R/V *Tioga* Cruise #725
Cruise Report

January 10th, 2014

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Report available at:
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National Science Foundation Ocean Acidification Grant # OCE- 1316040 (PIs: Lawson, Maas and Tarrant) *"Ocean Acidification: Seasonal and ontogenetic effects of acidification on pteropods in the Gulf of Maine"*

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2. Acknowledgements

The success of this cruise would not have been possible without the flexibility, proficiency and expertise of Captain Ken Houtler and mate Ian Hanley of the *R/V Tioga*. This cruise was supported by a grant from the National Science Foundation OCE-1316040 (Lawson, Maas, and Tarrant).

3. Background

As a result of increases in atmospheric carbon dioxide (CO_2), the ocean is taking up extra CO_2 and becoming more acidic, in a process referred to as ocean acidification (OA). Certain coastal regions, such as the upwelling system along the U.S. West Coast, are more susceptible to the effects of ocean acidification than others, because their waters are episodically or seasonally naturally higher in CO_2 concentration and lower in pH and saturation of aragonite (a calcium carbonate mineral). In such OA 'hot-spots,' continued anthropogenic perturbations to the carbonate chemistry will quickly push the system towards a more corrosive (aragonite under-saturated, $\Omega A < 1$) environment that many calcium carbonate shell-forming organisms may not tolerate. Coastal acidification in the Gulf of Maine (GoME) has generally not been considered to be a pressing concern, but new data (Wang et al. 2013) suggest that in the deep waters of the GoME low seawater pH may cause aragonite saturation states (ΩA) to be close to a chemical and ecological threshold (i.e. $\Omega A = 1$).

This cruise was part of an ongoing seasonal time series of carbonate chemistry measurements in the Gulf of Maine. It was also an auxiliary cruise for the collection of the local population of thecosome pteropod, *Limacina retroversa*. Keeping these animals in the laboratory is an integral part of a project which is the metabolic, gene-expression and calcification response of these animals to CO_2 exposure. Individuals from this cruise will be used to test water, bubbling, feeding and carboy type to ensure that animals are in good condition for subsequent cruises.

4. Cruise Objectives

The central goal of this cruise was to capture individuals of the thecosome pteropod *Limacina retroversa*. The secondary goal was to sample the carbonate chemistry profile in the GoME in the well mixed winter season. The long-term goal of this research is to understand forcings by climate, enhanced atmospheric CO_2 levels, and coastal eutrophication on seasonal and inter-annual variability in carbonate chemistry of the Gulf of Maine and to understand how variations in the natural environment impact the local planktonic calcifiers, specifically the thecosome pteropods. The specific goals of the overall research are to:

1. Quantify seasonal variations of carbonate system parameters and buffer intensity in deep waters of the Gulf of Maine in order to evaluate the sensitivity of these waters in response to acidification due to anthropogenic forcing, such as increase in atmospheric CO_2, freshening of the GoME (decrease in total alkalinity) and increases in water-column respiration due to eutrophication. We will test the hypotheses that deep waters of the GoME are already seasonally under-saturated with respect to aragonite saturation state, and that these waters have low buffer intensity compared to overlying water, which would cause them to be more susceptible to acidification pressures and to reach critical ecological thresholds ($\Omega_A < 1$) more readily.
2. Quantify seasonal patterns in the abundance of the pteropod *Limacina retroversa* and its vertical distribution relative to concurrent measurements of water column chemical properties, testing the hypothesis that this species is absent in the acidic waters of the near-bottom nepheloid layer.
3. Test whether there are seasonal patterns of gene expression, shell quality and metabolic rate linked to seasonal exposure.

4. Determine how experimentally enhanced levels of CO_2 influence the gene expression, shell quality and metabolic rate of *Limacina retroversa* that are exposed for a period of 1-14 days in the laboratory and explore whether these responses are mediated by seasonal exposure.

The specific goals of this particular cruise were to:

1. Measure the carbonate chemistry of the water column at sites in the Gulf of Maine, targeting the sites which were sampled during Tioga cruises 668 (May 2013), 700 (August 2013), and 715 (October 2013) to provide a seasonal contrast in the measurements.

2. Collect *L. retroversa* to bring back to the lab for husbandry experiments and to supplement the current population of breeding animals at WHOI.

3. Collect surface water and *L. retroversa* for live animal laboratory experiments.

5. Survey Design

On Thursday January 9th the R/V Tioga was packed at WHOI and left the dock with scientists and crew to head to Provincetown. On Friday January 10th it left Provincetown harbor at 7:09 and traveled to standard station 3. At this station the CTD was cast, water was drawn from depth using the "Little Giant" pump, and Reeve nets were conducted to collect animals. At ~16:00 the ship started to head back to WHOI and reached port at ~20:00. The water was craned off in the black crate. The animals were transferred in coolers.

Full information about casts and stations can be found in the Event Log (Appendix 1).

6. Cruise Narrative

The boat left from WHOI Thursday 9th at ~4:00. Departure was delayed by the need to fill up on water. Arriving in Provincetown the scientific crew went to the hotel at Angel's Landing.

Day 1: Friday January 10th

Friday morning the scientific crew assembled at the boat, which departed from the floating ferry harbor at ~7:00. Due to weather concerns, the decision was made to only go to standard station 3 (Figure 1). When station was reached, ~1 ½ hrs after leaving shore, the CTD was deployed. To defrost the unit it was held submerged at the surface for awhile before sending down. Almost immediately upon descent, the O_2 sensor began to behave oddly, reading -2. The temperature, oxygen and beam transparence were constant throughout the 125 m water-column causing us to doubt their validity as well. The CTD was brought back onto deck where the pump was examined to ensure it was working properly and there was no ice in the line. The CTD was then re-deployed with similar results. We chose to take water samples irrespective following the procedures laid out by Wang's lab. Nutrient samples were placed in the freezer associated with the standard refrigerator. While the CTD was being processed the "Little Giant" pump was deployed. The function of the pump was sporadic and upon examination it was observed that there had become a kink in the underwater splice, causing a bad connection. Ian re-spliced the line, successfully repairing the pump, and water was filtered from ~30 m through a 64 µm sieve into 6 trash cans. A number of 1-L jars were filled with this filtered water and stored in the fridge. To wedge them securely they were packed in with a few of the extra flotation devices. Once the pumping was completed we began to Reeve to collect animals. After 5 tows we had only retrieved a small number of individuals (~300 largish animals) but the weather was getting worse and the light was fading. We headed back to WHOI directly and reached port by ~20:00. The water was offloaded by crane onto a truck and the full water jars were put in coolers for transport to ESL. Leo and Ali helped allocated the animals at ESL while the rest of the crew moved the Reeve net into the staging room for cleaning. Demobilization occurred on Monday.

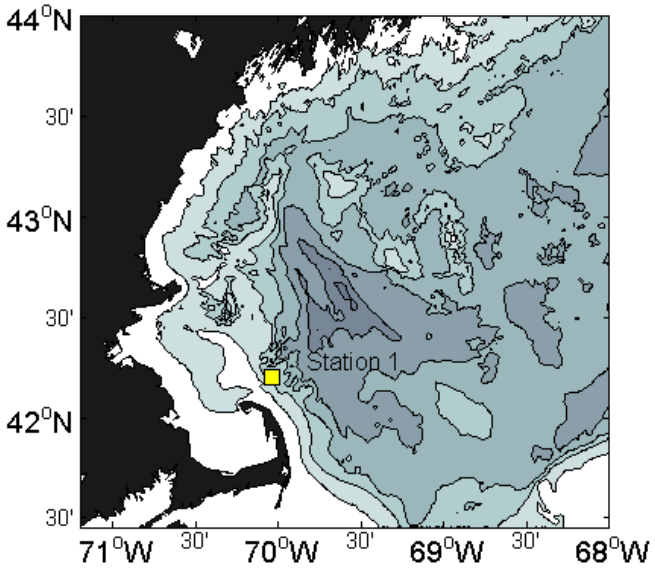


Figure1 – Gulf of Maine Map. We sampled extensively at Station 1 (standard station 3) due to inclement weather.

7. Equipment Configuration

7.1. *Deck configuration*

The collapsible plastic crate with 4 garbage cans was strapped down to the starboard side of the back deck. The Reeve net was stowed on the port aft railing and. The CTD was positioned mid-ship. We used the same cable for all deployments. There was a table with a built in sink bolted down at the forward port portion of the back deck. The two extra trash bins were strapped to the middle port side, catty-corner to the sink/table. The refrigerator was strapped down forward of the winch.

7.2. *Lab configuration*

The main lab aft counter housed the laptop which was used for event logging. The starboard counter had chemistry sampling equipment. On the floor was sampling equipment. The rest of the backup supplies, foul weather gear and personal belongings were at mid-ship and in the port bunk space.

8. Hydrography: CTD

8.1.1. Introduction

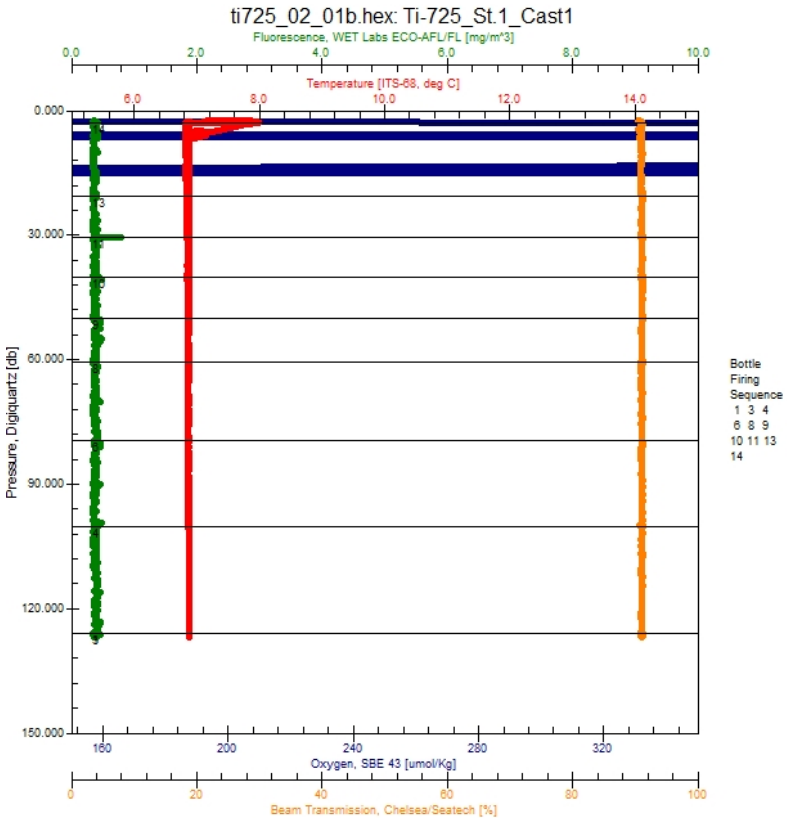
CTD rosette casts were conducted to provide profiles of the water column and to take water samples. Hydrographic parameters give insight to the environmental conditions that the pteropods experience and can help guide Reeve net capture.

8.1.2. Methods

The R/V *Tioga* CTD rosette had a16 bottle rosette with 3-L Niskins, and a SBE3/SBR4 sensor set. Niskin bottle sampling provided water for the carbonate chemistry analysis. Depths for bottle sampling generally are chosen to characterize the bottom nepheloid layer (BNL) and then to continue at pre-designated intervals throughout the rest of the water column. The typical protocol for the CTD is to sample upper 100m at 10 m intervals, 100-200m at 20 m intervals, and less frequently below. At the near-shore station (i.e. Standard Station #3) the water depth necessitated only the firing of 10 bottles, following the pattern established on previous cruises (Appendix 2).

8.1.3. Preliminary Results

The O_2 sensor broke during the deployment, but later dockside maintenance indicated that all other sensors were fully functional. The water column was just mixed fully to the bottom.



9. Chemistry

9.1. *Introduction*

Dr. Zhaohui Aleck Wang's group from the Department of Marine Chemistry and Geochemistry at WHOI provided bottles and sampling instructions for later measurement of carbonate chemistry parameters. Measuring these parameters will allow us to calculate pH, the carbonate compensation depth and the calcium carbonate saturation state, three important variables that may influence the formation of aragonite shells by pteropods.

9.2. *Discrete Measurements of Dissolved Inorganic Carbon and Total Alkalinity*

9.2.1. Methods

Discrete dissolved inorganic carbon (DIC) and total alkalinity (TA) samples were collected from the surface to near-bottom. Depths were chosen to follow previous sampling patterns (See CTD Methods). DIC and TA samples were collected in 250mL Pyrex borosilicate glass bottles after being filtered with a 0.45um in-line capsule filter. Each bottle was rinsed three times, filled completely, and then the sample was overflowed by another one and one half bottle volume. Air head space of about one percent of the bottle volume (~3 ml) was left in each sample bottle to allow room for expansion. Each sample was then poisoned with 100uL of saturated mercuric chloride, capped with an Apiezon-L greased stopper, thoroughly mixed, and then tied with a rubber band over the glass stopper. Duplicate samples were

collected at random depths of selected stations to evaluate the precision of the measurements. These samples will be measured for DIC and TA back in the Wang Lab at WHOI.

9.3. *Discrete Nutrient Measurements*

9.3.1. Methods

Nutrient samples were collected in acid cleaned Kimble 20mL plastic bottles. Before the cruise, the bottles were soaked in 10% hydrochloric acid for four hours, rinsed three times with de-ionized water, and then dried in the oven at 50°C for 48 hours. During collection, the sample was filtered with a 0.22um Pall capsule filter. The bottle was rinsed three times with the sample and then filled. Collected samples were put into the plug-in freezer aboard ship immediately upon collection. When the R/V *Tioga* reached WHOI these samples were taken to the WHOI Nutrient Analytical Facility for analyses. Concentrations of ammonium, nitrate plus nitrite, nitrite, orthophosphate, and silicate will be determined by a Lachat Instruments QuickChem 8000 four-channel continuous flow injection system, using standard colorimetric methods approved by U.S. Environmental Protection Agency.

10. Zooplankton Sampling

10.1. *Reeve Net*

10.1.1. Introduction

The objective of Reeve net sampling was to gently collect live specimens to be sampled for culturing experiments. These trawls were short in duration and aimed to maximize pteropod catch.

10.1.2. Methods and Approach

A 1-m diameter Reeve net with a 150-um mesh net was deployed via the A-frame. The book-clamp to attach the net was borrowed from the rigging shop. Ship speed during tows was ~1-1.5 knots. We followed a similar tow profile as had been maximized on the previous cruise (Tioga_715, October 2013) but with less success. We did, however, manage to maximized wire time to coincide with the amount of time it took to pick thecosome pteropods from the catch. The tactic was to send the wire out fast (15 m/min) to shorten the catch time and then to park the net at 80, 70 , 60 and sometimes 50 or 90 for ~10 or 5 min at each depth, and then pull 15 m/min to the surface. (See Appendix 3 for more details).

On the bench installed on the back deck, the cod end was promptly divided among a number of buckets. Since pteropods tend to sink, the bottom buckets were examined first. The contents were swirled and the pteropods sucked up of the center of the bottom using a plastic pipette. These were then sorted through and “large” animals (big enough to do respiration experiments with) were put into pre-cleaned glass jars at densities of ~30 individuals/L while the “small” were put into pre-cleaned glass jars at higher densities. All jars were kept in the fridge (set to ~8° C) before and after filling to maintain ambient temperature.

10.1.3. Preliminary Findings

The most successful tow trawled from 90-60 m, with a total of 90 large sized animals (see Reeve sheets, Appendix 3). Most of the tows resulted in only ~60 animals. In conclusion the large animals totaled ~300 individuals and there was a similar number of small sized class animals to be kept for breeding.

11. Pump

11.1. *“Little Giant” Pump*

11.1.1. Introduction

The objective of the “Little Giant” pump was to retrieve water from depth for animal culture. This goal is to have large amounts of water of the appropriate salinity, DIC/TA and temperature to replicate the

conditions the animals experience in situ. To achieve this, six “pteropod ptransporter” garbage cans were brought onboard and held in a plastic cage.

11.1.2. Methods and Approach

First 100 feet of heavy duty hose (2 lengths) was lowered into the water attached to the winch line. At intervals a carabineer had been attached to the hose with electrical tape and then affixed to the hose to keep the hose close to the line. Once the hose was at depth (we aimed for 30 m) the pump was attached. The pump was a “Little Giant” sub pump with a watertight spliced extension cord. The cord had gotten a kind during transport and had to be re-spliced during the cruise. Another hose was attached to the outflow of this unit. The pump was lowered until just below the surface using a safety line, lashed down and then turned on. The outflow hose was strapped to the trash barrels using clamps and filtered through a 63 micron sieve.

12. Cruise Participants

Science Party

1	Amy Maas	Chief Scientist	WHOI	Biology
2	Mike Lowe	Postdoc	WHOI	Biology
3	Robert Levine	Guest Student	WHOI/Cornell	Biology
4	Shannon Crosby	Guest Student	WHOI/Depauw	Biology

Officers and Crew

1	Ken Houtler	Captain
2	Ian Hanley	Mate

13. References

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