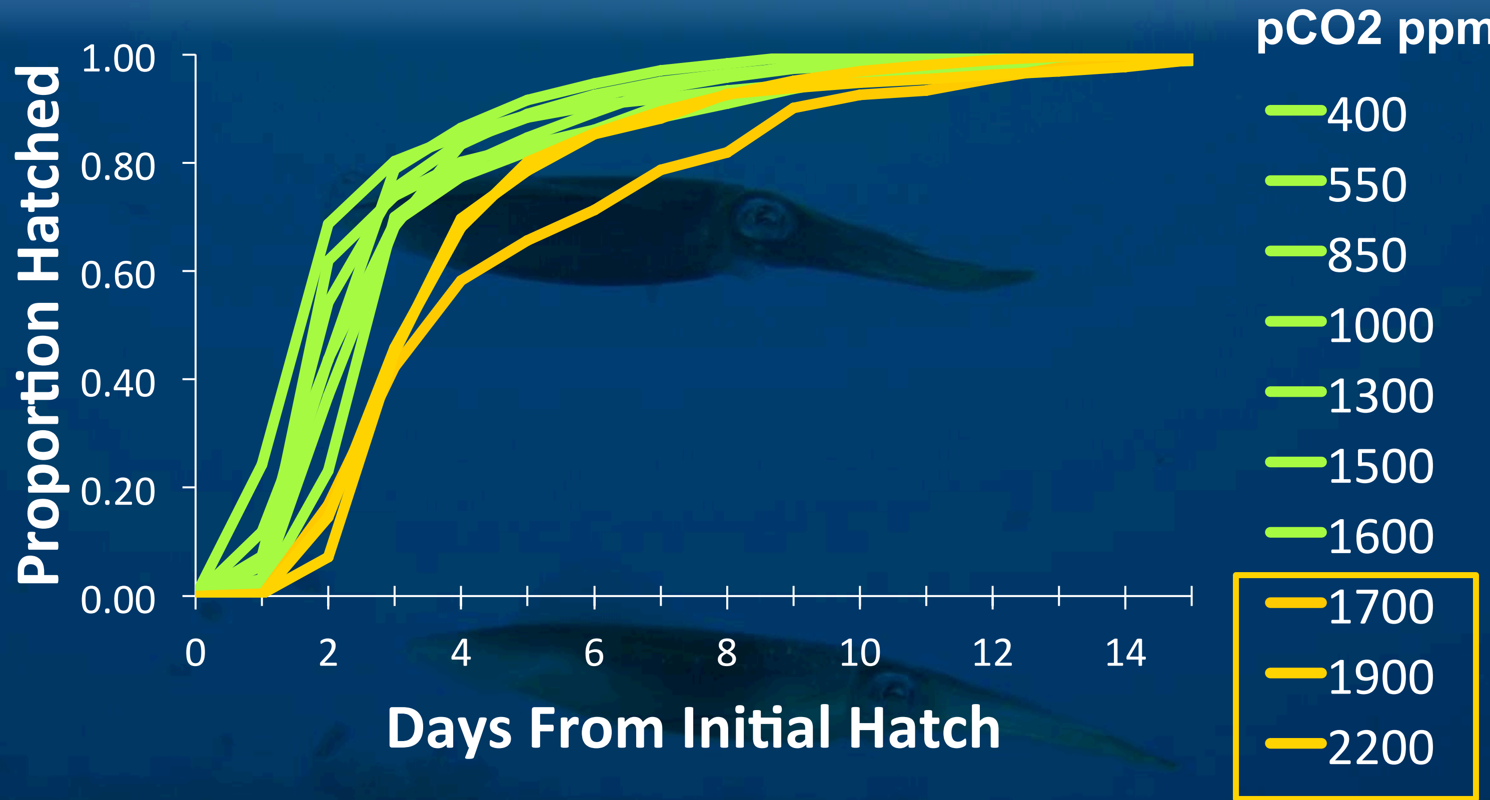
- pCO2, pH, and profiles of temp and salinity
  Total depth, 23-25 m
- <u>Water column</u> (1, 10, 20 m depth) generally consistent (but benthos not measured)

Hatching Delays



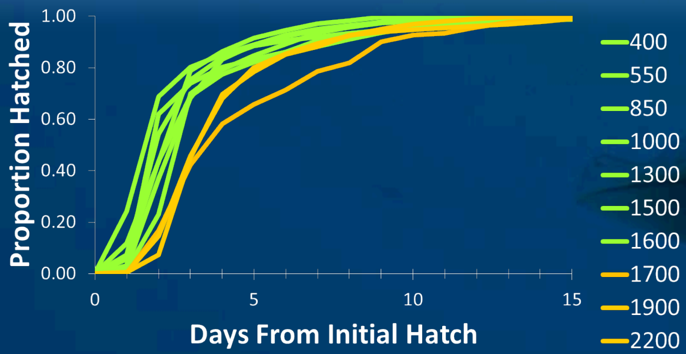
A delay in hatching time of about 1 day occurs in all CO_2 levels above 1600ppm.

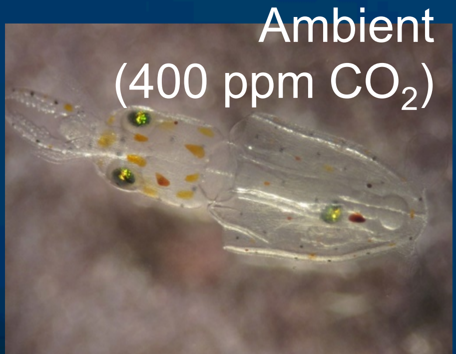
Hatching Delays



- A delay in hatching time of about 1 day occurs in all CO_2 levels above 1600ppm
- Development appears slowed

Survival

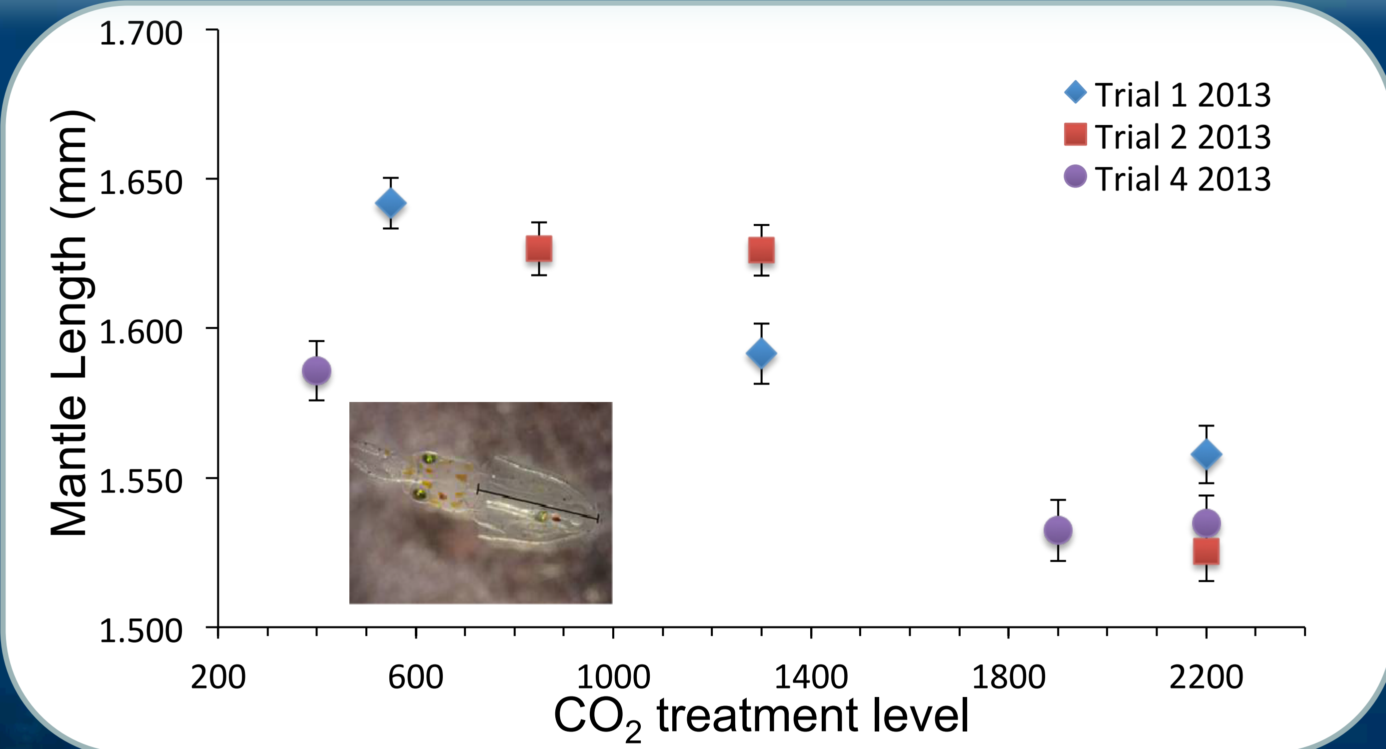




Treatment (ppm CO2)	Embryos Survived to Hatching (%)
400	96.2
550	87.4
850	93.6
1300	95.5
1500	98.4
1700	94.2
1900	93.4
2200	94.7

- Survival does not appear impacted

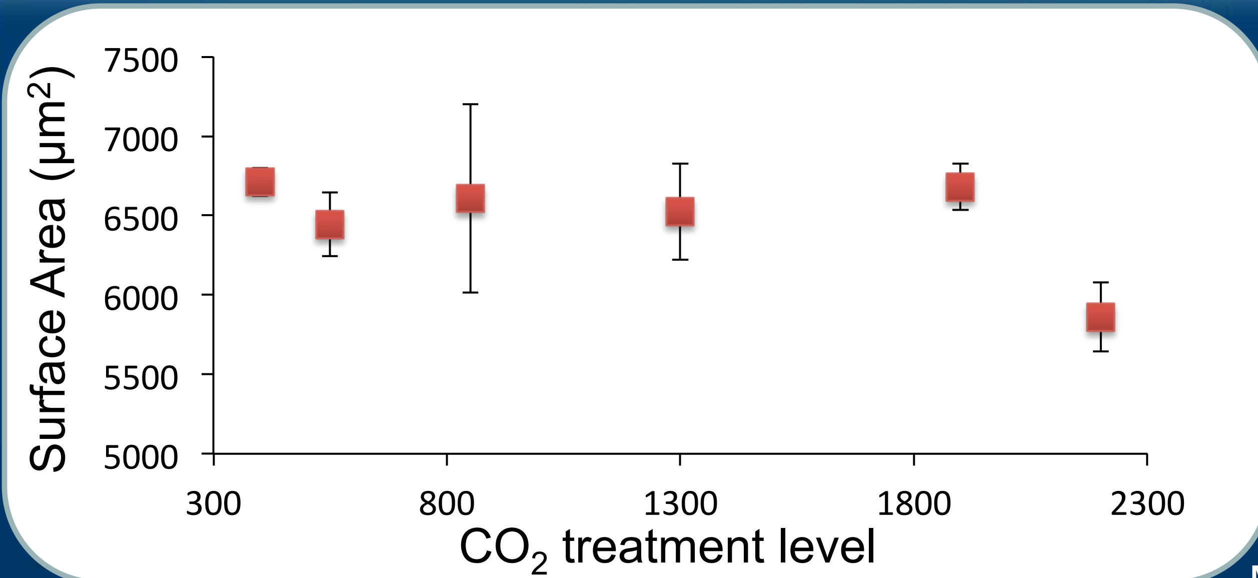
Mantle Length Changes



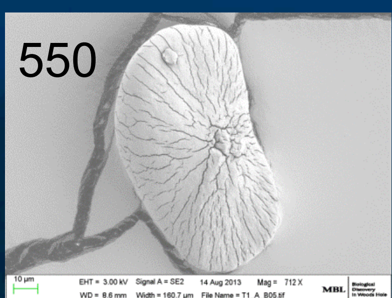
Mean ±SE
K-W $p<0.001$

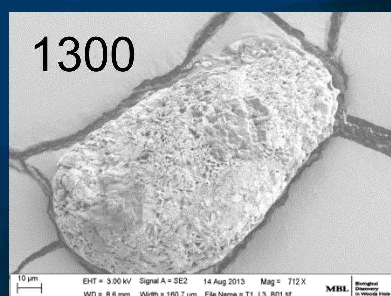
- A ~6% decrease in dorsal mantle length, a metric of somatic growth, from 400 to 2200
- Effect near 1600-1800…

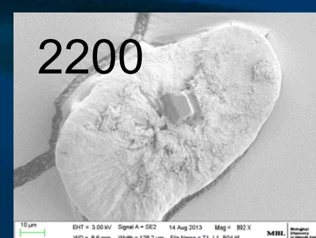
Statolith Surface Area (2013 data)



Mean ±SE
K-W p<0.0001



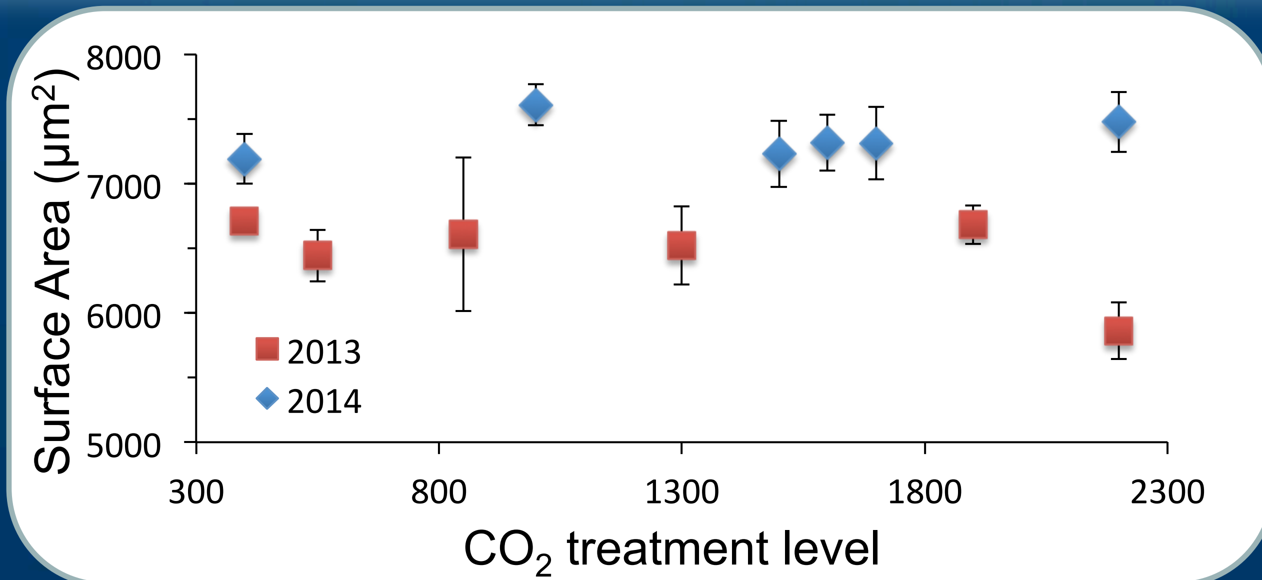


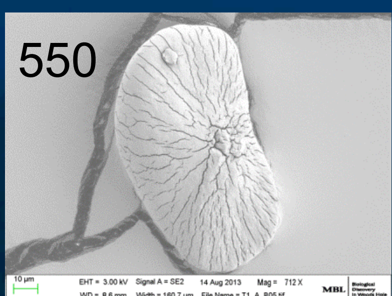


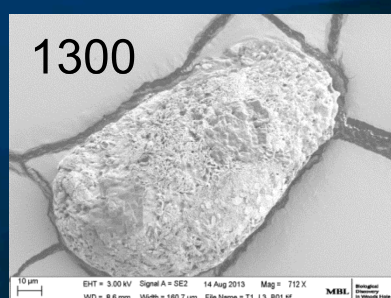
SEM of statoliths from one trial; (photos to scale)

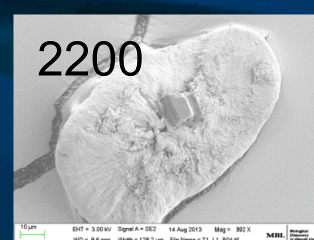
- Reduction in statolith size is seen in CO_2 exposures at highest levels

Statolith Surface Area (2013-2014)







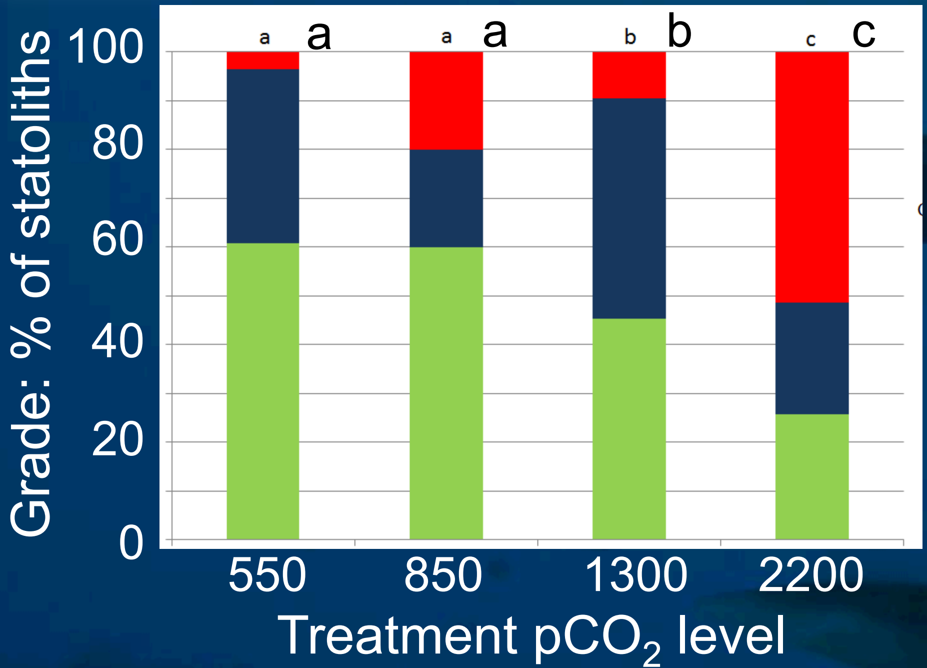


Mean ±SE
2013: K-W $p<0.0001$
2014: K-W $p>0.05$

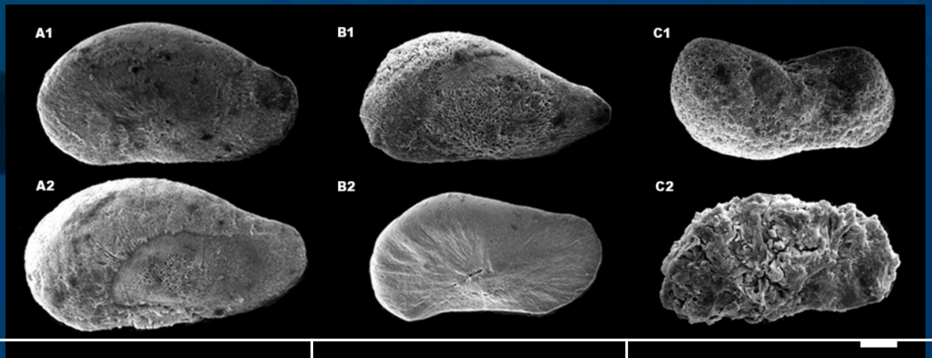
SEM of statoliths from one trial; (photos to scale).

- Trend does not hold with 2014 data
- (so only 2 of 3 years show differences)

Statolith Changes



Statolith porosity grades

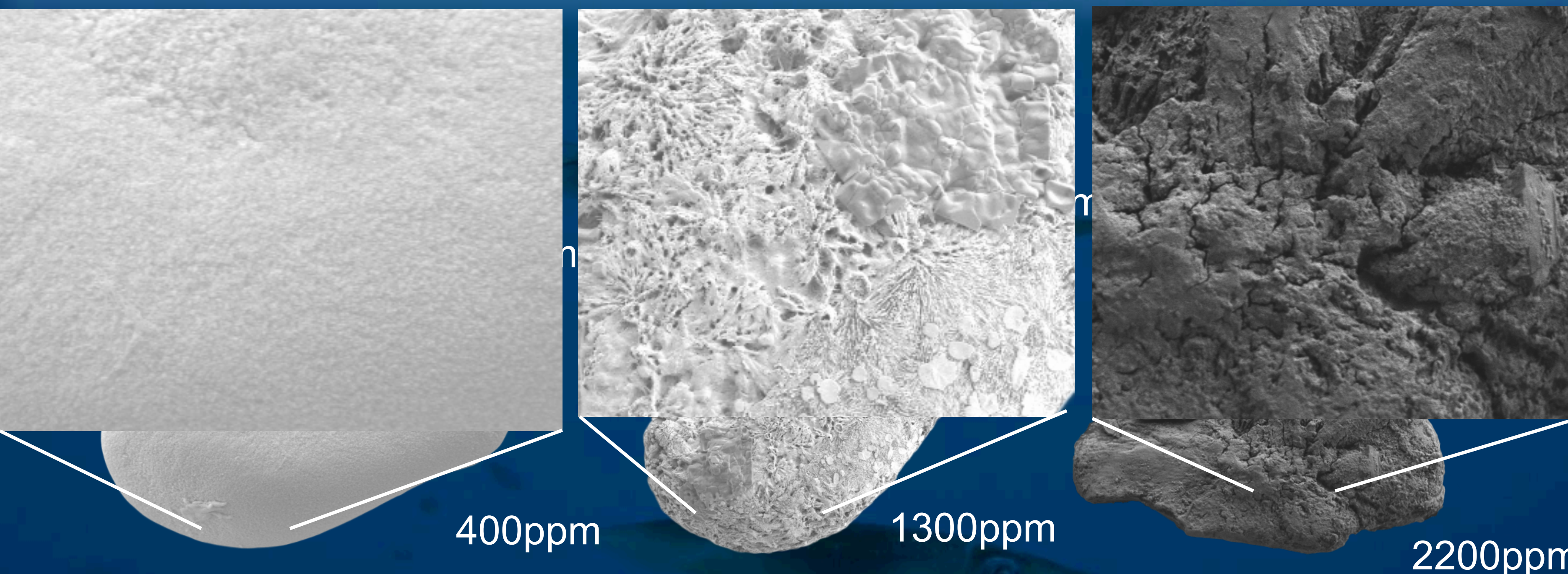


Grade 1: standard shape Standard-minimal porosity	2: standard shape with some abnormalities porous	3: abnormal shape, porous

- Statoliths were graded based on size, structure, and porosity
- Changes occurred linearly with increase pCO_2 levels

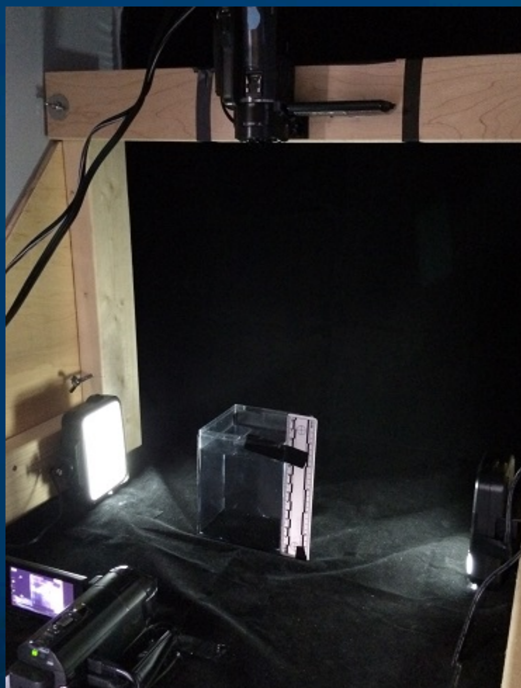
letters indicate statistically different groups

Statolith Changes



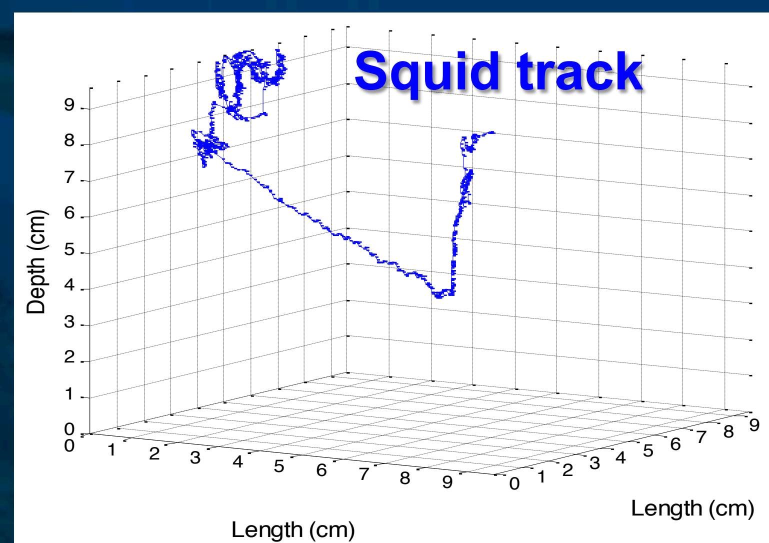
- Aragonite crystals and statolith structure seems in be substantially disorganized with increasing CO_2 levels

Swimming behavior

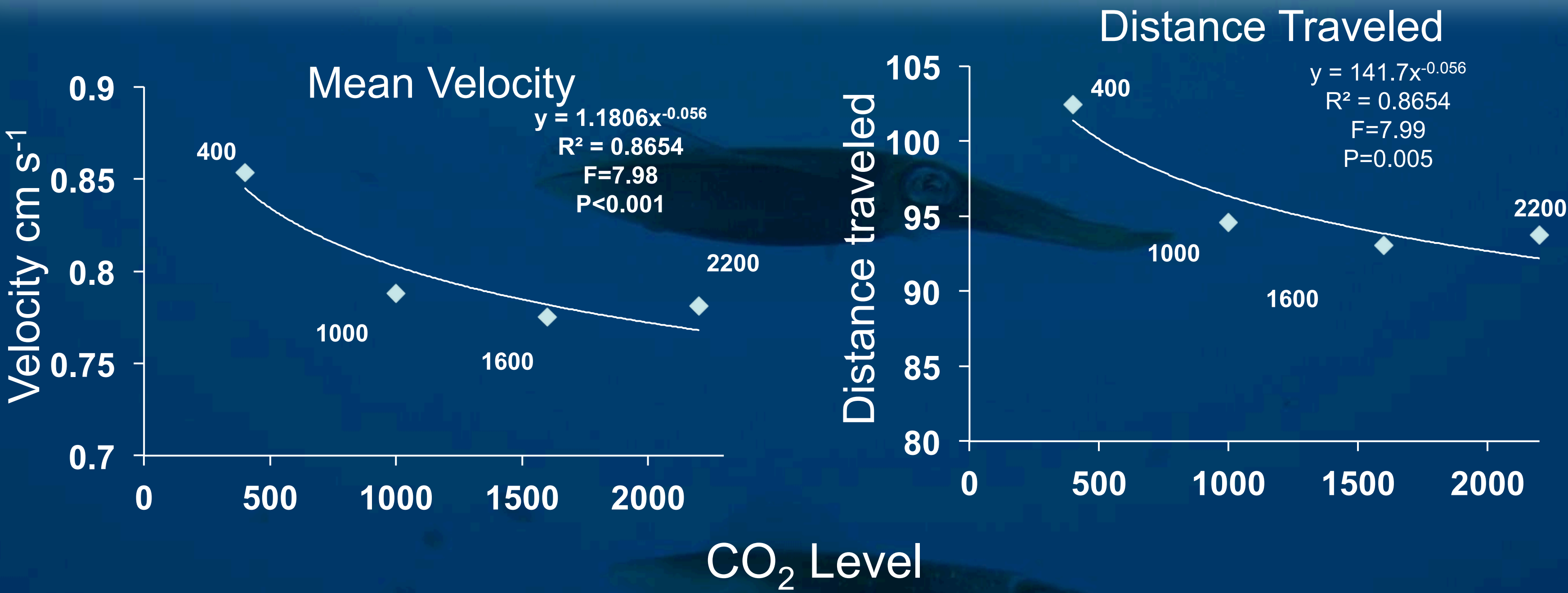


- Swimming behavior tracked with overhead and front HD video
- MatLab track reconstructed in 3D for 2 min (after 30 sec acclimation)

- Velocity, distance, ethology (swim vs. rest), turn rates, location in chamber



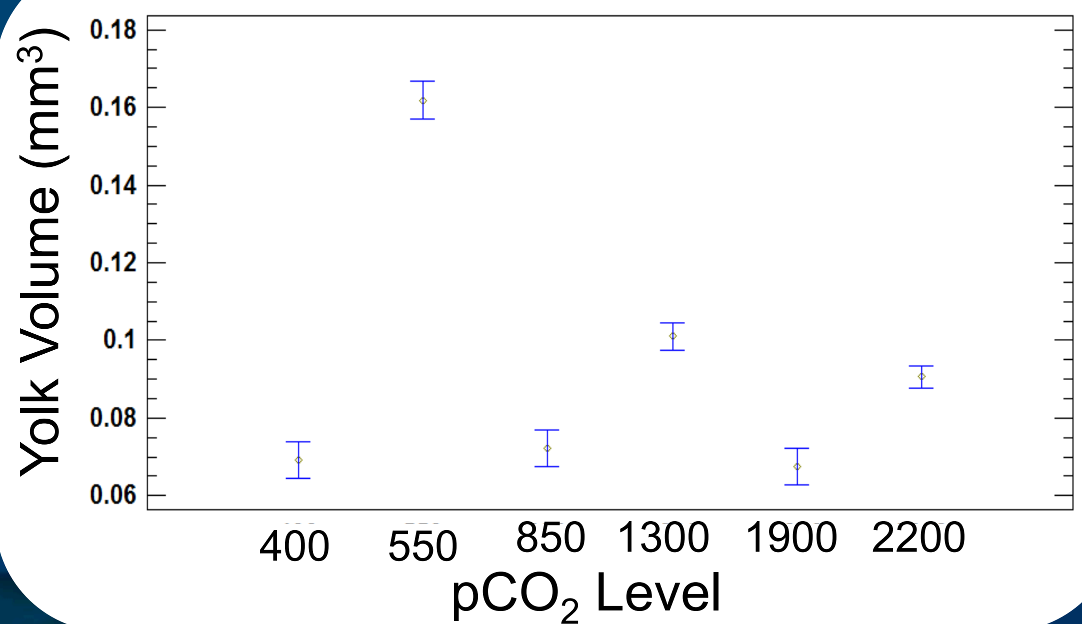
Swimming behavior



- Decrease in velocity and distance traveled
- But variance was high and medians were not significantly different

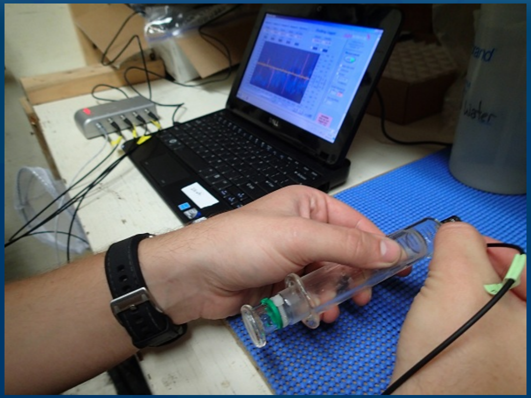
→ Sensory experiment (directed swim)

Metabolic Changes: Yolk sac consumption

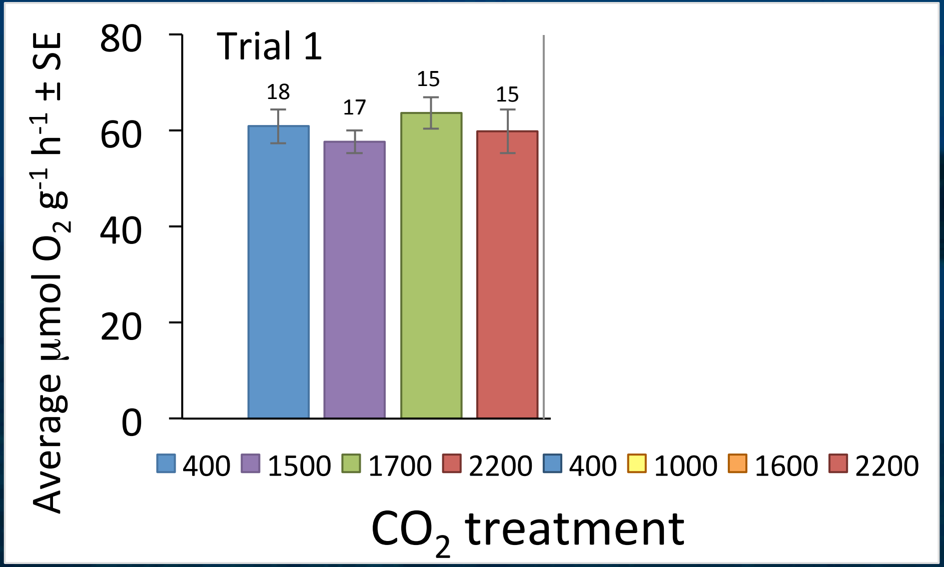


- No significant difference between yolk sac volume and CO_2 exposure
- Animals may not be consuming more energy

Metabolic Changes: Respriometry



- Hatchlings from each treatment → gas-tight glass respiration chambers with seawater of that treatment level
- Oxygen concentration measured using a FireSting fiberoptic oxygen meter with optical sensor (3-5 hr record, >70% O_2)

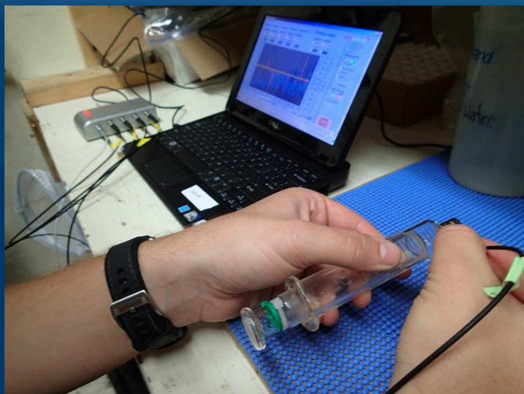


With former WHOI PD: Amy Maas

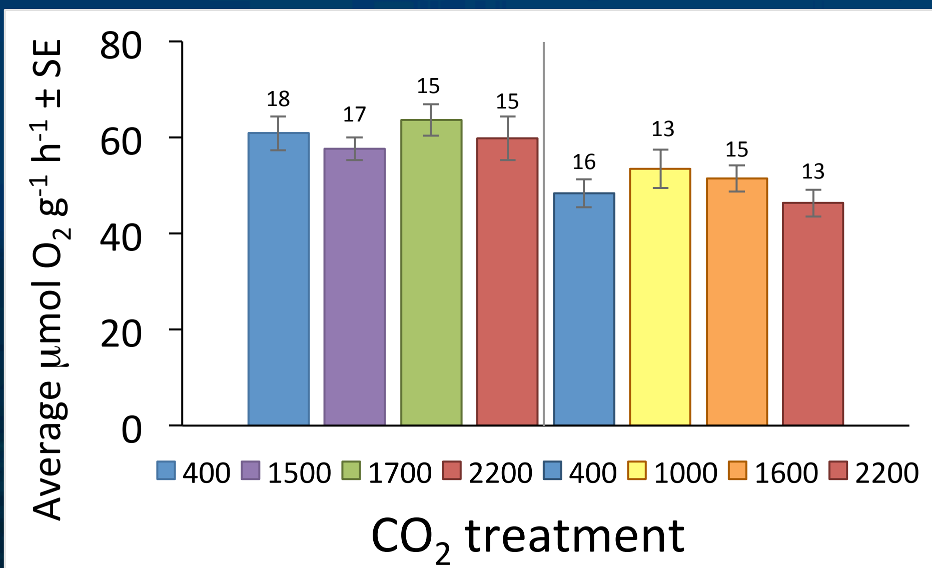
- No diff. in oxygen consump vs. CO_2 treatment
- Diff based on Trail

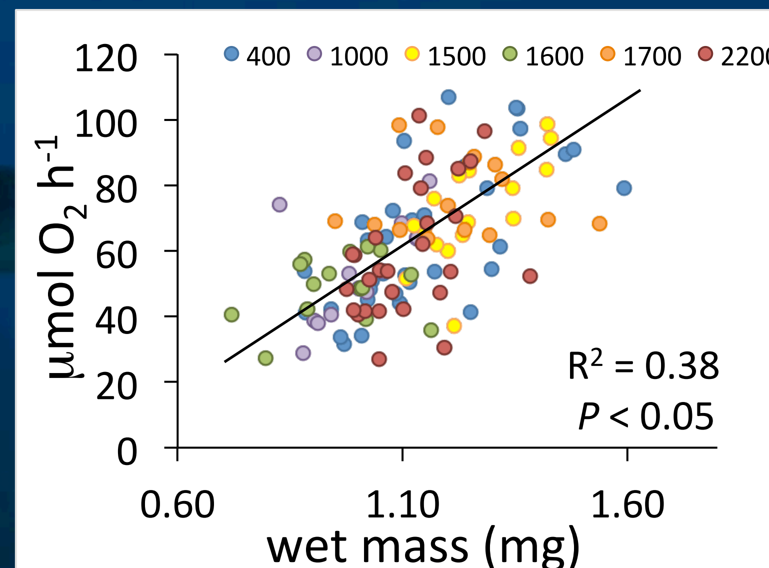
(No effect: $F_{5,114} = 0.737$, $p = 0.597$)
(Effect: $F_{1,113} = 12.185$, $p = 0.001$)

Metabolic Changes?



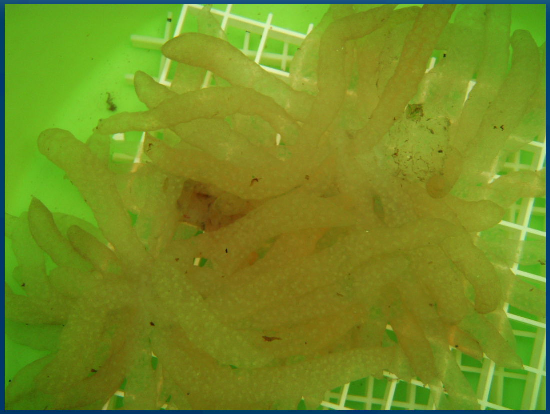
- Effect of mass of the individuals, not CO_2

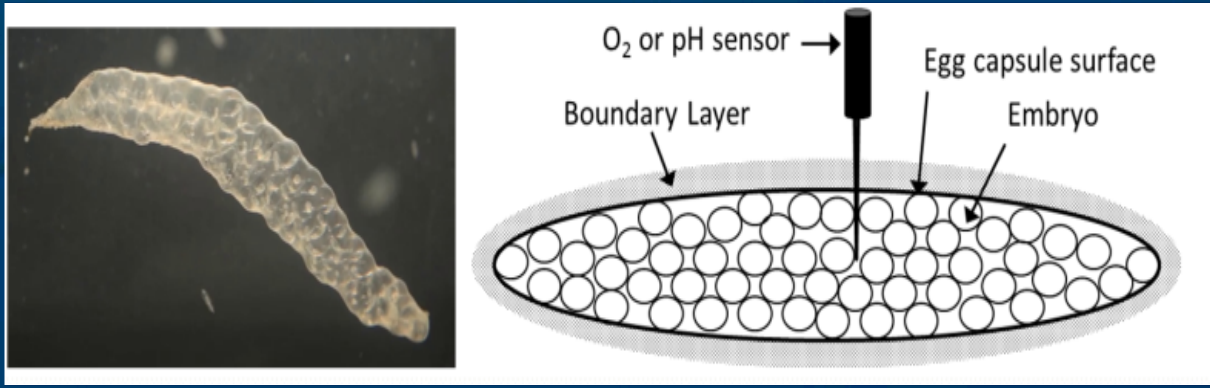




$(F_{1,114} = 38.492, p < 0.001)$

Profiling egg capsule and boundary layer





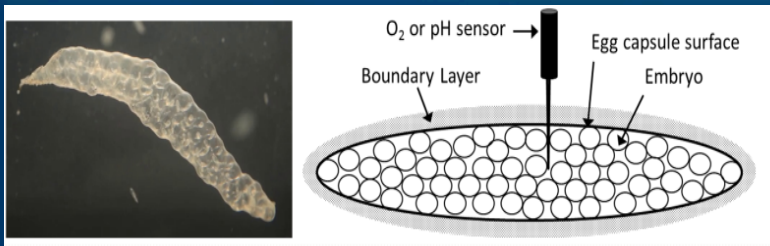
- Oxygen and pH profiles of ambient capsules measured using a FireStingO_2 optical O_2 sensor and liquid ion exchange (LIX) pH sensors

(Gieseke & de Beer 2004)

- Profiled through the water, to a 'boundary layer' at the capsule surface into the capsule center
- Ha: Respiration and capsule membrane will influence local O_2 and pH

WHOI Post Doc Scholar Matt Long

Profiling egg capsule and boundary layer

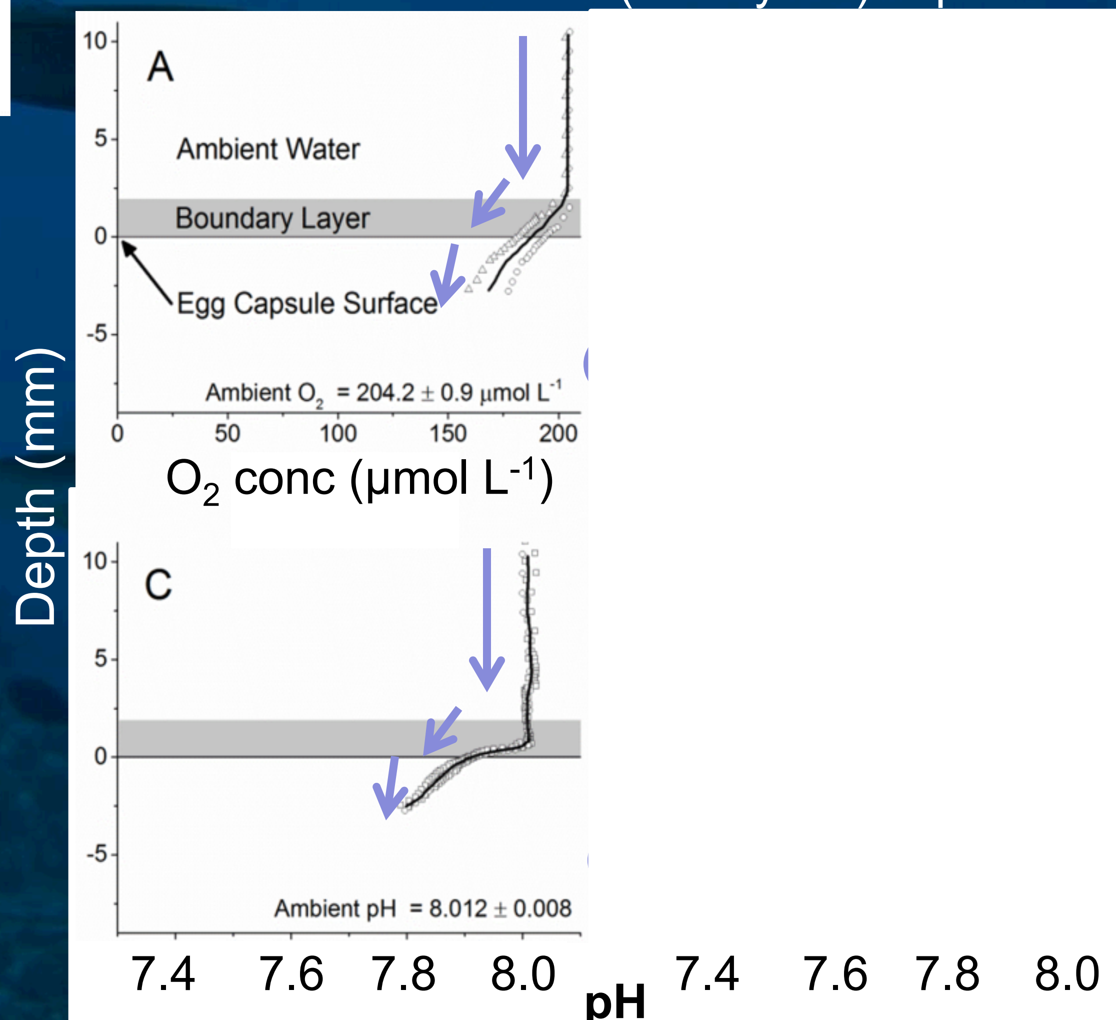


- Near term capsules reached 7.34 pH;
- Capsule O_2 approach zero (1.9) μmol L^{-1}
- Below conc. of Atlantic OMZs
- Below the 50% (7.6) O_2 blood affinity in adult squid…

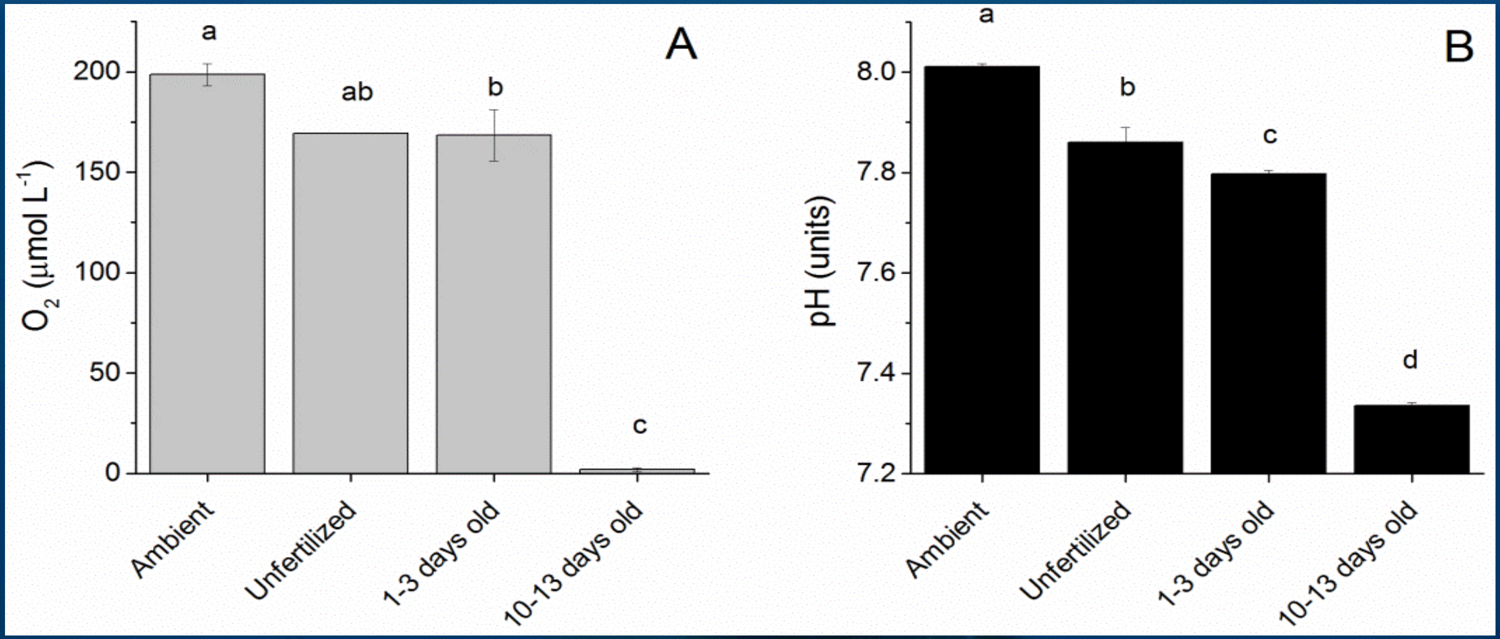
(Long et al submitted)

Newly laid (1-3 day old) capsules

Near-full-term (almost hatched) (13 day old) capsules



Profiling egg capsule and boundary layer



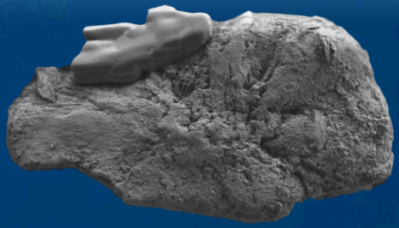
[O_2 (p <0.0001) and pH (p <0.0001); letters indicate differences between groups (using Tukey post-tests)]

- O_2 concentrations (A) and pH (B) dropped significantly from ambient water by capsule age
- Near limit of stress (in capsule)? Increased resilience once hatched?
- Hatching cue?

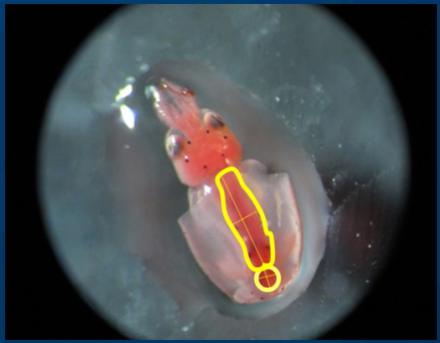
(Long et al submitted)

Summary

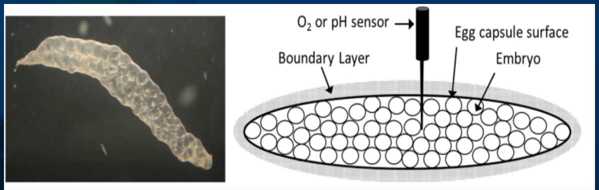
- Effects seen but variation by year (and cohort?)



- Development differences not seen in energy expenditures



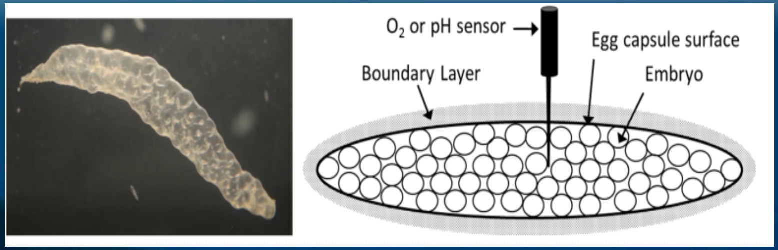
- Capsules ‘naturally’ face low pH and low O_2



- Suggests some resiliency to CO_2 conditions (and much to understand)

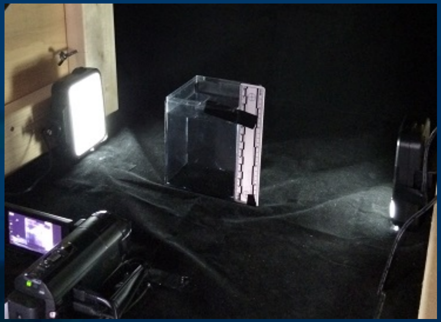
Upcoming Work

- Egg capsules in an acidified environment and within flow



- Cohort differences (across the summer) and parental contribution

- Statolith composition changes and sensory behavior

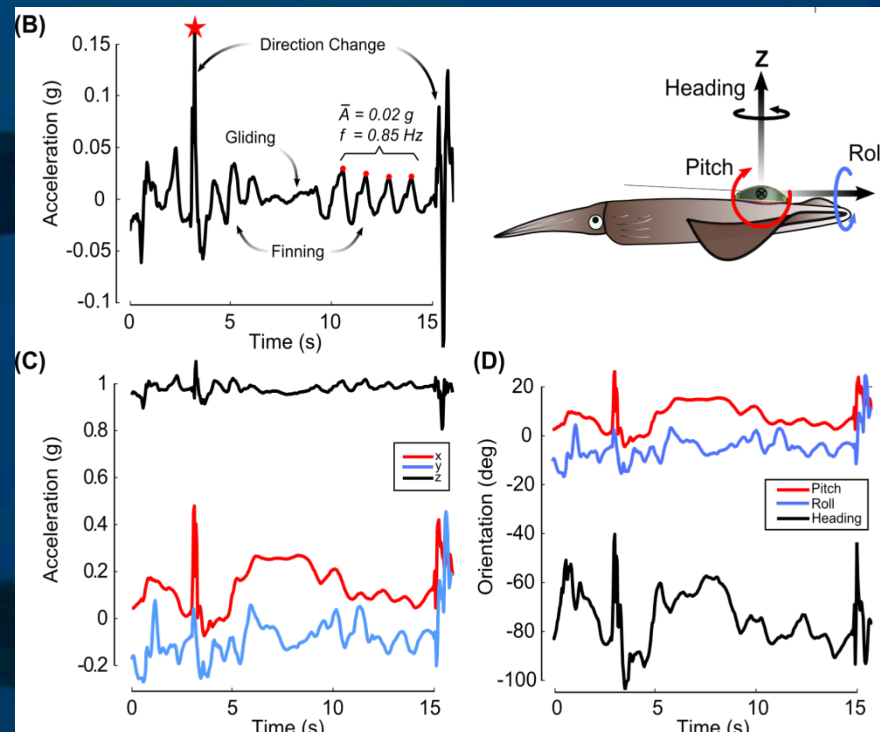


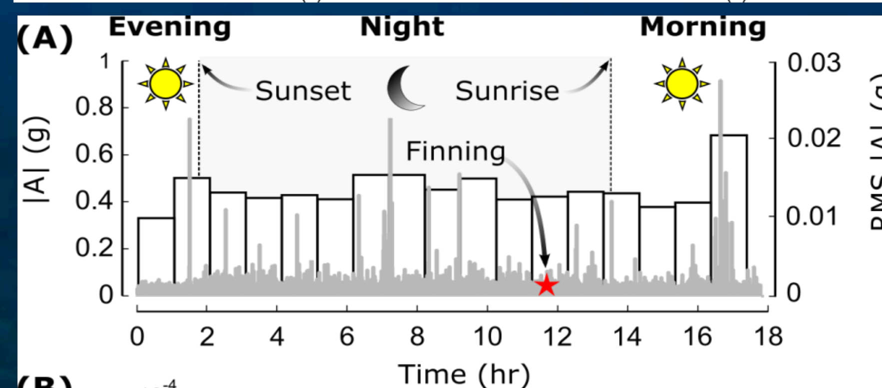
- Natural environment at eggs

Upcoming Work

- ITAG: High-sample rate adult vital rates and activity patterns + local environment

- With Aleck Wang – Adding mini-O_2 and salinity sensors





(Mooney et al *in press* Animal Biotelemetry)

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