

FIELD AND LABORATORY STUDIES OF PTEROPOD ECOLOGY AND PHYSIOLOGY IN RELATION TO NATURAL VARIABILITY IN CARBONATE CHEMISTRY

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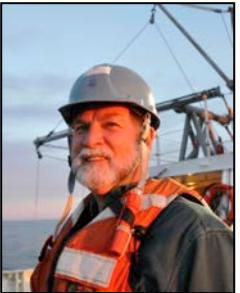
The Pteam

2

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Peter Wiebe



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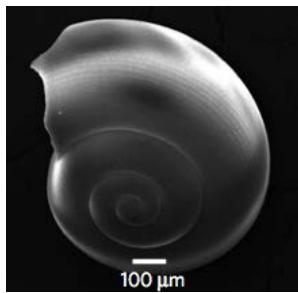


Camille Pagniello
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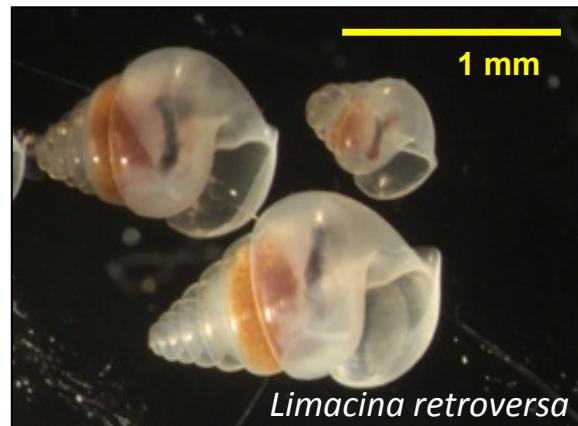
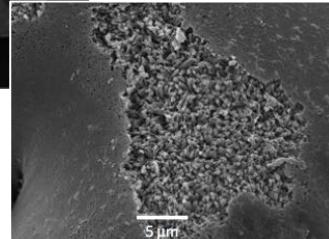
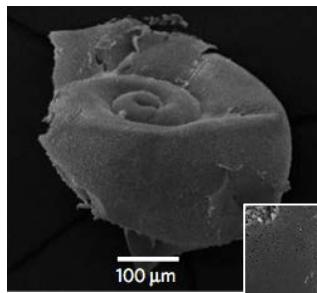
Thecosomatous Pteropods

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- Aragonitic shell-forming planktonic gastropods
- Important to biogeochemistry and as prey item for many commercial fish (e.g., salmon)
- Lab and field studies have shown shell dissolution in response to under-saturation ($\Omega_A < 1$)



Limacina helicina



Limacina retroversa

Overall Approach

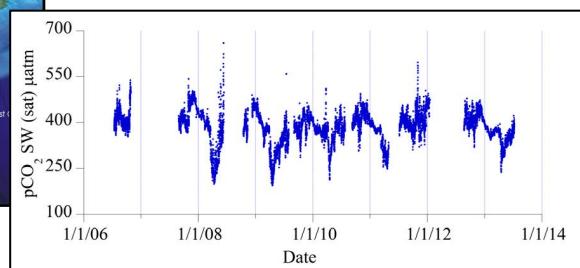
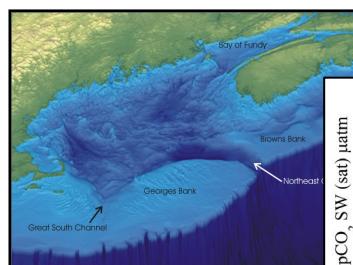
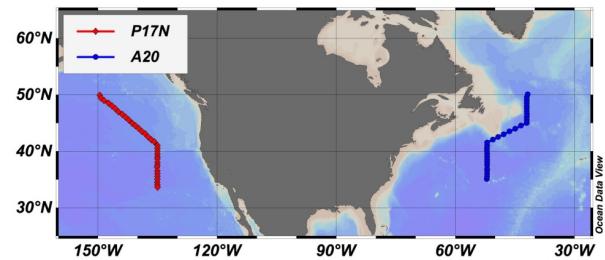
- Combine field studies with shipboard and lab experiments
- Capitalize on natural variability in carbonate chemistry (spatial and seasonal) as natural experiments
- Goals are to gain improved understanding of thecosomatous pteropod biology in order to understand potential response to OA



SHIPS!

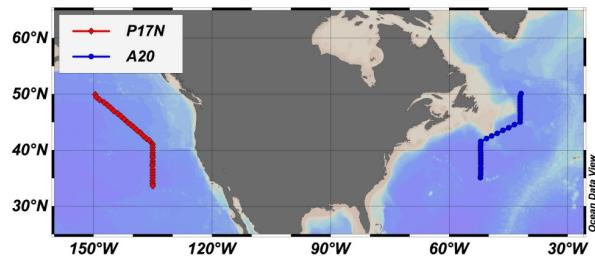


LAB!

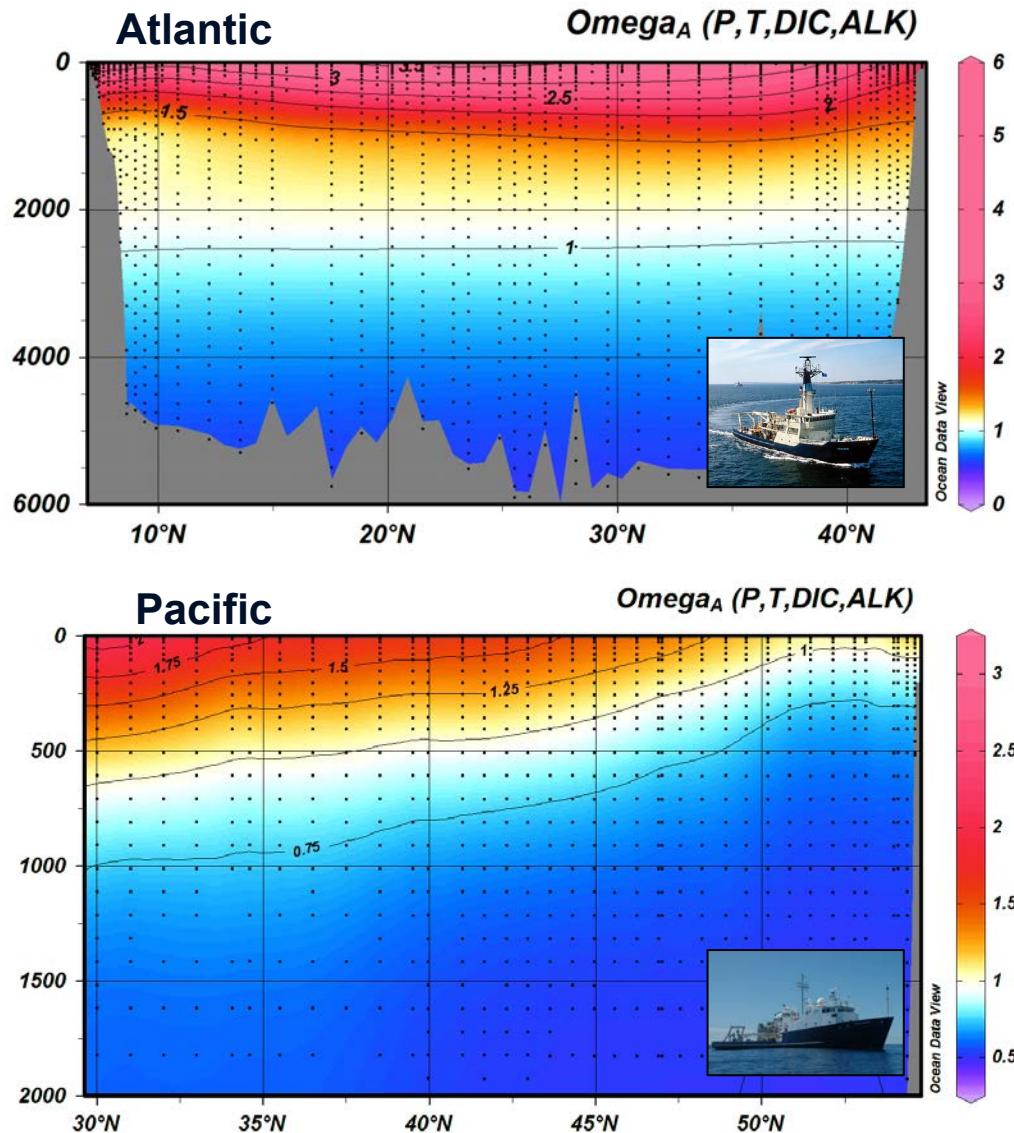


Ocean Acidification Pteropod Study (OAPS)

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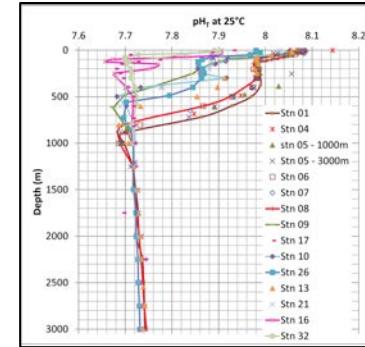
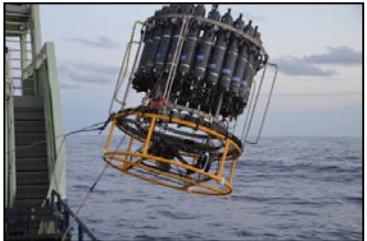
- Differences exist between and within the modern-day Atlantic and Pacific Oceans in the Aragonite Compensation Depth (ACD)
- Goal is to examine:
 - Carbonate chemistry
 - Natural distribution, behavior (including DVM), species diversity, shell condition
 - Physiology (metabolism and gene expression)
- Open-ocean cruises in August of 2011 (Atlantic) and 2012 (Pacific)



Sampling Methods

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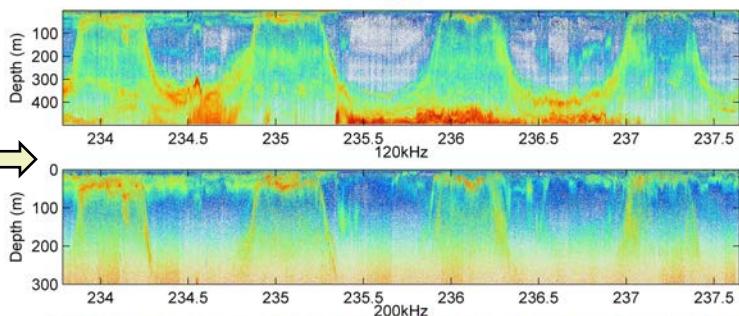
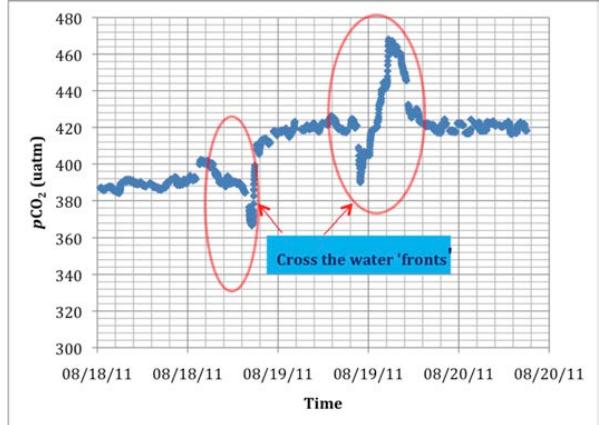
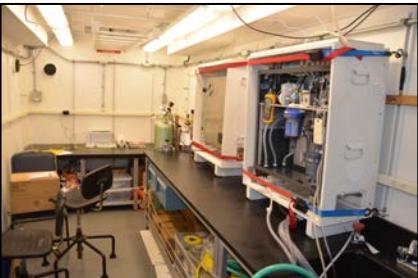
- CTD to characterize physical environment (to 1000-3000m)
- Niskin bottle sampling to measure pH, DIC, alkalinity, nutrients, salinity
- Depth-stratified net system and Video Plankton Recorder to sample directly the zooplankton (to 1000m)
- On-board CO₂ exposure experiments of animals sampled by Reeve net



Underway Sampling

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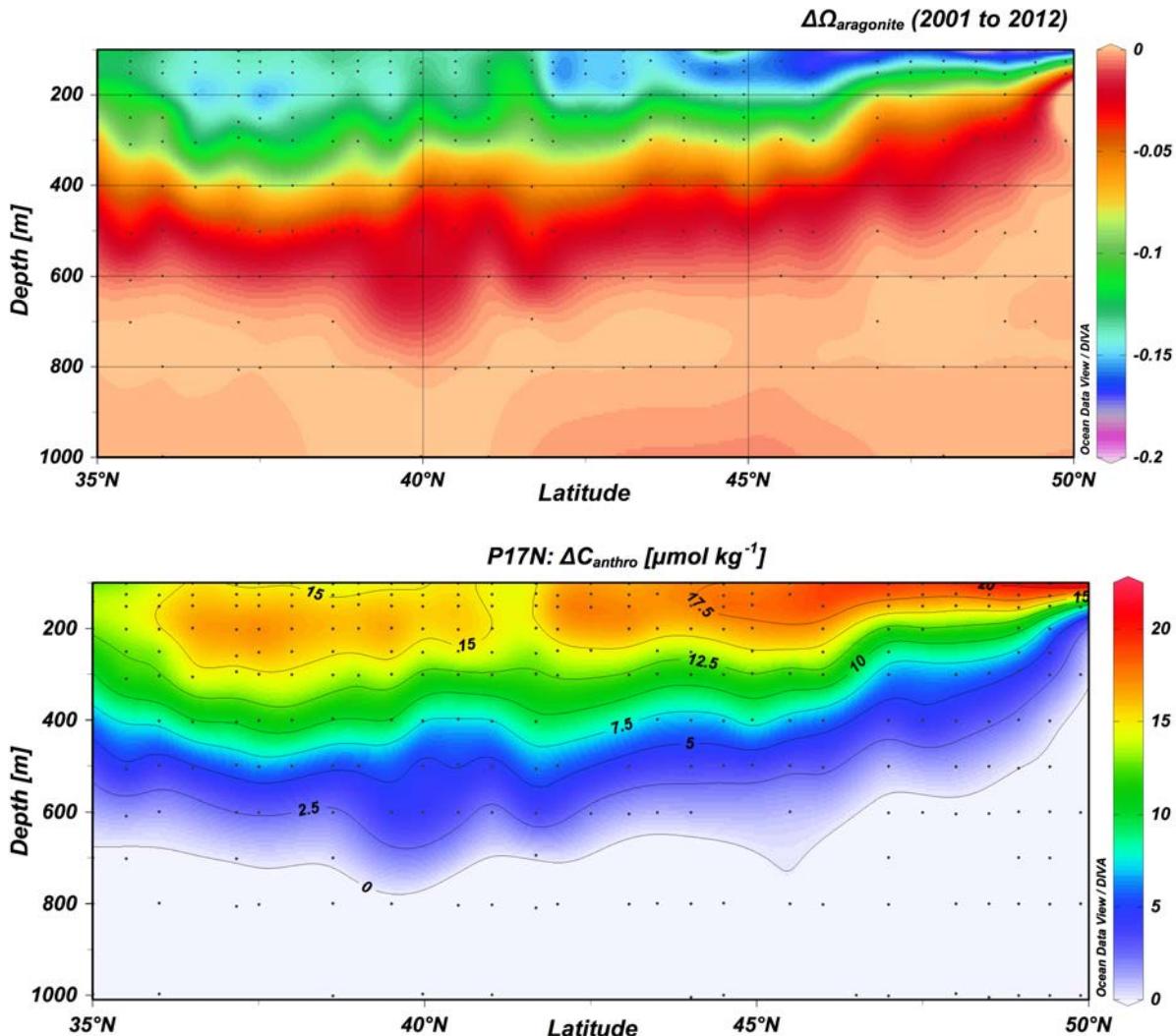
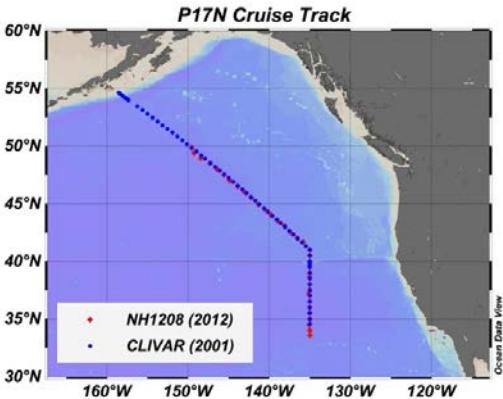
- Measurements of surface sea water $f\text{CO}_2$, DIC, pH, and air $p\text{CO}_2$
- Multi-frequency and broadband echosounders to map the zooplankton and fish scattering
- Visual surveys for surface-associated predators (seabirds, marine mammals, large fish)



Pacific Carbonate Chemistry

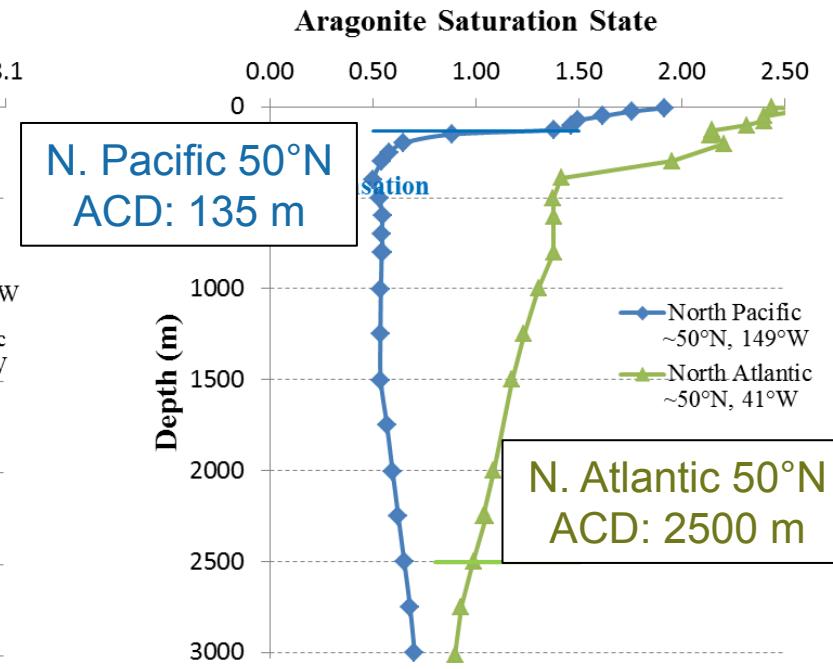
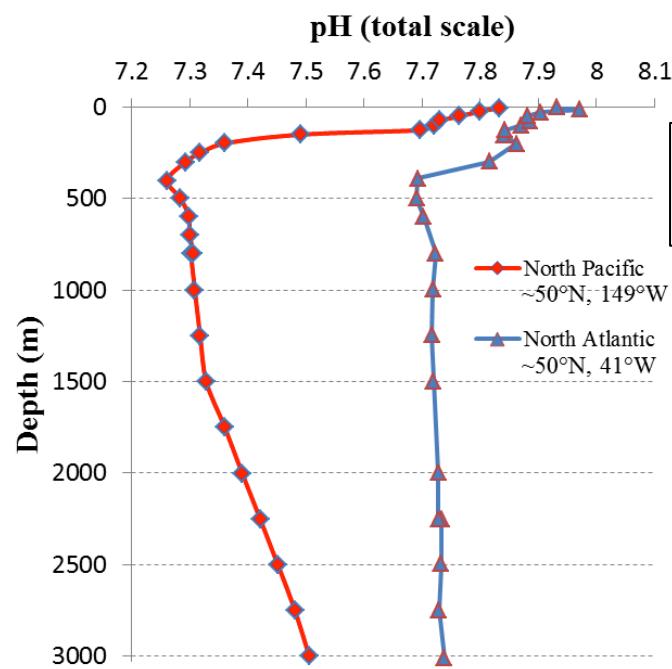
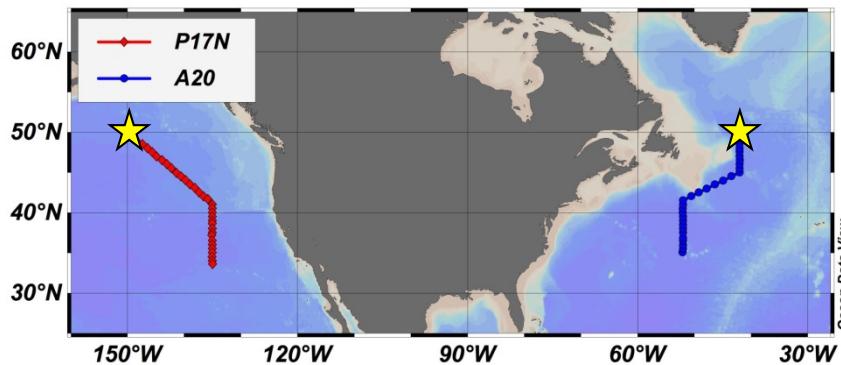
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- Decrease in pH since 2001, the last time CLIVAR/WOCE line P17N was occupied
- Shoaling of aragonite saturation state contours



Natural Experiment

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Diversity and Abundance

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species

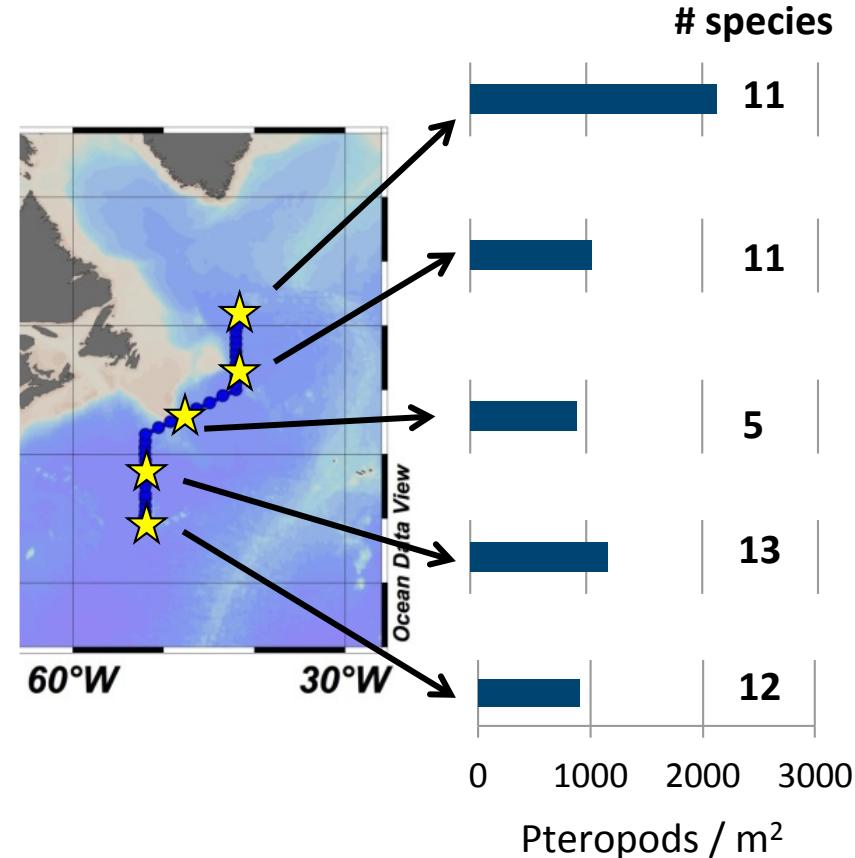
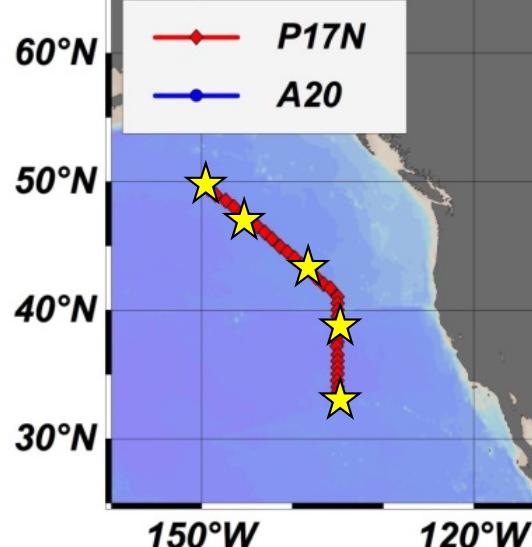
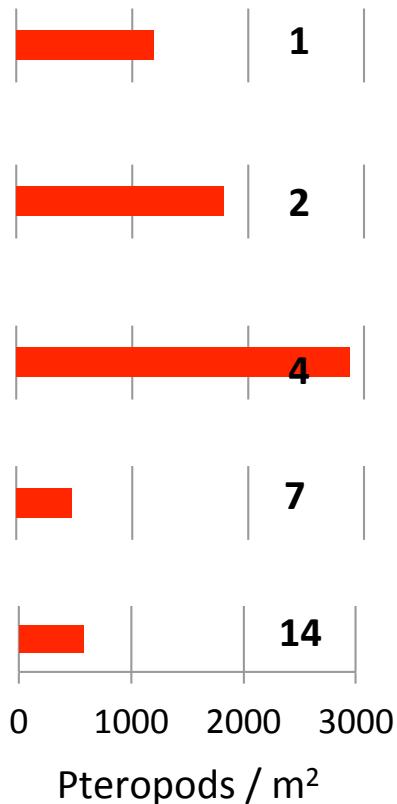
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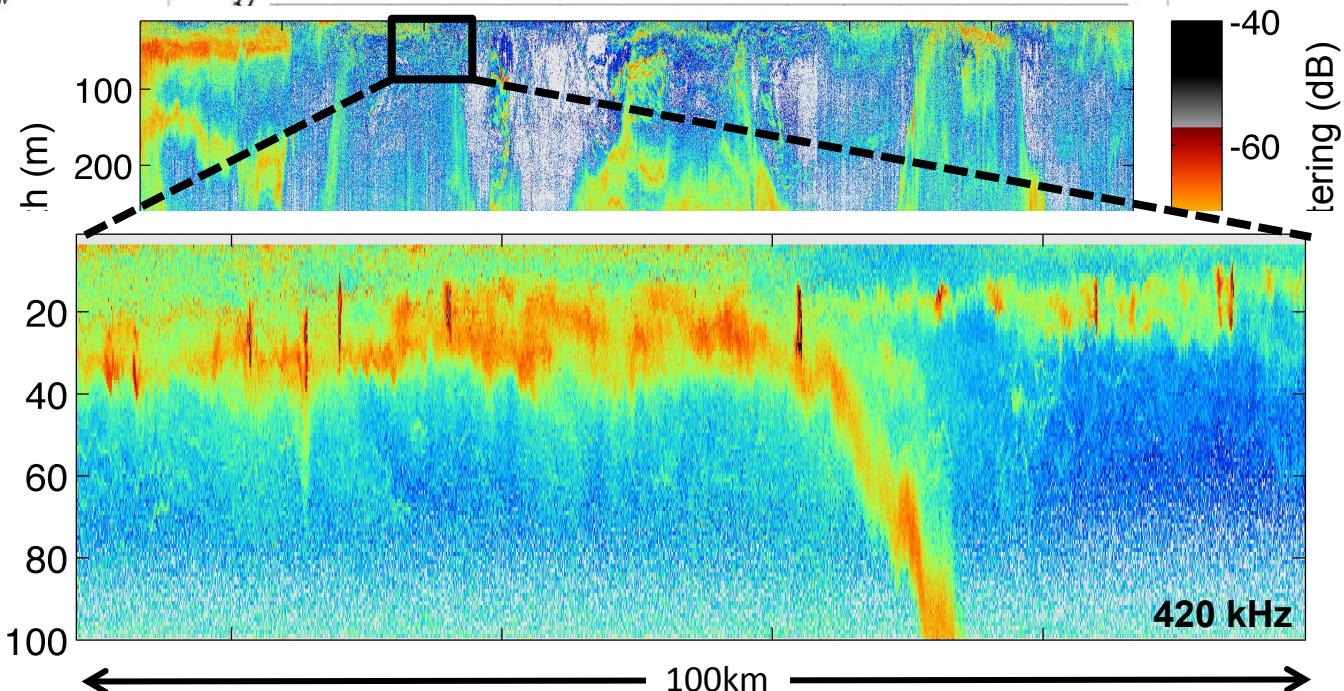
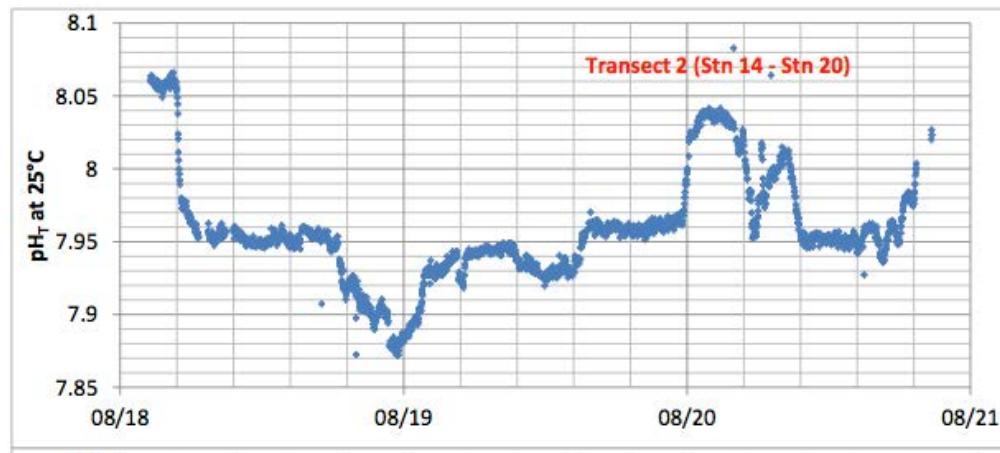
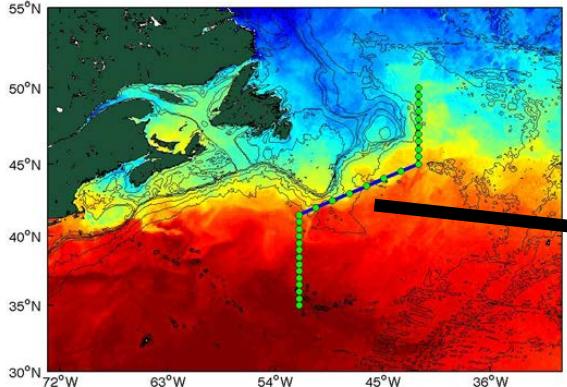
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Acoustic Observations of Pteropod Swarms

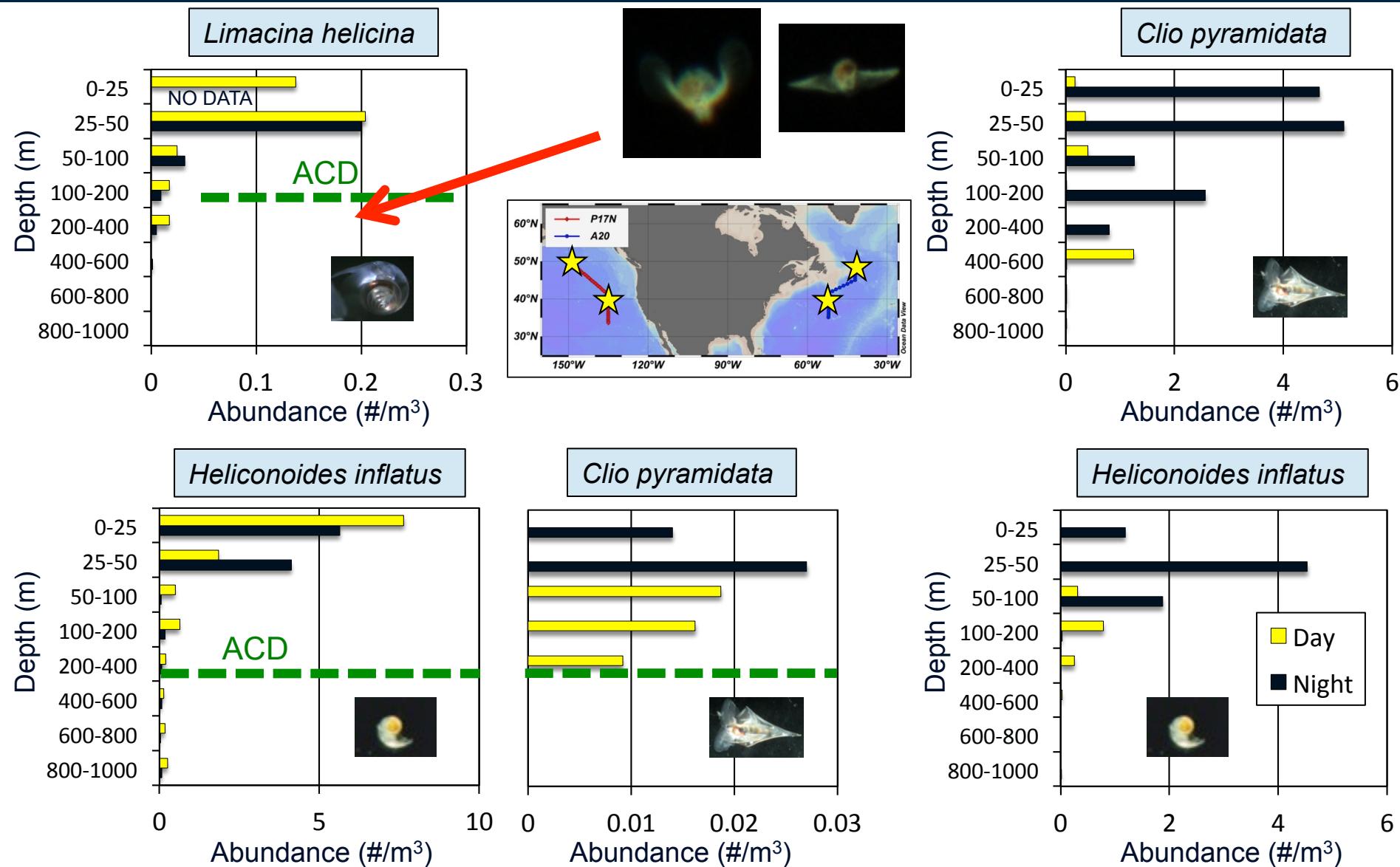
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- Tongue of low salinity waters off the Grand Banks
- Dense swarms of *Limacina retroversa* on average 630 m in extent, 7.9km apart, mean densities of ca. 3000 inds.m⁻³

Vertical Distribution

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Shell Condition

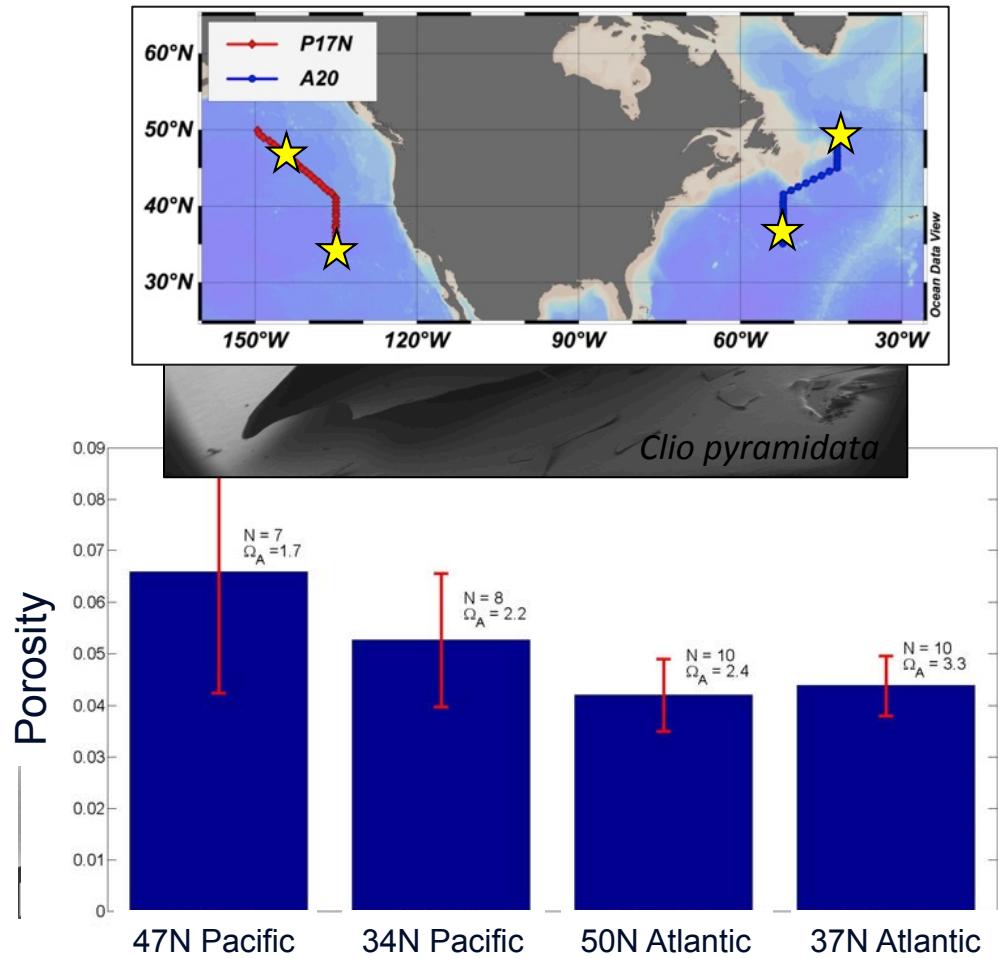
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Alexander Bergan
PhD Student

Scanning Electron Microscopy

- Used to measure “porosity” and thickness of leading edge
- Differences in the porosity of the adult shell of *Clio pyramidata* between regions but not significant

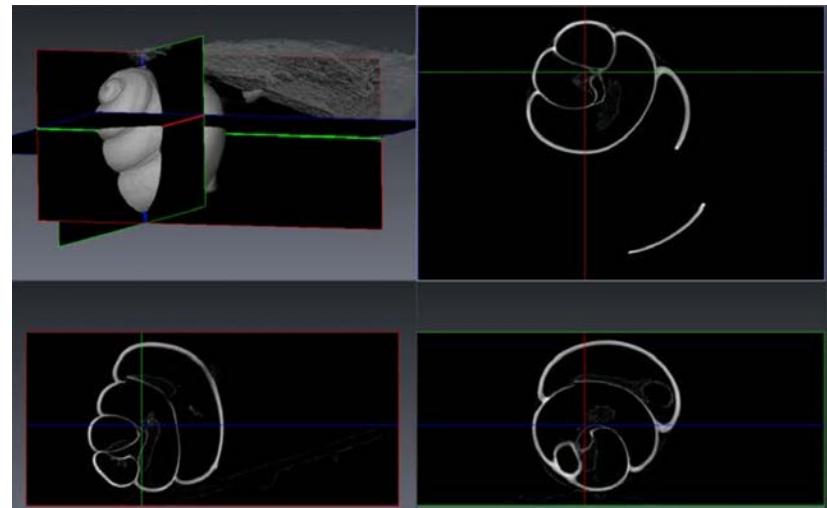
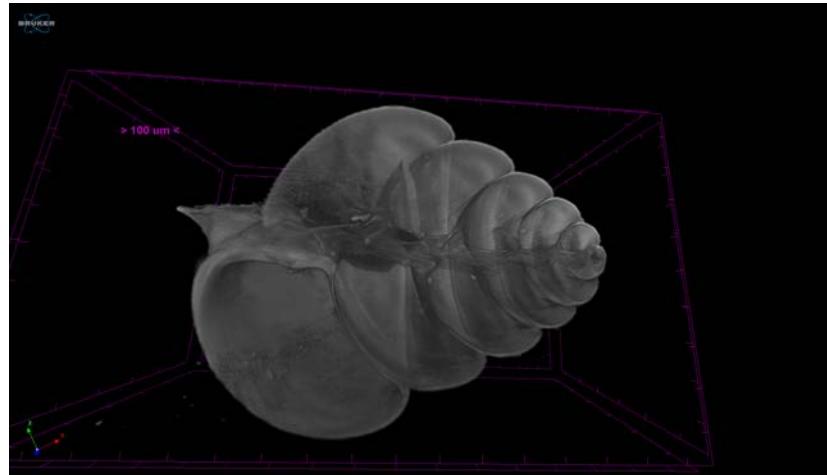


Shell Condition

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Micro CT

- X-ray micro computed tomography shows inner structure at fine resolution ($<1\mu\text{m}$)
- Developing methods for quantifying shell thickness and density



Physiology

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Dr. Amy Maas

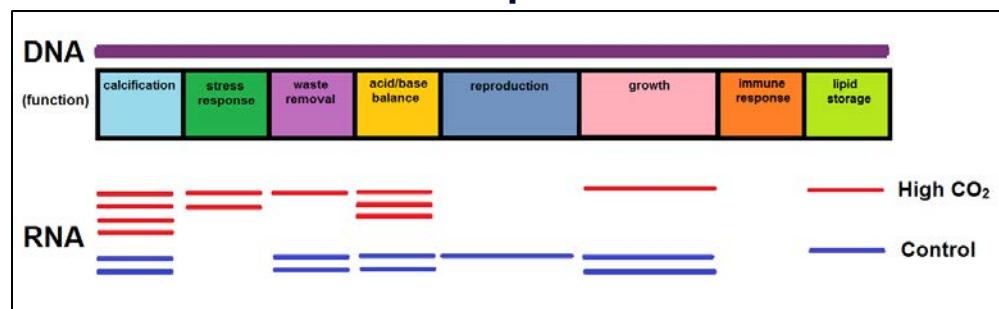
Short-term (3-18 hr) experimental exposure:
High CO₂ (400 vs. 800 PPM)
Low O₂ (21 vs. 10%)



Oxygen Consumption

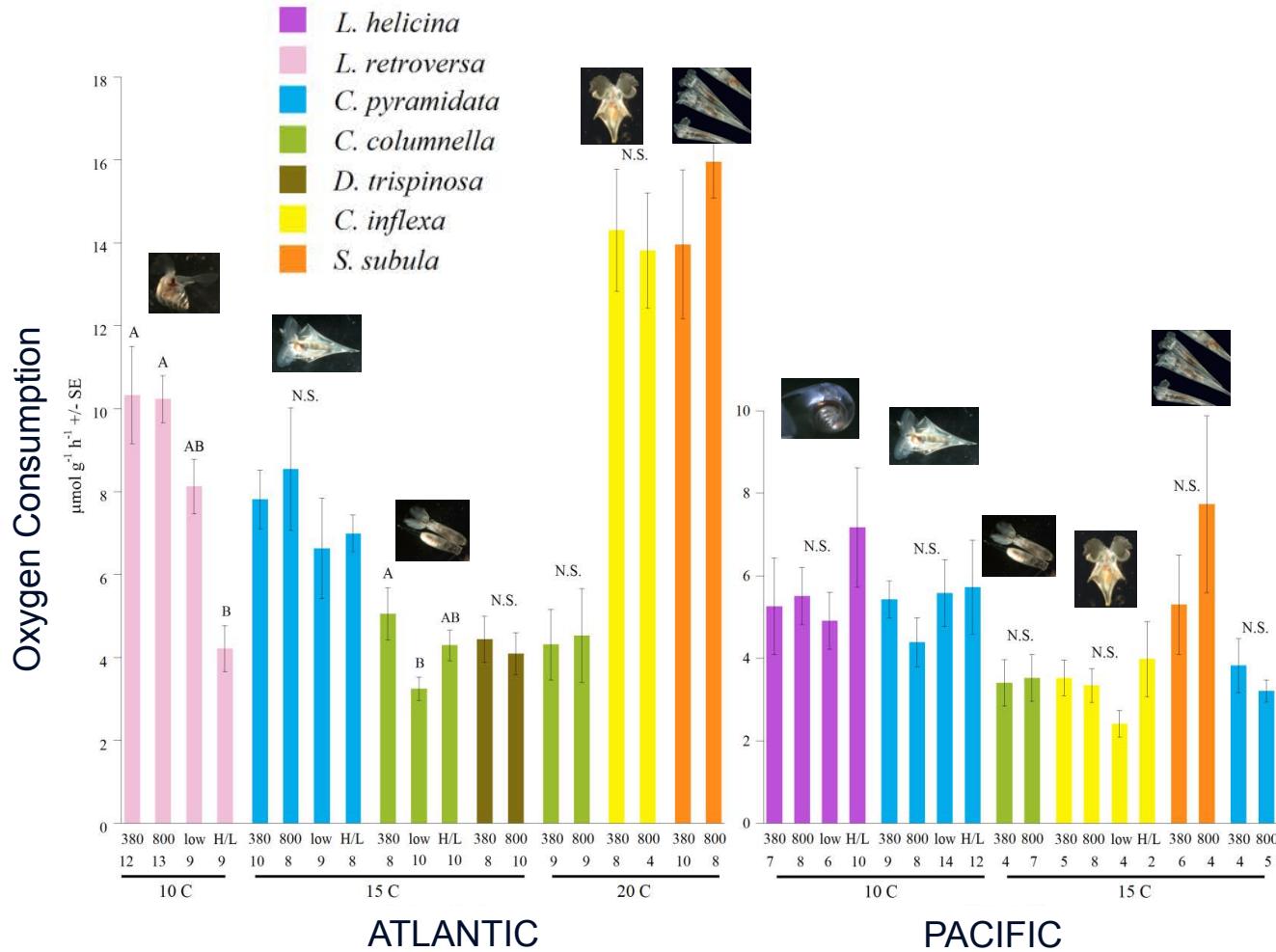


Gene Expression



Physiology – Metabolism

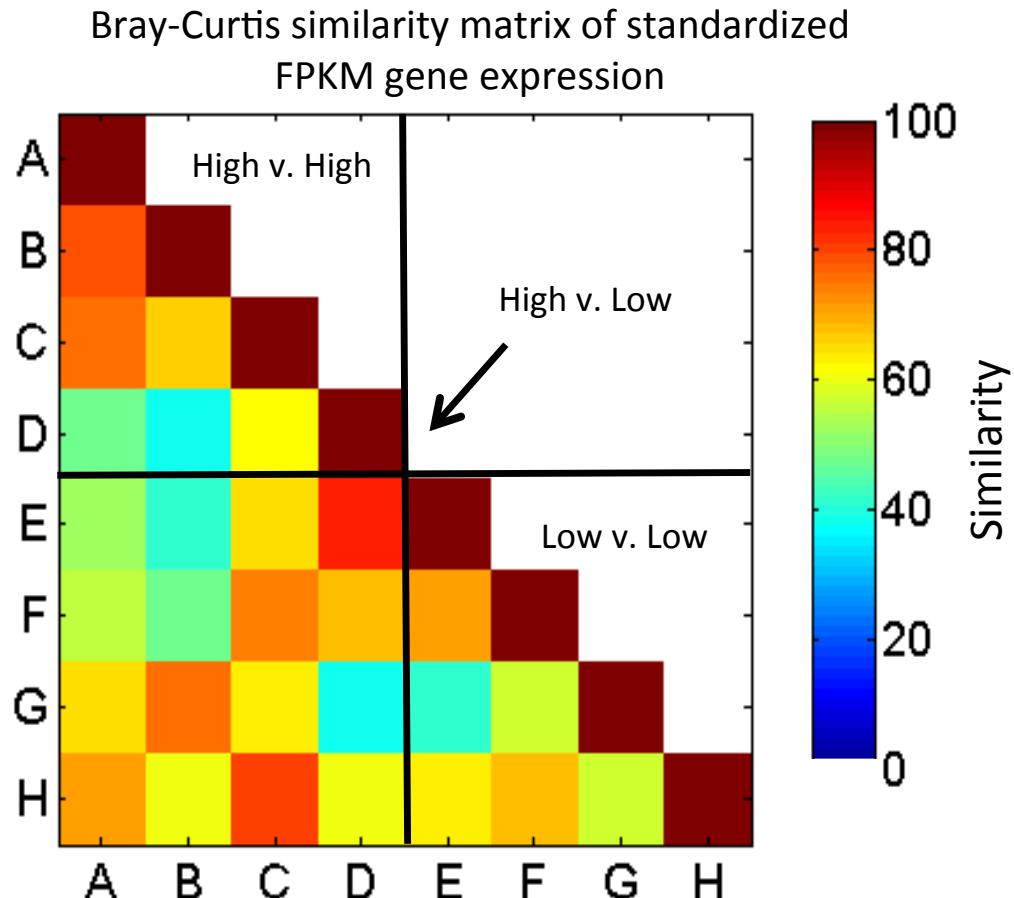
16



- No effect of CO₂ alone on metabolism for any species in either basin
- O₂ (alone or in concert with high CO₂) only affected two Atlantic species

Physiology – Gene Expression

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- *Clio pyramidata* transcriptome included homologs of genes with known biomineralization role in other molluscs
- High variability but some significant patterns of differential expression
- Down-regulated some genes associated with aerobic respiration in response to high CO₂
- Up-regulated some genes associated with biomineralization

Biogeography and Taxonomy

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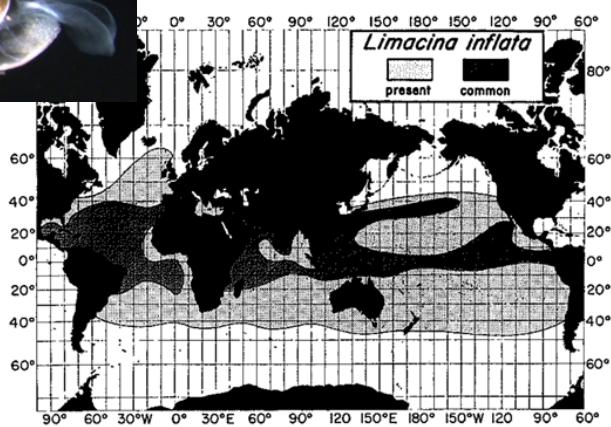


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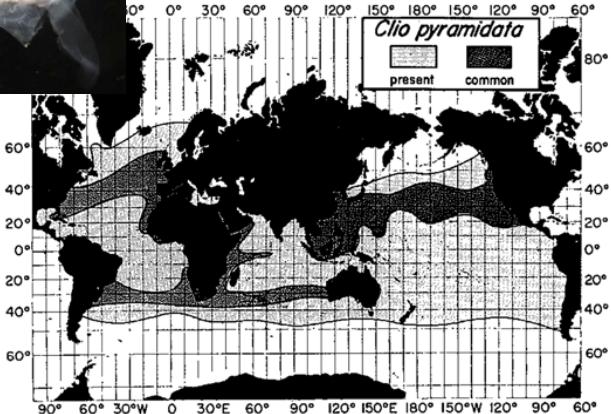
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Heliconoides inflatus



Clio pyramidata



Clio pyramidata

N Atlantic Ga01.21.1
Ga01.21.2
Ga01.21.3

S Atlantic Ga01.11.1
Ga01.11.2
Ga01.11.3

N Pacific Ga01.31.1
Ga01.31.2
Ga01.31.3

S Pacific Ga01.37.1
Ga01.37.2
Ga01.37.3



Heliconoides inflatus N Atlantic Ga11.15.1
Ga11.15.2

S Atlantic Ga11.07.2
Ga11.07.1

N Pacific Ga11.16.2
Ga11.16.3

S Pacific Ga11.24.1
Ga11.24.2
Ga11.24.3

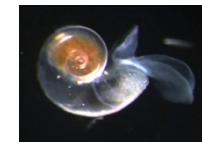


Clio pyramidata N Atlantic Ga11.15.1
Ga11.15.2

S Atlantic Ga11.07.2
Ga11.07.1

N Pacific Ga11.16.2
Ga11.16.3

S Pacific Ga11.24.1
Ga11.24.2
Ga11.24.3

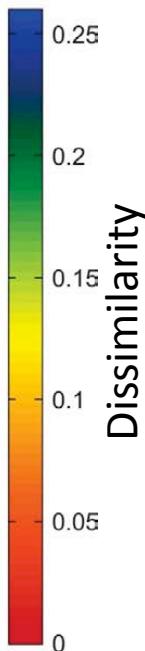


Heliconoides inflatus N Atlantic Ga11.15.1
Ga11.15.2

S Atlantic Ga11.07.2
Ga11.07.1

N Pacific Ga11.16.2
Ga11.16.3

S Pacific Ga11.24.1
Ga11.24.2
Ga11.24.3



Summary

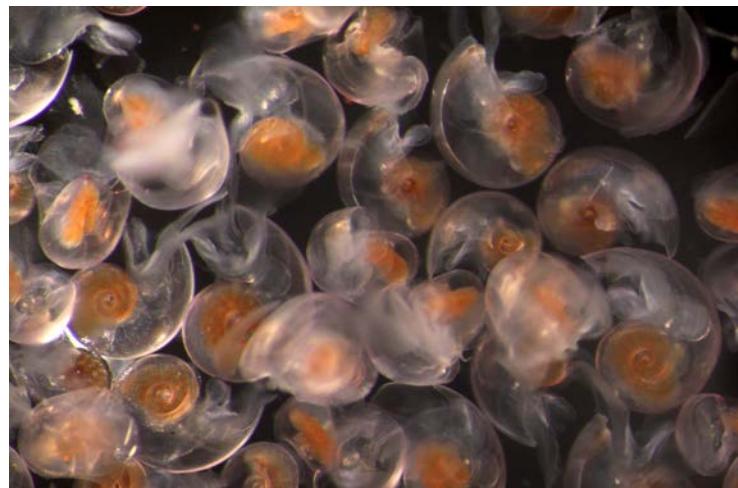
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CONCLUSIONS

- Inter-disciplinary sampling and approach
- Value in natural experiments
- Variability in diel vertical migratory behavior and physiological response to high CO₂/low O₂
- Check out our posters on Gulf of Maine pteropods (acclimation, effects on early life stages and fitness)

OUTSTANDING QUESTIONS

- Trans-generational effects, adaptive capacity
- Ecosystem/foodweb effects
- Biogeochemical implications



Acknowledgements

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Clio polita

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 - Pickman Foundation
 - Coastal Ocean Institute



Gulf of Maine

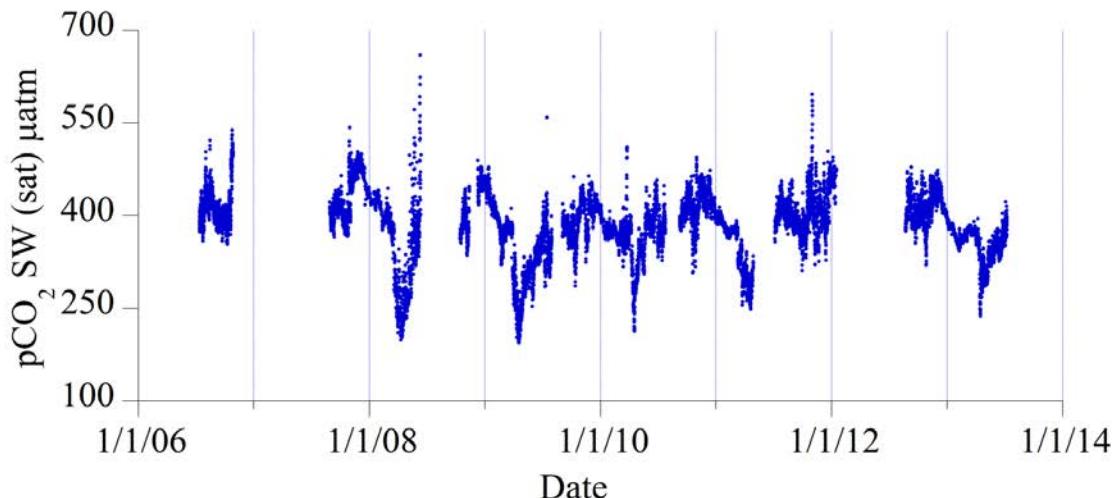
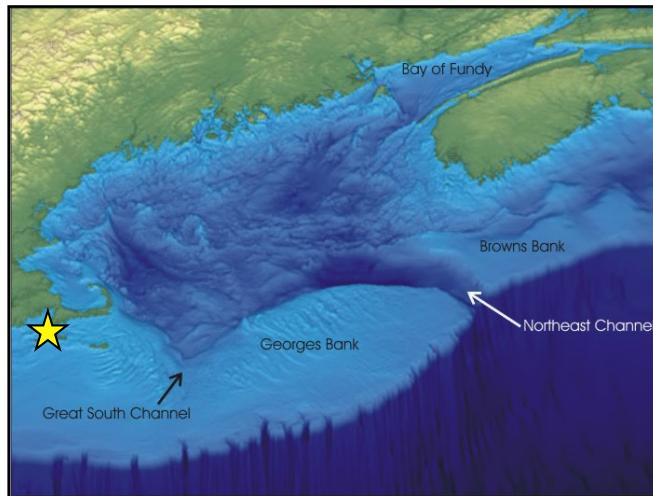
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- Seasonal variability in surface conditions
- Examining carbonate chemistry through the water column
- Implications to thecosomatous pteropods and possibility for acclimation
- Using *Limacina retroversa* as a lab rat for OA studies.



Limacina retroversa

The Gulf of Maine



Laboratory Exposure Experiments

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Seasonal field sampling



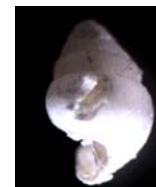
Ambient
400 ppm



Medium
800 ppm



High
1200 ppm



**14+ day exposure
to ambient (400), medium (800)
and high CO₂ (1200 ppm)**

Laboratory Studies

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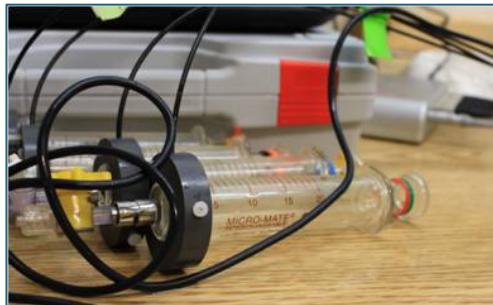
Early life stages & development



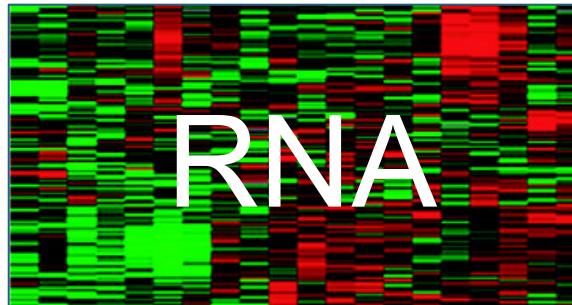
Culture Protocols



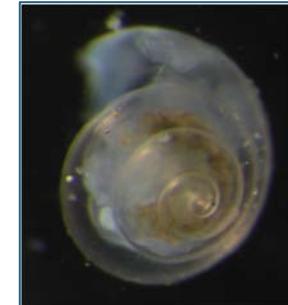
Sinking / Swimming



Respiration rates



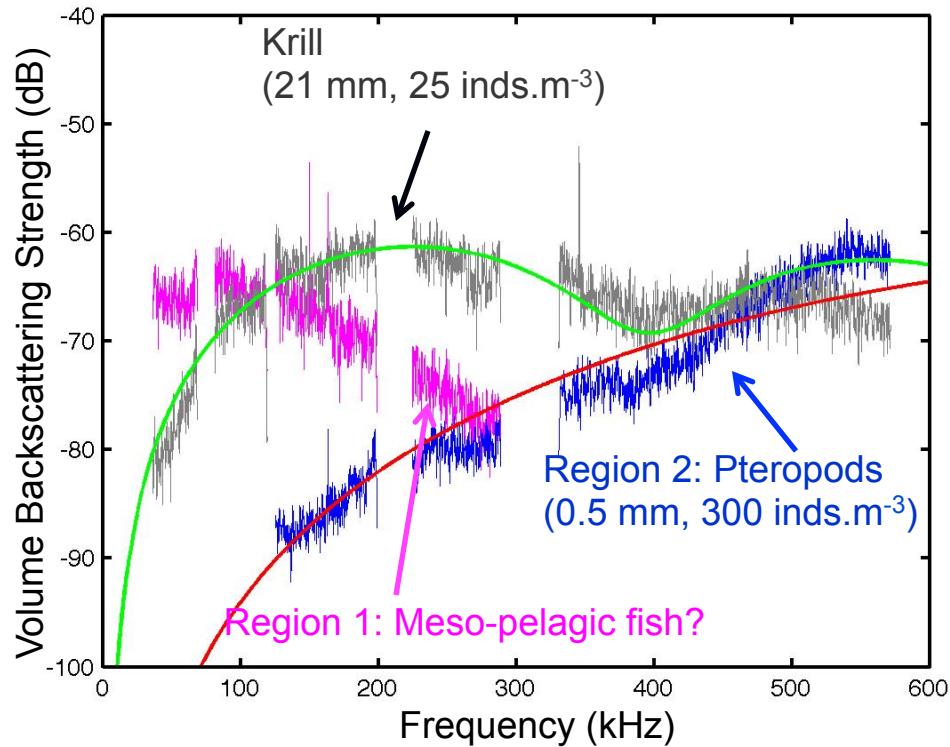
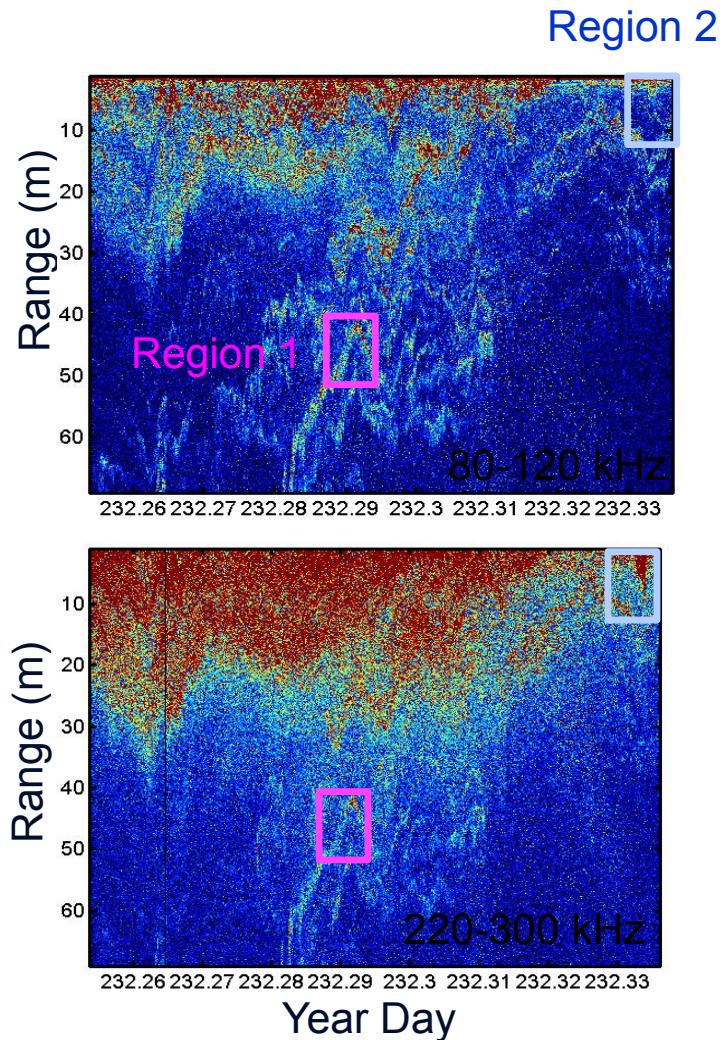
Gene Expression



Shell condition

Broadband Acoustic Sampling

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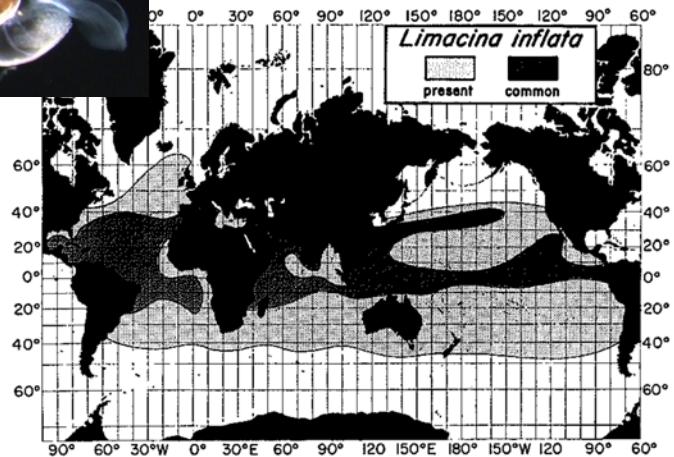
- Broadband acoustic system measured scattering spectra consistent with thecosomatous pteropods, euphausiids, and mesopelagic fishes

Biogeography and Taxonomy

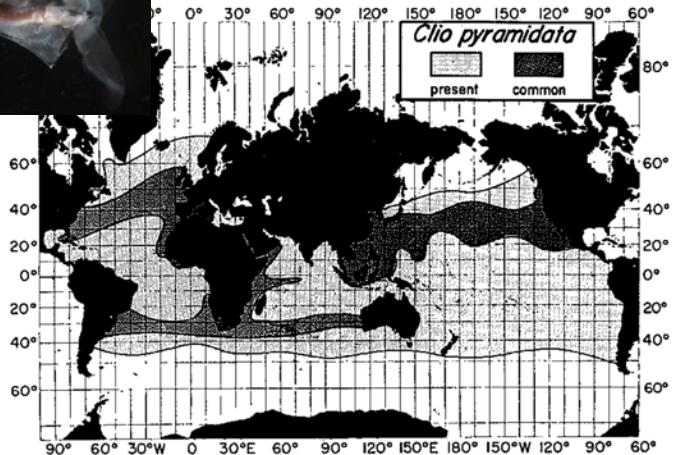
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Heliconoides inflatus



Clio pyramidata



Dr. Amy Maas

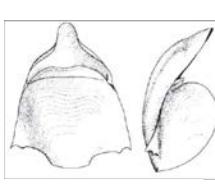


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D. elegans



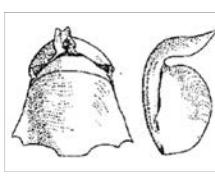
D. atlantica



D. strangulata



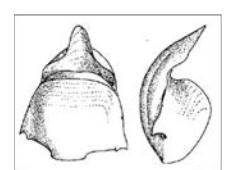
D. robusta



D. juvenile



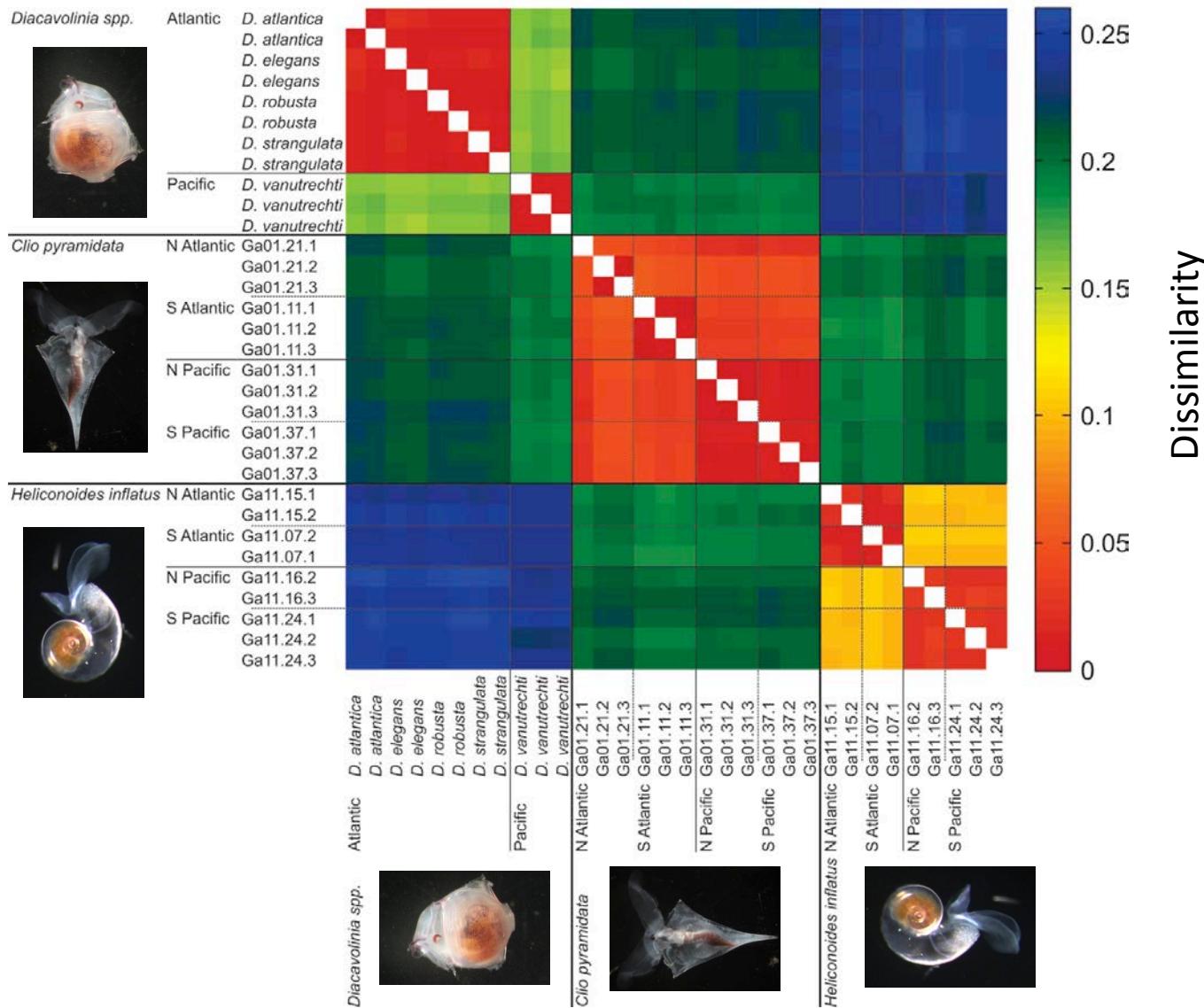
D. vanutrechti



Diacavolinia spp.

Biogeography and Taxonomy

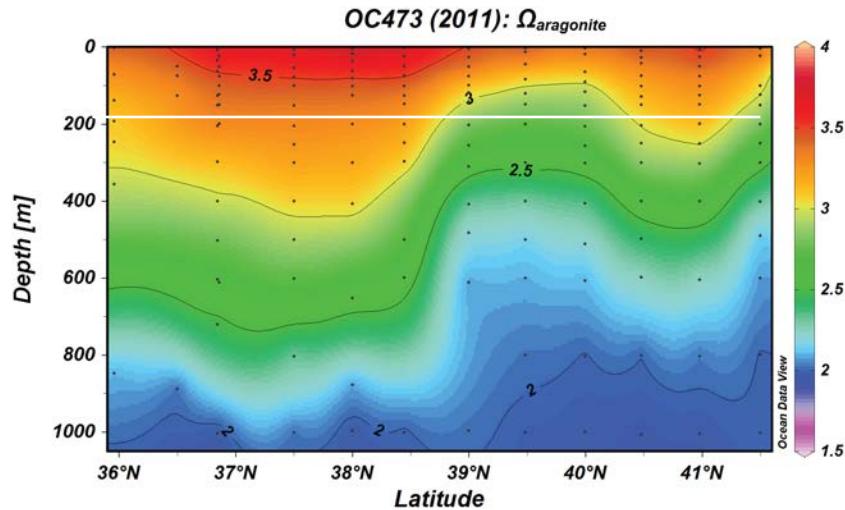
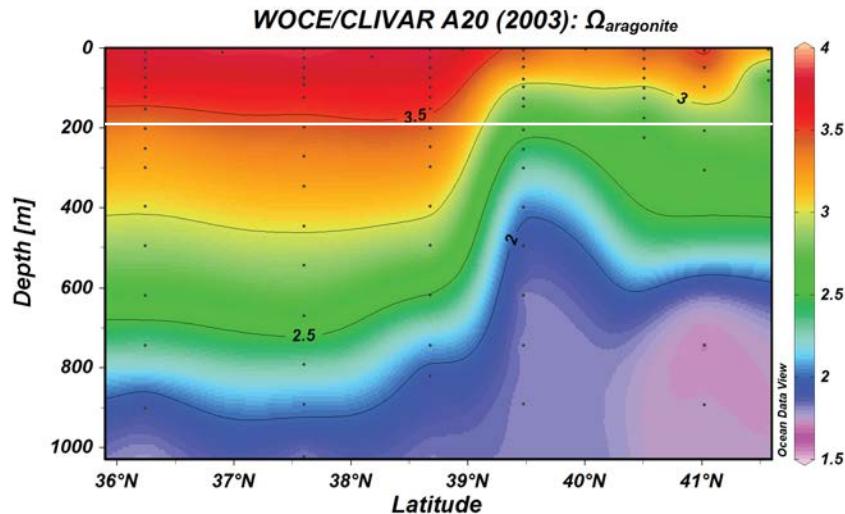
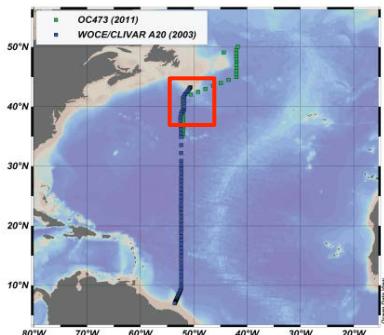
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Atlantic Chemistry

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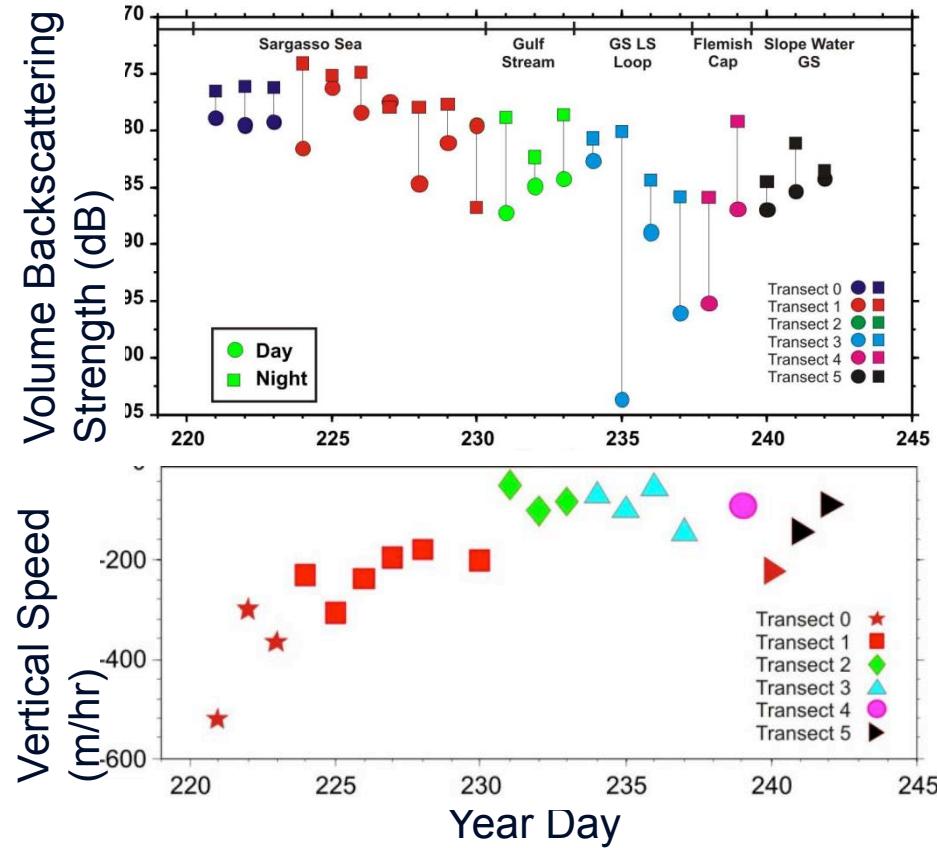
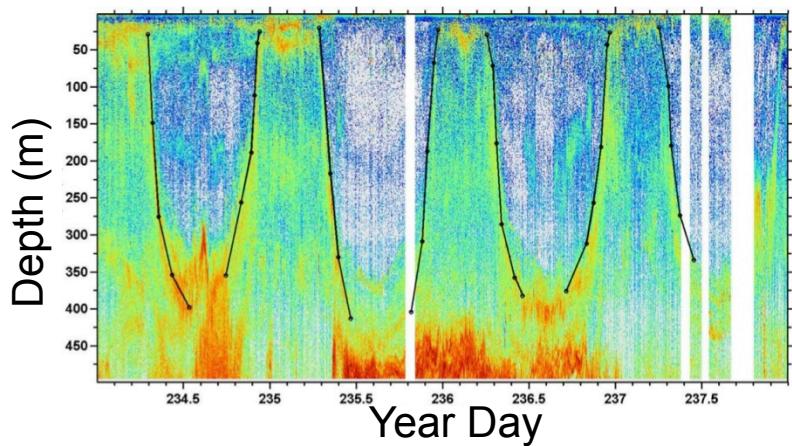
- Decrease in pH since 2003, the last time CLIVAR/WOCE line A20 was occupied
- Aragonite saturation state contour of $\Omega_a = 3.5$ has shoaled by ca. 100m
- At northern end of survey lines, compensation depth ca. 2500m in Atlantic and 135m in Pacific



Underway Acoustic Observations

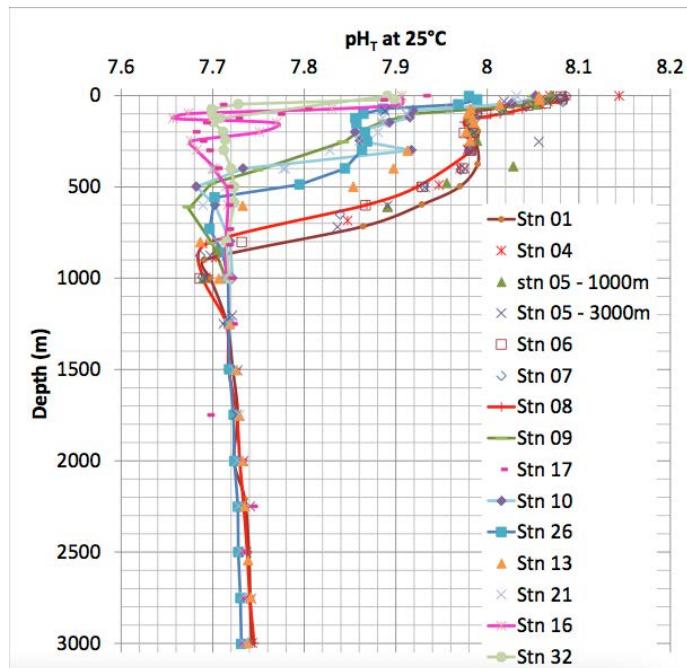
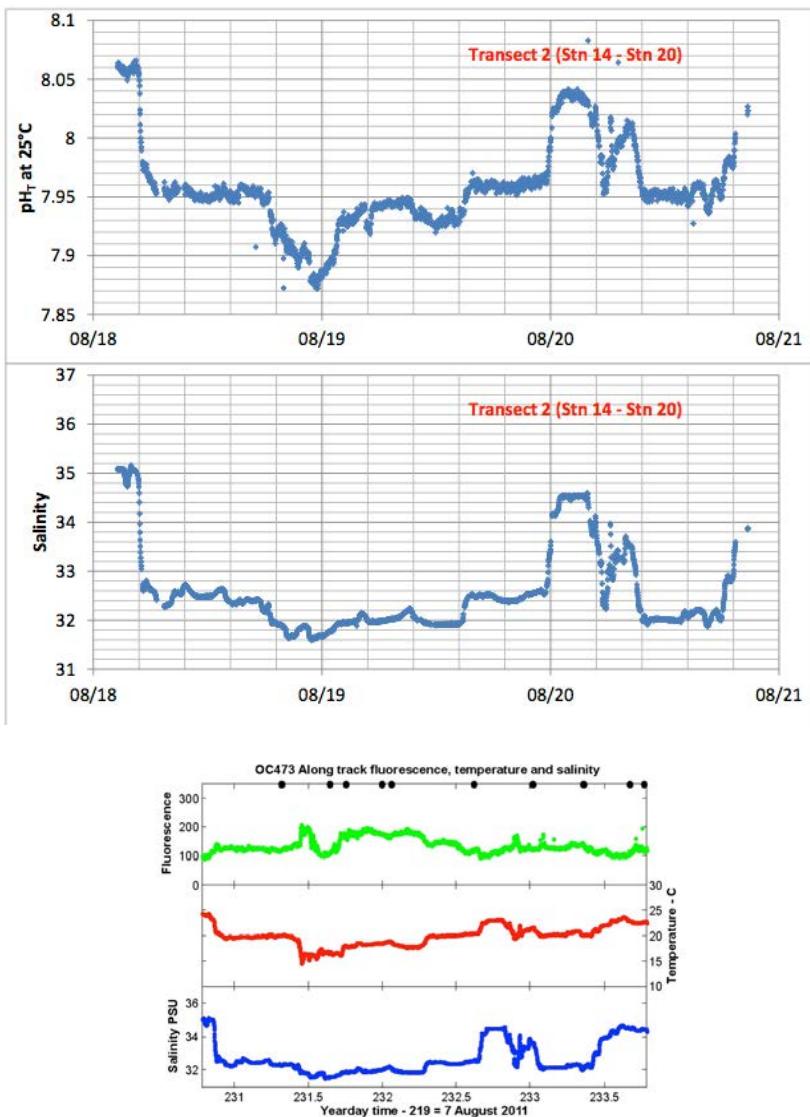
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- Multi-frequency acoustics to characterize Deep Scattering Layer



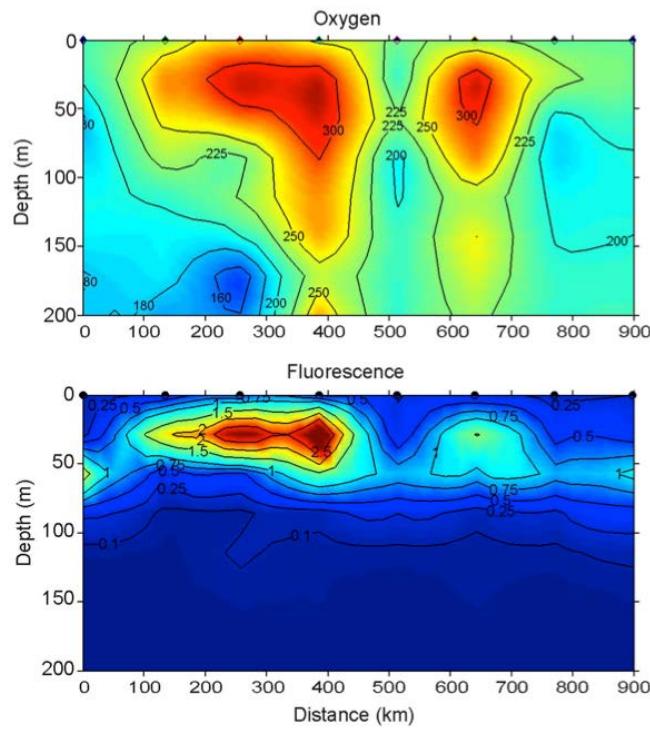
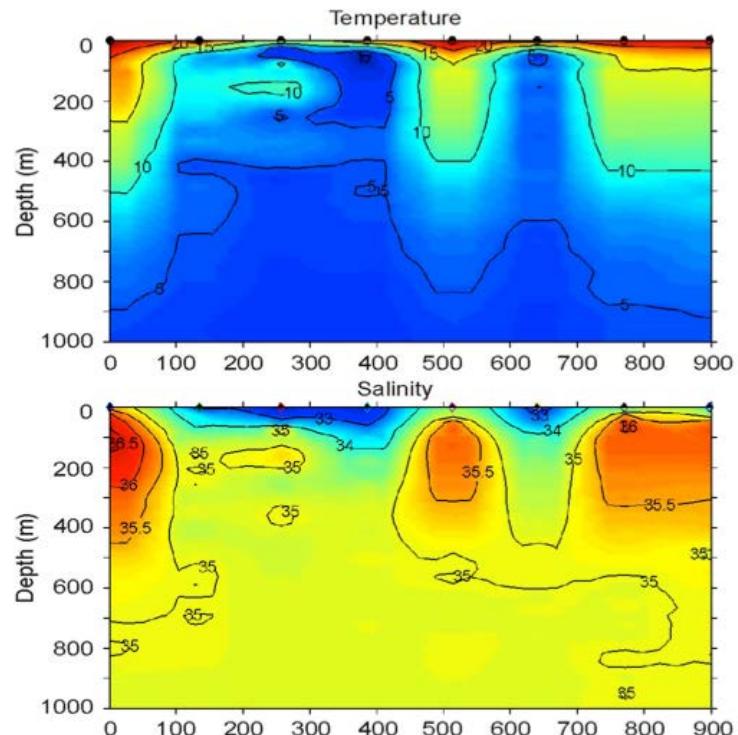
Underway Observations

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Underway Observations

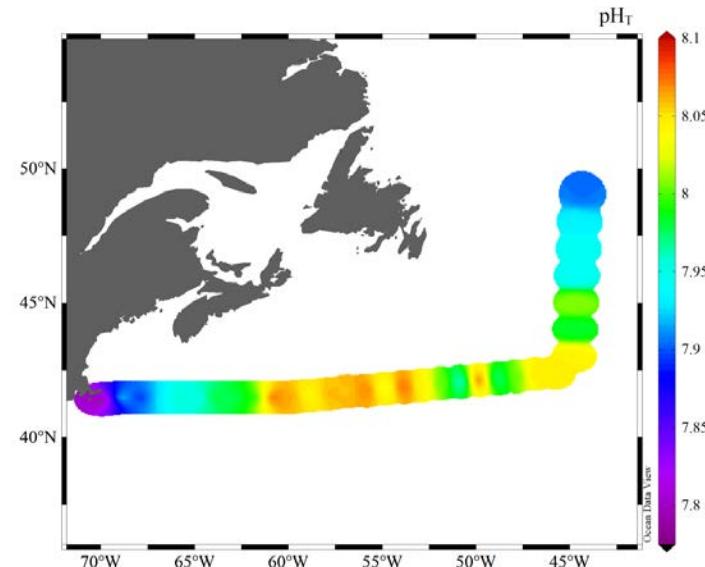
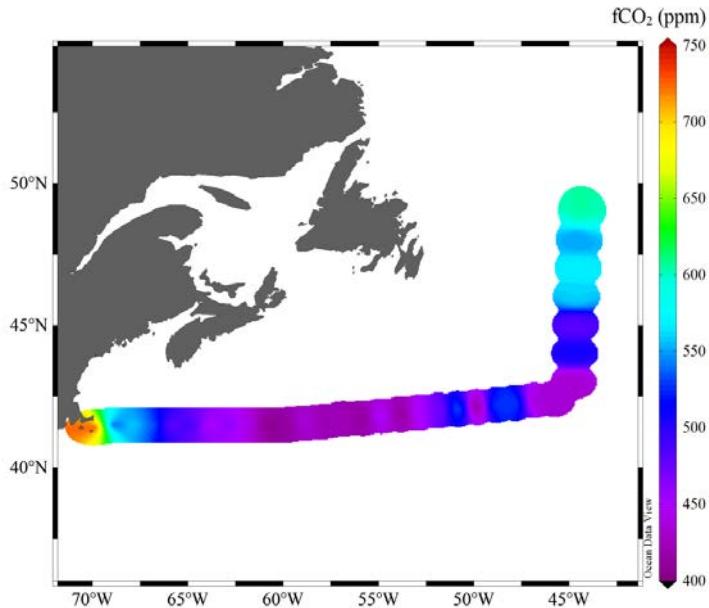
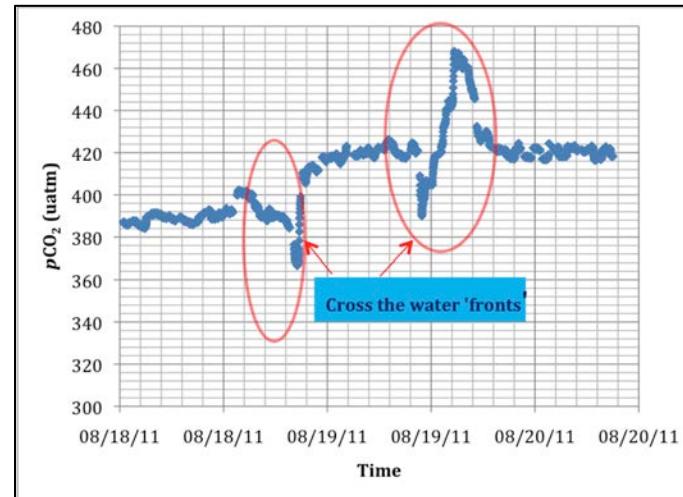
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Underway Chemistry Observations

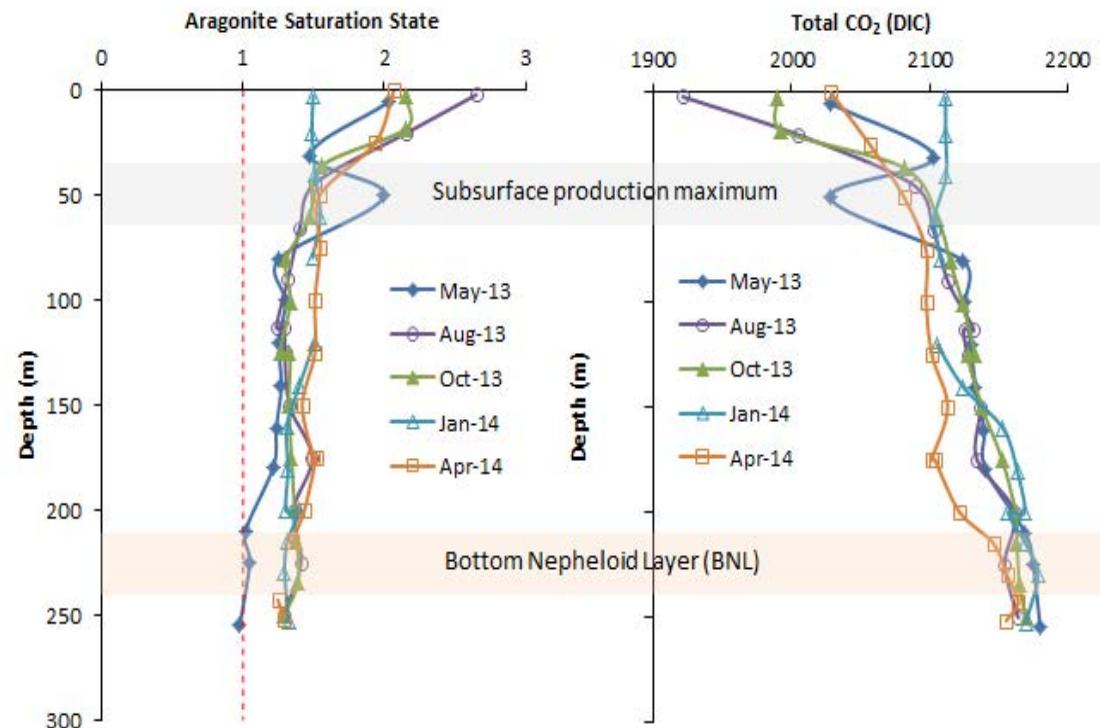
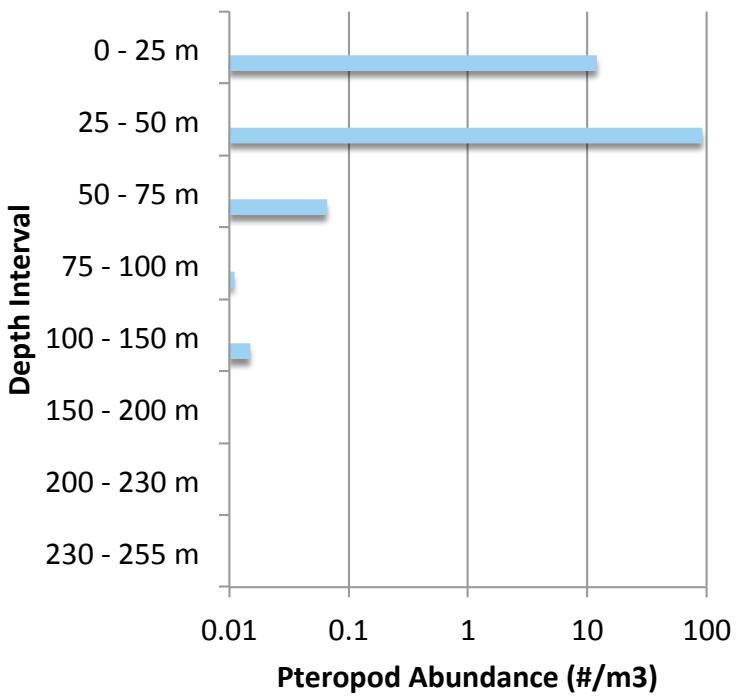
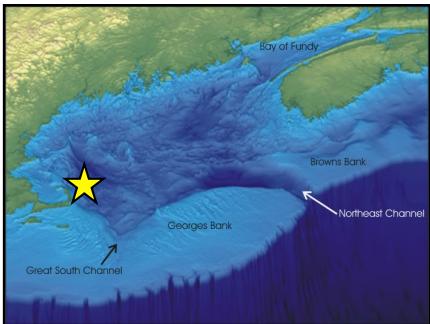
31

- Flow-through systems for carbonate chemistry



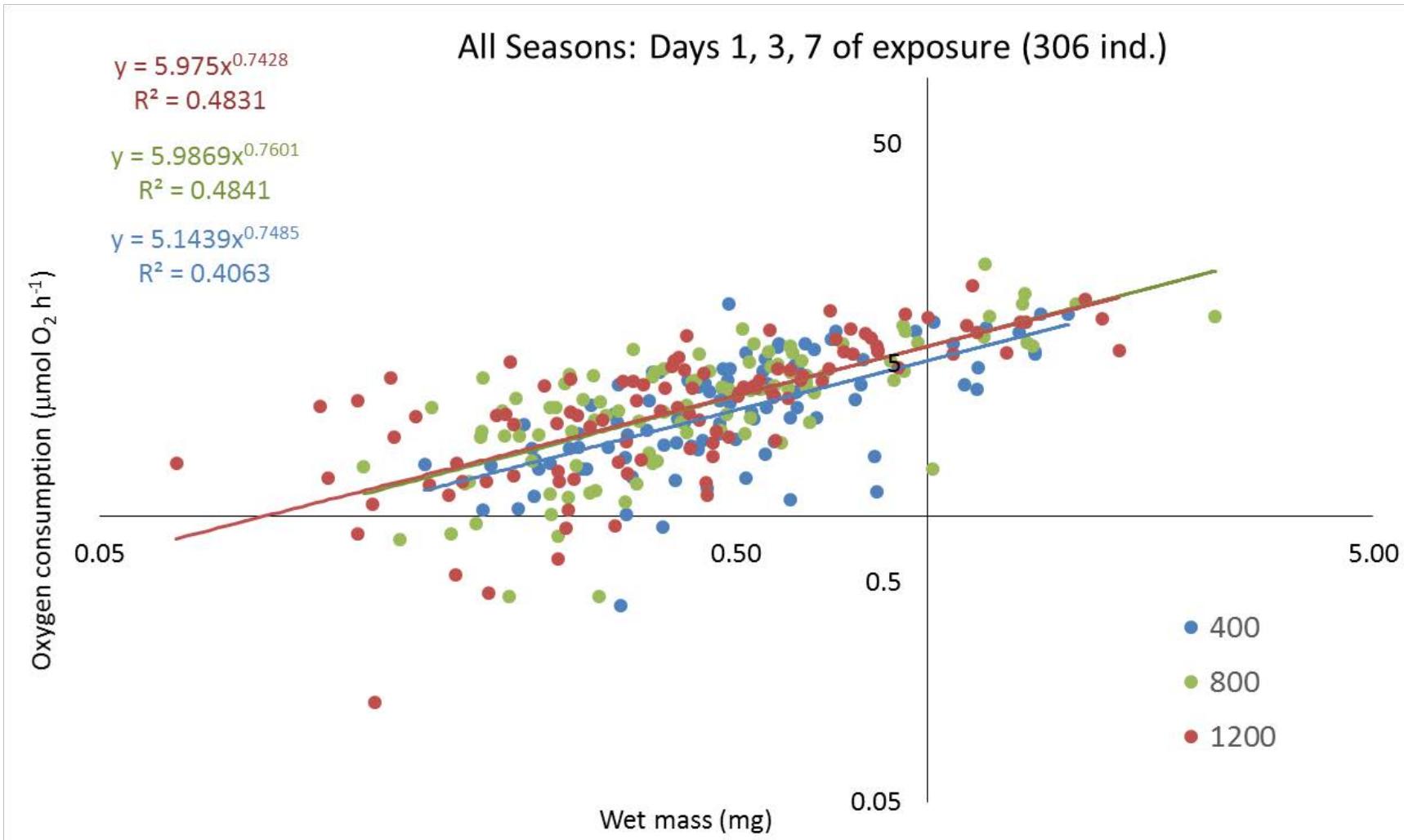
Field Studies

32



Differences in rate due to CO₂ exposure

33



Shell Condition

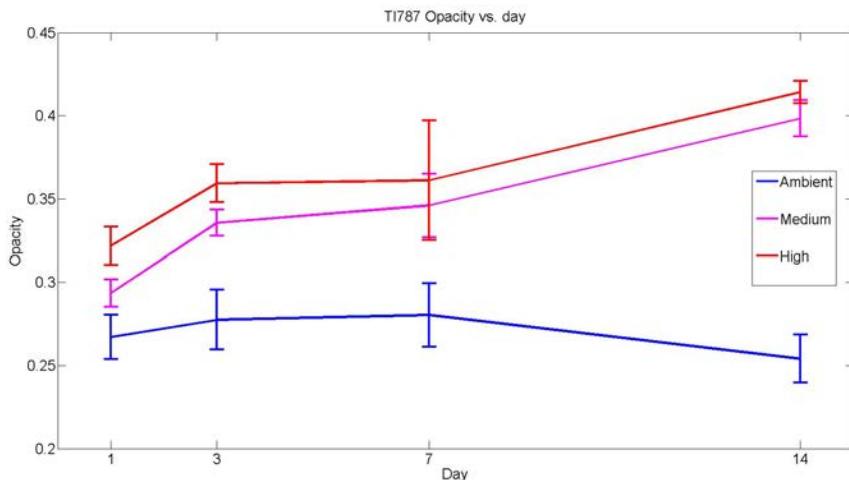
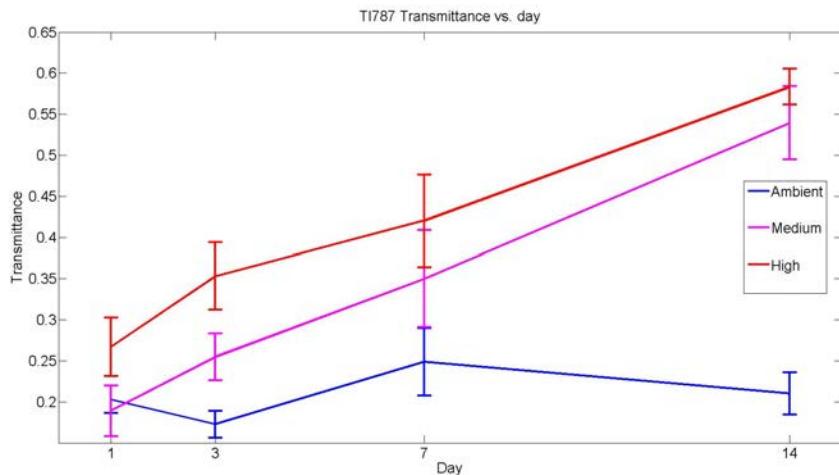
34

Transparency and Opacity

- A quantitative interpretation of the Limacina Dissolution Index (eg. Gerhardt *et al.* 2000)
- Images from a dissecting microscope at 2.5x magnification with transmitted and opaque lighting
- Cropping is used such that the operculum and any holes are removed from grayscale analysis (any single layer of shell)



Alexander Bergan
PhD Student



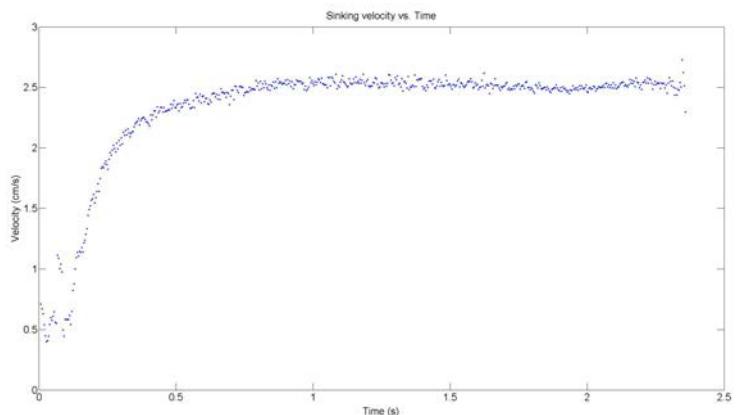
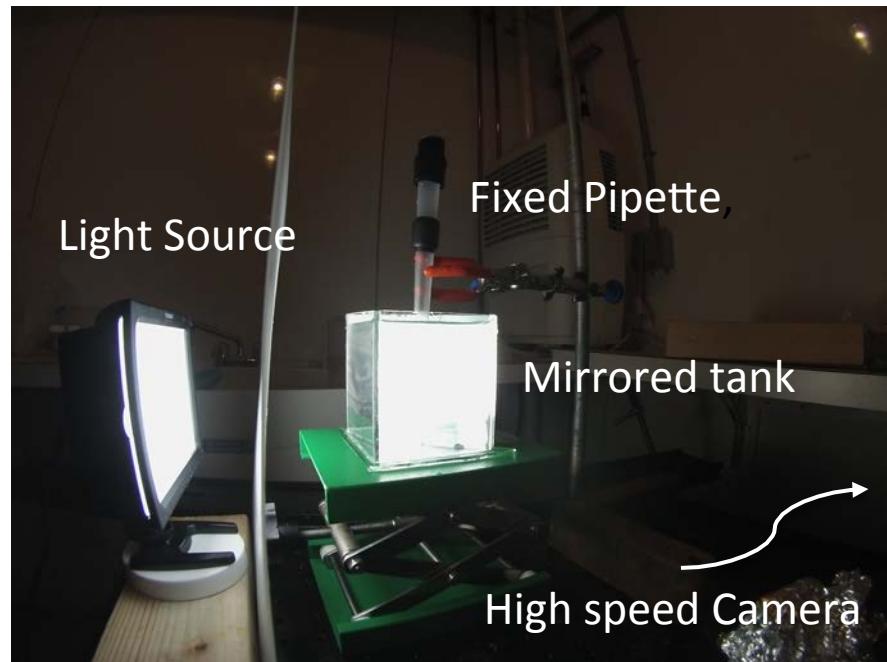
Sinking

35

- *Limacina retroversa* reared in seawater bubbled with 400 ppm CO₂, 800 ppm CO₂, and 1200 ppm CO₂
- Video taken in mirrored tank at 500 frames per second, giving 3D path and velocity of the animals

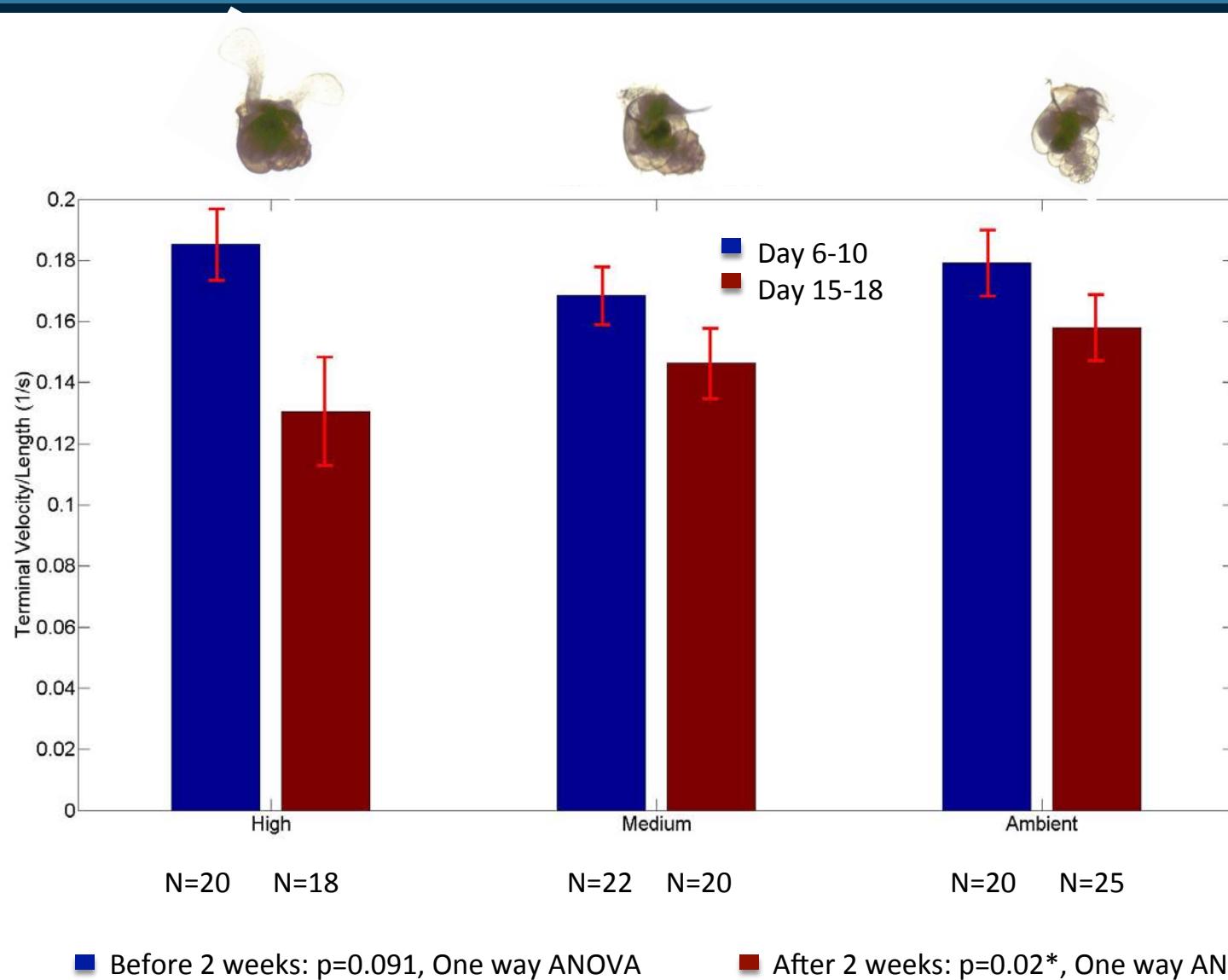


Alexander Bergan
PhD Student



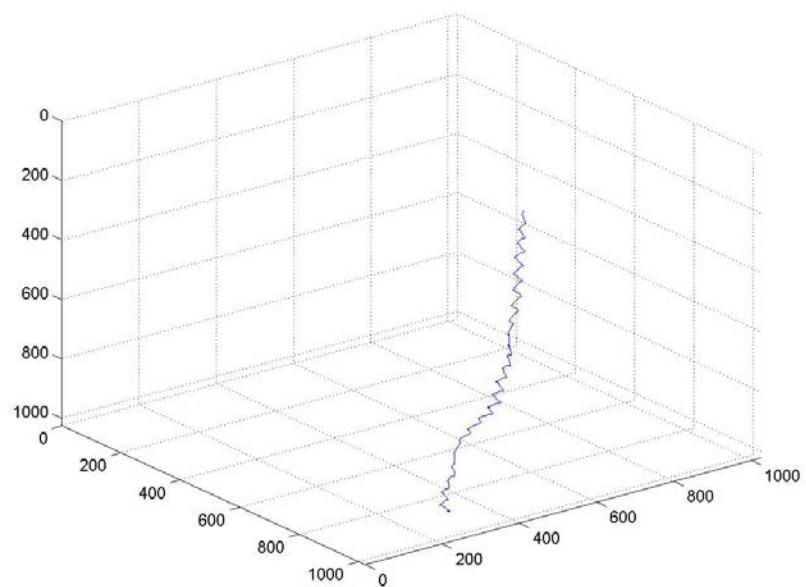
Sinking

36



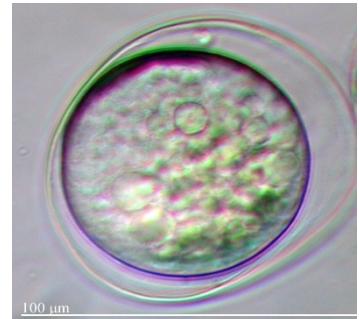
Swimming

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Early Life Stages

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1- cell



2- cell



4- cell



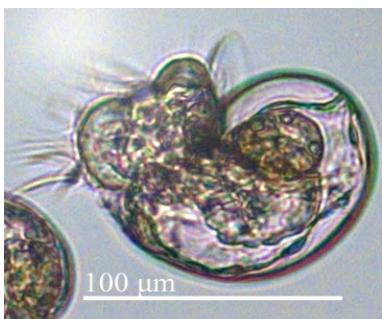
8- cell



Late gastrula



Trochophore larva



1 week veliger larvae



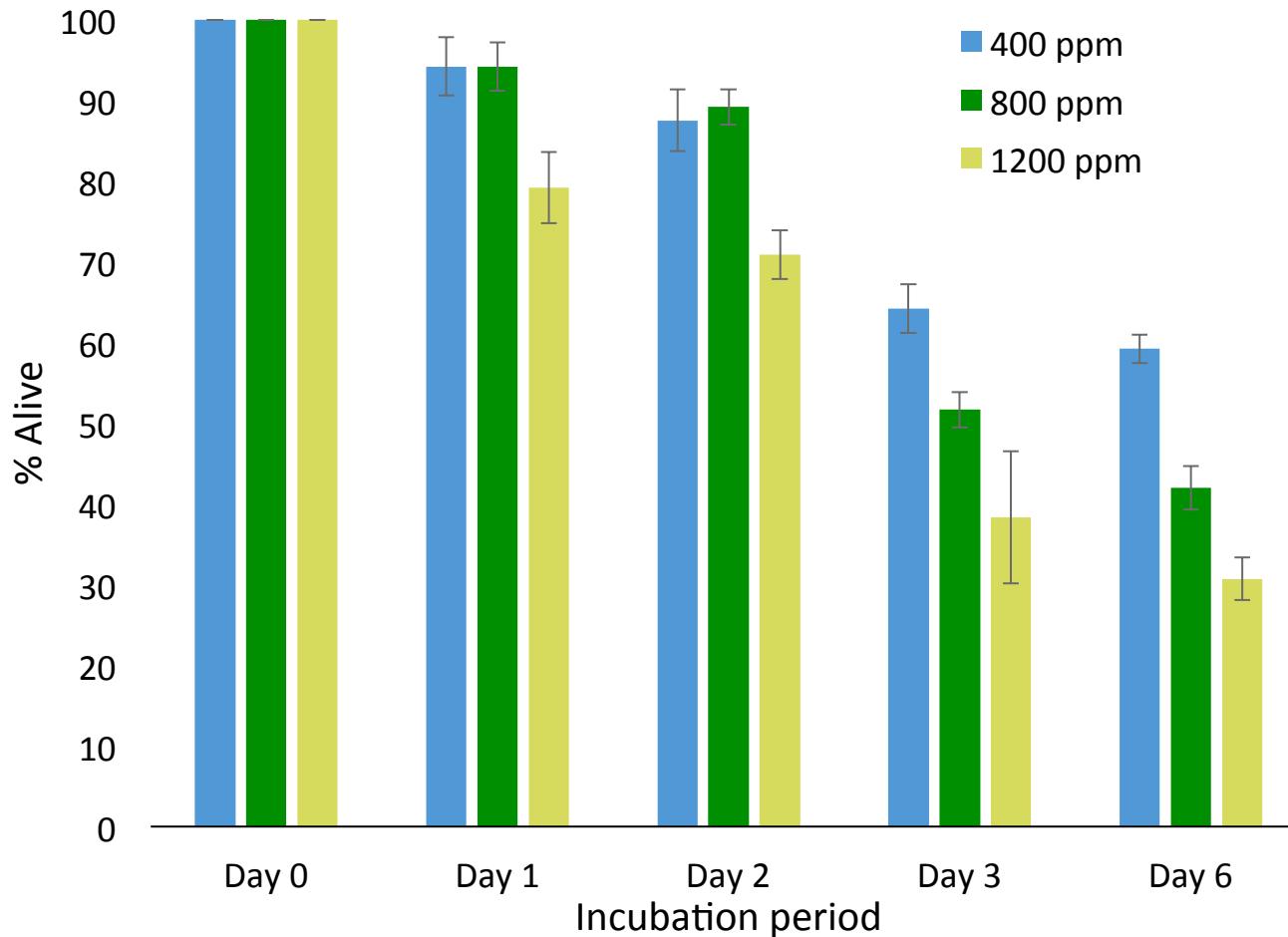
3 week veliger larva



Stage	Time
Spawning	0 h
2-cell	4 h
4-cell	6 h
8-cell	9 h
16-cell	11 h
Blastula	16 h
Gastrula	24 – 72 h
Hatching	3 days
Trochophore	3-6 days
Veliger	6-7 days
Juvenile	1-2 month
Reproductive Adult	3 months
Life Span	6 months

Early Life Stages

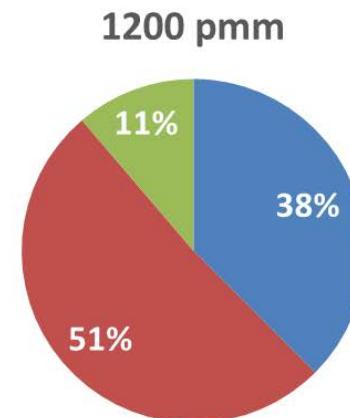
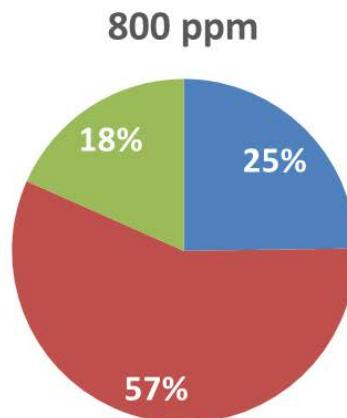
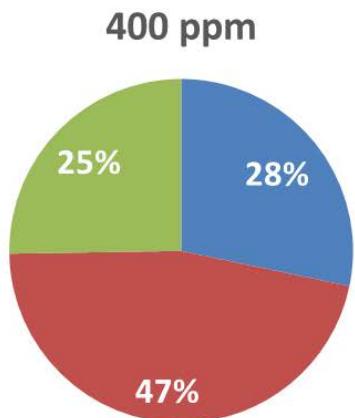
39



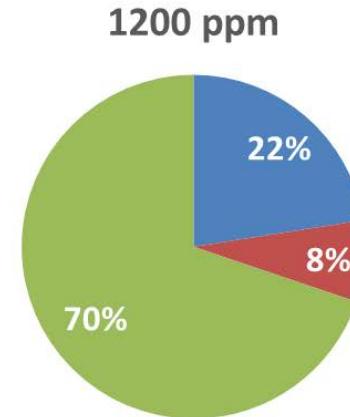
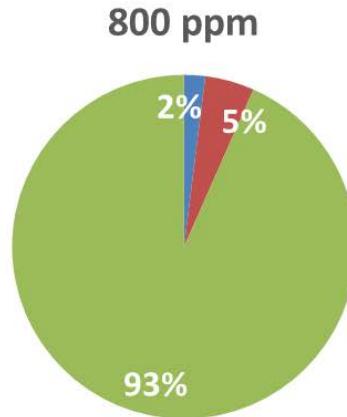
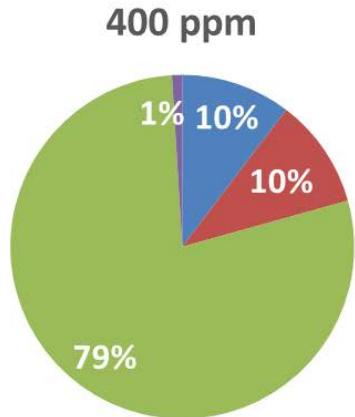
Early Life Stages

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Day 2



Day 6



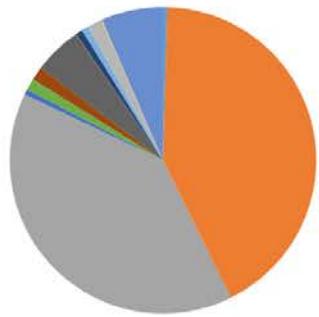
■ Gastrula (non-moving)
■ Gastrula (moving)

■ Trochophore
■ Veliger

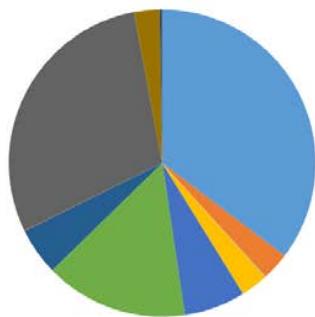
Limacina retroversa transcriptome

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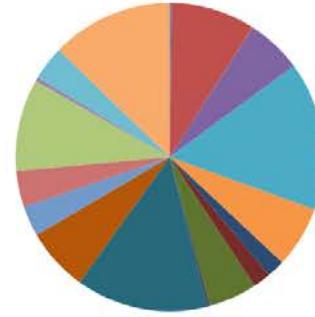
	<i>Limacina retroversa</i>	<i>Clio pyramidata</i>
Bioproject	PRJNA260534	PRJNA231010
Transcriptome	GBXC00000000	GAWL00000000
# transcripts (isoforms)	270,317	45,802
# components (genes)	125,222	30,853
N50	1,699	851
% Blast ID	~31%	~23%



Molecular Function

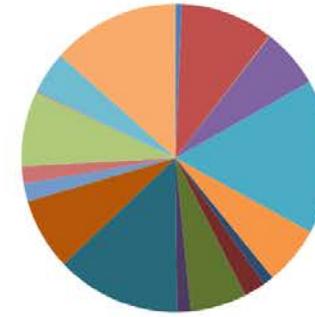
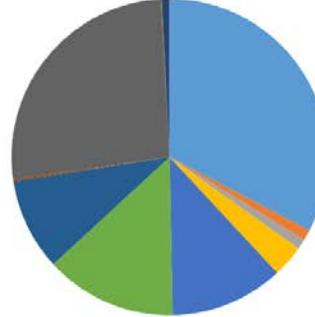
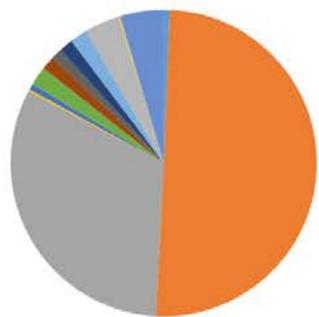


Cellular Component



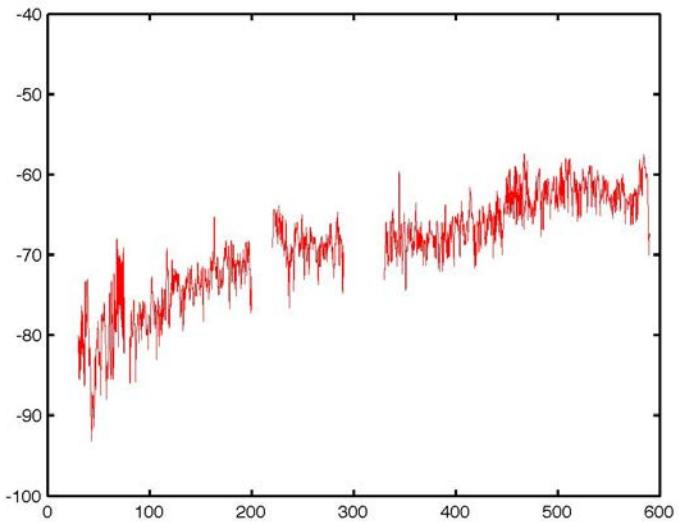
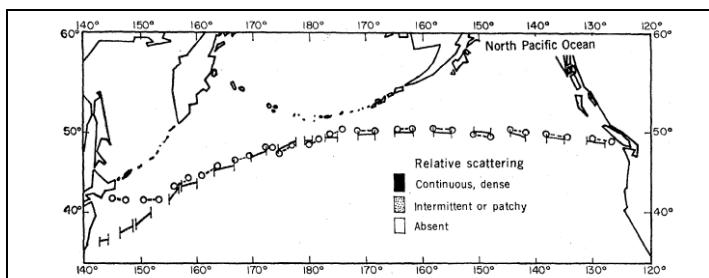
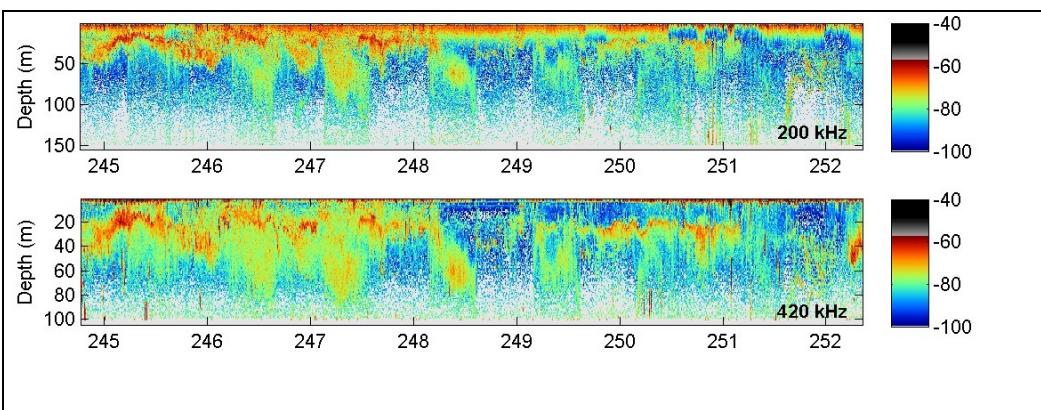
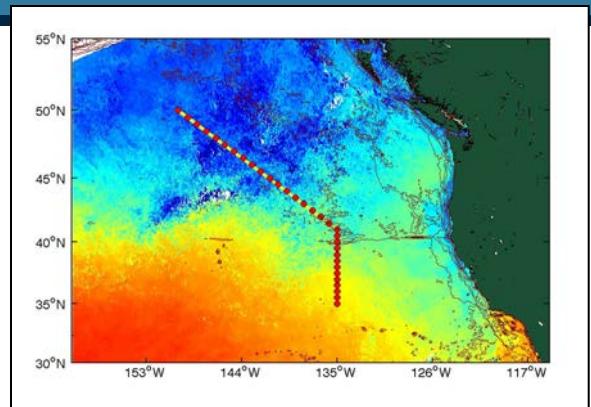
Biological Process

**GO term
classifications**

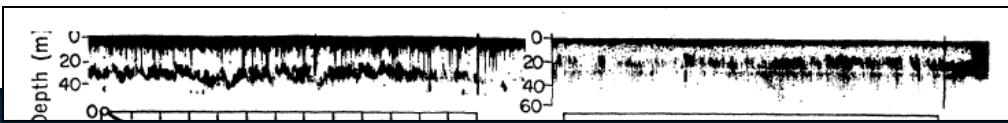


OAPS – Pacific 2012

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- Dominant scattering feature was a non-migratory shallow layer
- Additional analyses and comparison to net samples are required



Art-Science Collaboration

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THE PTEROPOD PROJECT
charismatic microfauna
Blue Mountain Gallery
May 22 – June 16, 2012

