R/V *Tioga* Cruise #700
Cruise Report

August 27th – 28th, 2013

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Report available at:
Biological and Chemical Oceanography Data Management Office
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http://bcodmo.org/

Coastal Ocean Institute Grant (PIs: Wang, Lawson) "*Acidification of the Coastal Ocean: Are deep waters of the Gulf of Maine already corrosive to pteropods?"*

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"*

1. Table of Contents

2. Acknowledgements

The success of this cruise would not have been possible without the outstanding efforts of Captain Ken Houtler and mate Ian Hanley of the *R/V Tioga*. We are grateful for the efforts of Ann Tarrant and Alex Bergan who assisted with transferring the water collected on the first day to Woods Hole Oceanographic Institution's Environmental Systems Laboratory. We further appreciate the efforts of Nancy Copley, Melissa Patrician and Alex Bergan for helping with the VPR and last minute cruise packing. This cruise was supported by a grant from the WHOI Coastal Ocean Institute (Wang, Lawson and Maas) and by NSF grant 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. $\Omega A = 1$). Currently, there are no year-round CO_2 system measurements to assess conclusively whether the deep waters in the GoME are already experiencing seasonal ΩA under-saturation. If seasonal under-saturation is present, however, this may have detrimental consequences to thecosome pteropods, a group of aragonite shell-forming zooplankton that are important members of the pelagic food web and key contributors to biogeochemical cycles. This cruise was the second in a project aims to assess seasonal variations of the CO_2 system in the deep GoME and the associated impacts on thecosome pteropods. It also supported preliminary efforts to bring pteropods back to the lab for experiments under various CO_2 conditions.

4. Cruise Objectives

The central goal of this cruise was to sample the carbonate chemistry profile of two sites in the GoME and to document the abundance and vertical distribution of the pteropod species *Limacina retroversa*. 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 the associated implications to planktonic calcifiers, notably pteropods. The specific goals of this project 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.

The specific goals of this particular cruise were to:

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

2. Measure the carbonate chemistry in the nepheloid layer to provide a seasonal contrast in the measurements.

3. Catch pteropods with a vertically stratified net system to quantify their size class, abundance and vertical distribution in the context of the carbonate chemistry and season.

4. Collect surface water and pteropods to test out methods for water maintenance in regards to live animal experiments, shell quality (70% ethanol), physiology (live) and gene expression studies (RNAlater).

5. Survey Design

On Monday August 26th the Tioga was packed at WHOI and left around 15:00 to preposition to Provincetown Harbor. The scientific crew followed by car and stayed at the Cape View Hotel south of Provincetown. On Tuesday we traveled to our deep sampling site in Wilkinson (Standard station 1) to conduct the full sampling regime (CTD/VPR, MOCNESS, Reeve) and collected water with the little pump for experiments. We started the transit back and paused at station 2 for a Reeve net. Upon return we were met at the dock by Ann Tarrant and transferred a 55 gallon barrel of water to a truck that she returned to WHOI. The next day we traveled to Murray Basin, an offshoot of western Wilkinson Basin (standard station 2/consecutive station 3 in Fig. 1; ca. 260 m), where we did a full sampling regime and collected some more water with the small pump. We then transited to our shallow station (standard 3/consecutive station 4 in Fig. 1) where we did our CTD. We started steaming back while we set up the big gas-powered pump, which was deployed at station 5. Of note: the boat left from the floating ferry harbor both days and was tied up there overnight as well. Although the scientists all returned to WHOI Wednesday night, the boat remained in Provincetown and returned to WHOI early Thursday morning. Full information about casts and stations can be found in the Event Log (Appendix 1).

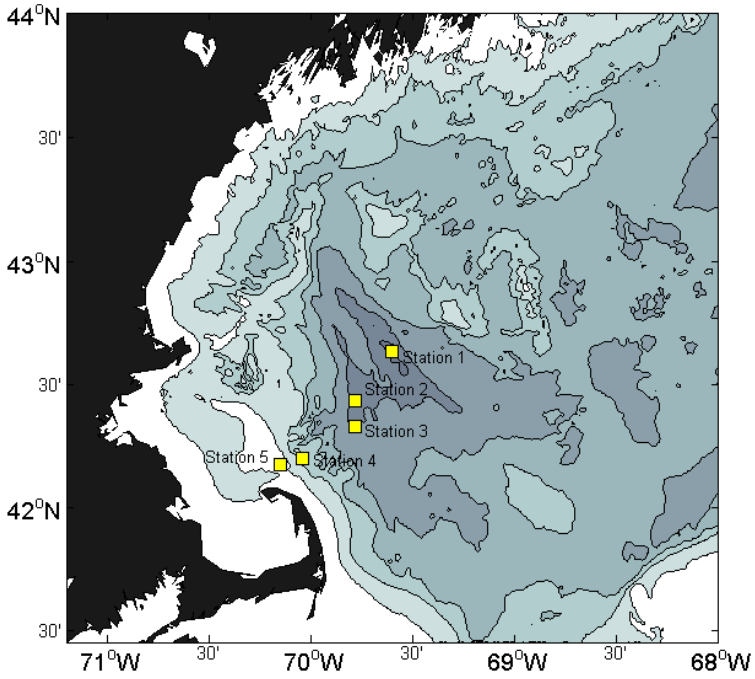


Figure1 – Gulf of Maine Map. We sampled extensively at Station 1 (standard station 1) and put in a Reeve at Station 2 on Day 1 (August 27th). On Day 2 (August 28th) we extensively sampled at Station 3 (standard station 2), and put in a CTD at Station 4 (standard station 3) and put in the pump at Station 5.

6. Cruise Narrative

Day 1: Tuesday August 27th Tuesday morning the scientific crew met the Tioga at the Provincetown dock and the *Tioga* left port at 8:25 on August 27th (delay due to faulty coffee maker) and reached the deep sampling site (Station 1 in Figure 1; ca. 300+ m) after ~ 3 hours. This site was chosen as it has been sampled previously for carbonate chemistry by Wang in 2007 and in May of 2013. We did our full sampling regime (CTD/VPR, Reeve, MOCNESS), then pumped two trash bins of water from ~30 m using the "Little Giant" pump setup. From the Reeve net there were only a very few spiral shell gastropods of a small size. Although some looked like they had a left-handed sinistral spiral (characteristic of thecosome pteropods) some looked suspiciously brown and dextrally spiraled (typical of other gastropods, often found in the plankton as larvae). These were put in RNAlater for examination upon return to land.

We left station 1 at around 16:30 and steamed towards Provincetown. Along the way we stopped at a second station to do a brief Reeve tow, which yielded larger and definitely dextrally spiraled individuals and only one identifiable *Limacina retroversa*, which were also placed in RNAlater. We then returned to Provincetown by ~20:25. At the dock we were met by Ann Tarrant and transferred water off the boat via the small pump finishing by 20:52. We went to the Squealing Pig for dinner. Ann drove the water back to WHOI where she and Alex Bergan transferred it into trash cans and carboys for temperature and gas acclimation (done by 00:30ish).

Day 2: Wednesday August 28th

The next day, August 28th, we left Provincetown at 7:07 and traveled out to a site in Murray Basin, an offshoot of western Wilkinson Basin (Station 2 in Figure 1; ca. 260 m), arriving at 9:11. This second site was chosen as it has been sampled for zooplankton by Lawson's lab in 2010, 2011, and 2013. Here we completed a full sampling regime (CTD/VPR, Reeve, MOCNESS), then pumped water from ~30 m using

the “Little Giant” pump setup (4 trash cans ~30 minutes). There were very few thecosomes in the cast, so Amy, Sophie and Taylor picked a number of juvenile gymnosomes from the Reeve cast for Amy to do experiments with. These were originally in three glass 1-liter jars, but were later diluted down to 6 jars to prevent stress during transport. On the way back into Provincetown we stopped at our previous more coastal station and did a CTD/VPR cast. While the cast was in the water we set up the big pump to test its capacity. While getting the CTD/VPR back onto the boat there was a failure in the block. Ken and Ian recovered the CTD/VPR with no harm done. This caused us to be unable to the big pump at depth, but we did some surface pumping to test flow rates and our plankton sieve. On the way in Taylor and Amy replaced the ethanol from the 1st days sampling. Upon reaching port cold pack were put into the one cooler with pteropods and transferred to Amy’s car, where they were then taken to ESL. Gareth and Amy put the animals into the incubation that evening and set up for the respiration experiment which was scheduled for the next day.

Day 3: Thursday August 29th, 2013
The boat arrived at ~9:00 and scientists began moving everything off.

7. Equipment Configuration

7.1. *Deck configuration*

The collapsible plastic crate with 4 garbage cans was strapped down to the port side of the back deck. The Reeve net was stowed on the aft side of the pallet with the ring through the band and the bucket bungeed on. The MOCNESS was tied down on the starboard aft, the CTD, with associated VPR on its new stand was positioned mid-ship (Fig. 2C). The generator for the large pump was tied down near the starboard side of the winch spool, while the rest of the pump, including the hosing, was tied down on the forecastle (fo’c’s’le; Fig. 2A). We used the same cable for all deployments. There was a table with a built in sink bolted down at the forward starboard portion of the back deck that could be plumbed with a seawater line from the Tioga’s sea chest. The ethanol was strapped down furthest aft of this table to allow for easy jar filling. Under the sink were the coolers full of jars for live animals and a tote containing the MOCNESS sample processing gear. During CTD processing this space also contained sample bottle totes.

7.2. *Lab configuration*

The main lab aft counter housed the laptop which was used for event logging and TDR attempted setup. The starboard counter had MOCNESS sampling supplies and chemistry sampling equipment. On the floor was more MOCNESS backup/sampling equipment. The rest of the backup supplies, foul weather gear and personal belongings were at midship and in the starboard bunk space. The MOCNESS computer unit was housed in the wheelhouse. There was quite a bit of extra space in the lab to support other projects. The only freezer present was part of a mini-fridge designated for science use in the aft part of the main lab, which may have led to problems with freezing and storing the nutrient samples.

Figure 2 – A) Configuration of the starboard side of the forecastle with the gear associated with the large pump, excluding the generator. B) The sink was set up on the port side of the back C) Configuration of the back deck featuring the VPR on its modified housing below the CTD, the ¼ m MOCNESS on the port side, and the collapsible pallet containing the four trash bins. The Reeve net was strapped to the aft part of the collapsible pallet (not shown here) Photos by: Peter Wiebe

8. Hydrography: CTD

8.1.1. Introduction

CTD rosette casts were the focus of the chemistry team aboard this trip as profiles of the water column provided a snapshot of the nepheloid layer and Niskin bottle sampling provided water for the carbonate chemistry analysis. These CTD measurements of environmental conditions provided key correlates of the distribution and abundance of pteropods. Depths were chosen to characterize the bottom nepheloid layer and then to continue at pre-designated intervals throughout the rest of the water column. At the deepest station (St. 1) this necessitated doing two CTD casts to allow for enough bottle sampling.
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. On the Tioga this needs to be modified as we have limited numbers of bottles,

and funds to analyze the samples. At the deep station (i.e. Standard Station #1, Station #1 on TI700), two casts are made, with a total of 16 bottles: only 4 bottles are fired on the first cast and then the full twelve on the second cast. The approach is to use 4-5 bottles for depths greater than 200m, including the benthic nepheloid layer (BNL), targeting the depths on the basis of the transmissometer data. One bottle is fired at the bottom of the cast (i.e. ca. 5 m off bottom), one at the top of the BNL and then 2-3 bottles at depths aimed at capturing regions of variability in transmission. In the case of the cast made during TI700, these were:

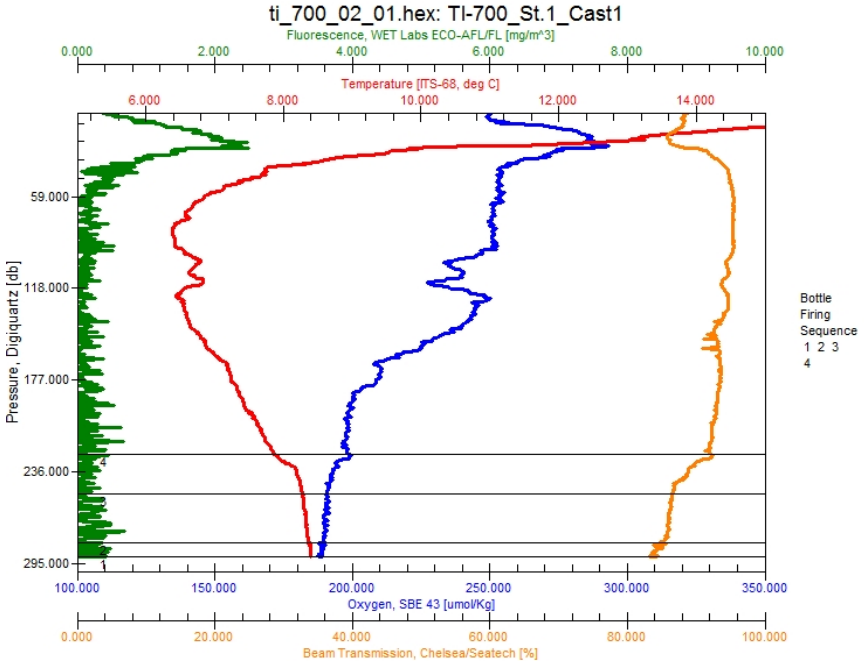
- bottom of the cast (293m)
- top of a region of changing transmission just above the bottom of the cast (283m)
- middle of the gradually changing part of the BNL (260m)
- middle of the steeply changing part of the BNL (250m)
- top of the BNL (225m)

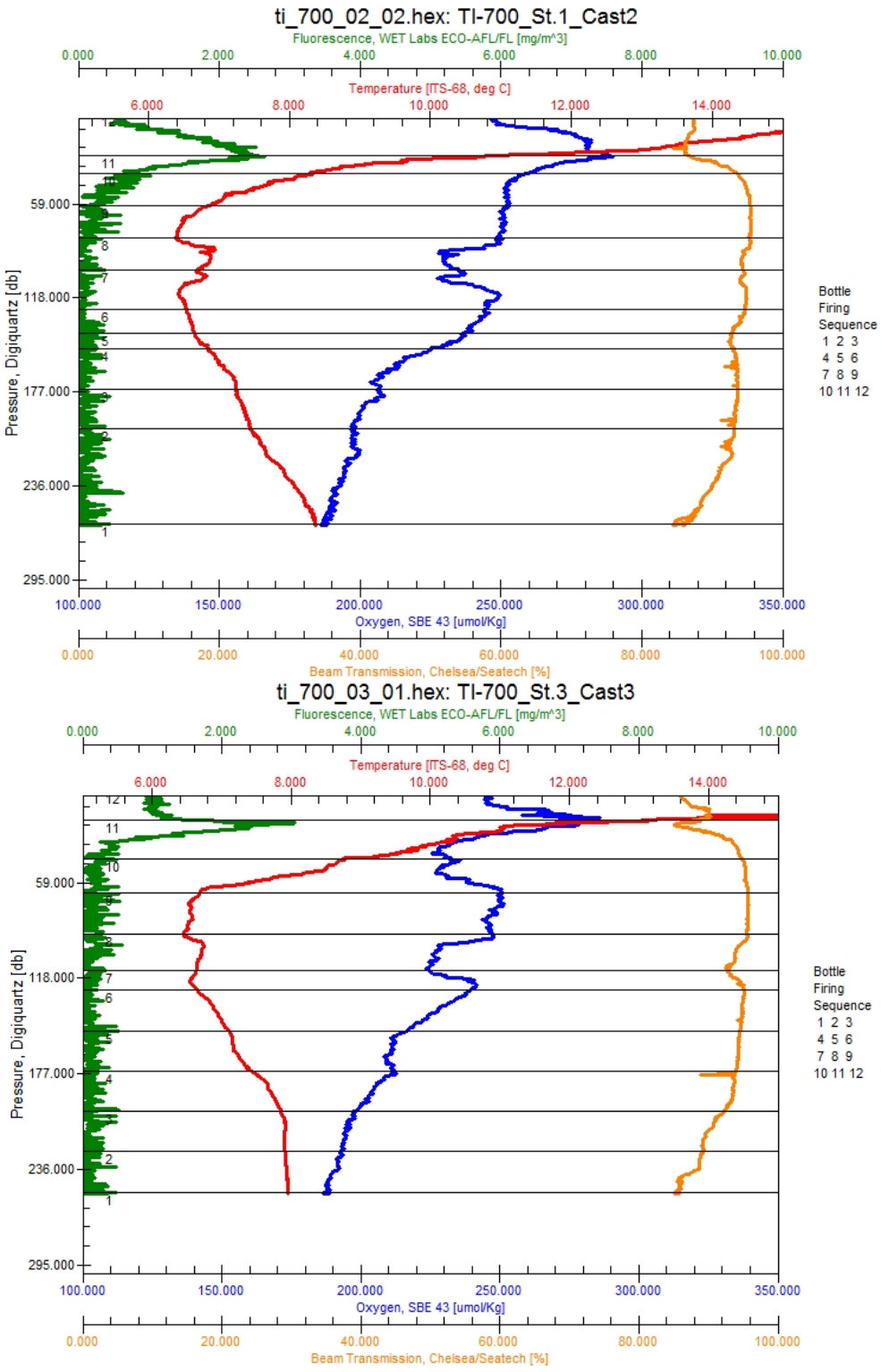
Then from 200m to the surface, bottles are fired in approximately 20m increments, with some discretionary changes in interval size to target interesting features in transmission or DO (or presumably salinity). At the shallower station (i.e. Standard Station Gareth/#2, Station #3 on TI700) one cast is made with 12 bottles. On TI700, bottles were again fired at regions of varying transmission within the NBL. To allow for a sufficient number of bottles in the NBL, the frequency of bottles shallower than 200m had to be reduced. This was done by targeting a region where transmission varied little over a large depth range (120 to 200m).

8.1.2. Methods

The *Tioga* CTD rosette had a12 bottle rosette with 3-L Niskins, and a SBE3/SBR4 sensor set. CTD casts were conducted of the full water column twice at station 1 on day 1 and once at station 3 and 4 on day 2.

8.1.3. Preliminary Results

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10.000
12.000
14.000
Pressure, Digiquartz [db]
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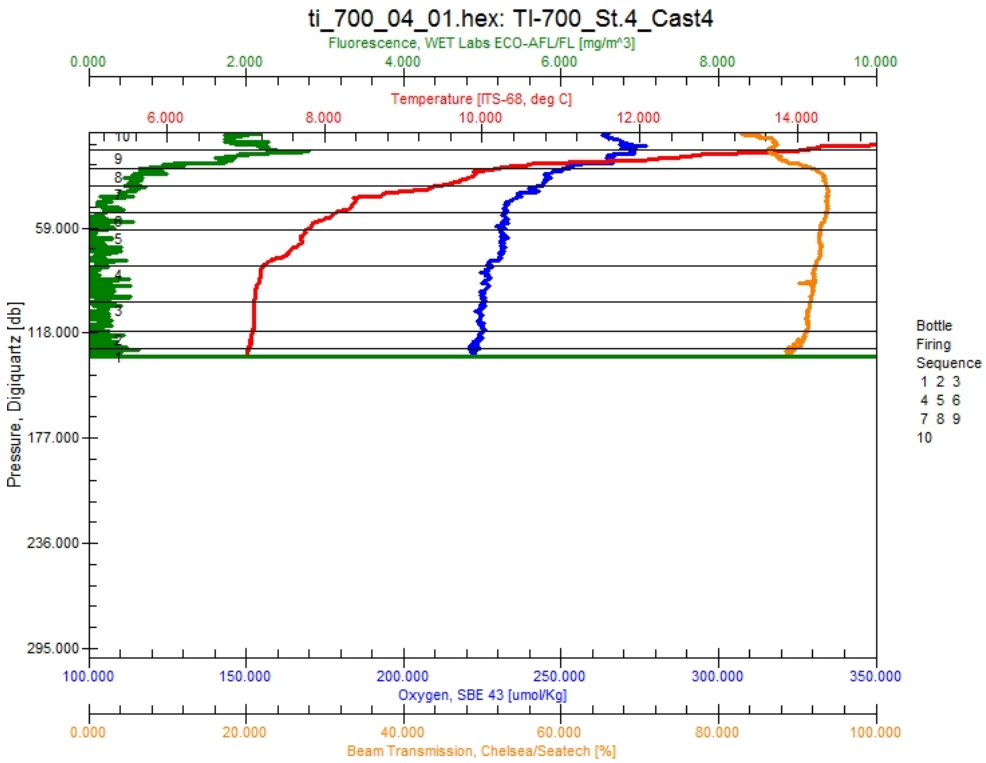

9. Chemistry

9.1. *Introduction*

Dr. Zhaohui Aleck Wang's group from the Department of Marine Chemistry and Geochemistry at WHOI collected discrete bottle samples of seawater for later measurement of carbonate chemistry parameters. Measuring these parameters allows 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 3 stations from the surface to near-bottom. Depths were chosen adaptively to target chemical features of interest while also attaining reasonably even coverage of the water column (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 80uL 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 deionized 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 mini-fridge freezer onboard the ship and were taken directly to a -20 freezer in the Wiebe lab when the *Tioga* reached WHOI then 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.

9.3.2. Difficulties

The mini-freezer was not large enough or cold enough to freeze all the samples full while out to sea. We will need to find an alternate method of freezing for further trips.

10. Zooplankton Sampling

10.1. *MOCNESS*

10.1.1. Introduction

A standard 1/4-m^2 Multiple Opening/Closing Net and Environmental Sensing System (MOCNESS; Wiebe et al., 1985) was used to collect zooplankton to determine the vertical distribution and abundance of the thecosomatous pteropods.

10.1.2. Methods

The MOCNESS was equipped with nine 150-um mesh nets (nets 0-8). The underwater unit used was #169; temperature probe was #535 and conductivity probe was #120. In addition to the standard temperature and conductivity probes the system also had a beta-type strobe-light unit for reducing avoidance of the nets by some zooplankton and possibly small fish. The strobe system has two units each with 12 LED sets (LUXEON Rebel LED) with peak output between 490-520 nm. The LEDs are powered by the MOCNESS battery and their pulse width, amplitude, flash rate period, and on/off are controlled by the MOCNESS software.

The MOCNESS was deployed from the aft winch and A-frame. Upon recovery the nets were all hosed down with seawater and the cod-ends were sequentially removed, placed in buckets, and transferred to the forward sink and table. Nets were preserved in 70% ethanol.

Oblique casts with the MOCNESS were made to ca. 5 m off bottom (based on the Knudsen echosounder on the ship's depth estimate) with a ship speed nominally of 2 kts. Sampling occurred consistently for the top 5 nets which were taken at 150-100, 100-75, 75-50, 50-25, and 25-0 m. The bottom two nets were chosen adaptively to cover the lower water column and ensure that the lowest net occurred exclusively in the nepheloid layer (see Appendix 2 for sampling sheets). Cast depth has been to 10m off bottom. Typically there has been a region of fairly constant or gradually changing transmission at the bottom of the BNL, with a region of steeply changing transmission at shallower depths, ending at the top of the BNL where transmission levels off. The protocol has been to sample with net 1 from the max depth to the top of the region of constant/gradually changing transmission and then with net 2 from there to the top of the region of steeply changing transmission (i.e. to the top of the BNL).

10.1.3. Trouble-Shooting

During deployment communication was lost with the MOCNESS at ca. 220m. De-selecting the strobe did not solve this problem, but communication was re-established by changing the BAUD rate to 1200. This suggests some issue with the connection through the winch/wire/wet end cable termination/dry end connections.

10.2. *Video Plankton Recorder*

Modified from OC473 *Nancy Copley, Alexander Bergan*

10.2.1. Introduction

The Video Plankton Recorder is an underwater video microscope system designed to record images of plankton ranging in size from less than one half millimeter up to a few centimeters. A strobe light flashing at 20 times per second captures images at this rate. A program called AutoDeck reviews the images at about 15 frames per second and extracts Regions of Interest (ROIs) that may be plankton based on certain parameters such as brightness and sharpness (see Settings for ROI Extraction below). We used the Video Plankton Recorder (VPR) in order to describe the abundance and vertical distribution of plankton taxa at station 1 on day 1 and station 2 on day 2. For this cruise a stand was adapted which allows the VPR to be strapped onto the *Tioga* CTD for simultaneous sampling.

10.2.2. Methods

The VPR was deployed simultaneous with the CTD. The hard-drive was removed following the set of two casts at Station 1 and after each cast at the other stations, and the data downloaded. Quick visual scrutiny of the data in Autodeck was performed ship-board to look for any depths of high thecosome abundance; no such depths were evident in the VPR data based on this preliminary examination, although gymnosomes were noted at 37m and 44m at Station 1.

<u>Recommended Settings for ROI Extraction:</u>
Segmentation threshold **0; 140** (brightness)
Focus: Sobel: **40**; Standard deviation: 1**0** (edge detection)
Growth Scale: **300** (extra area around object)
Minimum blob size: **10** (object size)
Minimum join distance: **1** (distance between objects)

10.3. *Reeve Net*

10.3.1. Introduction

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

10.3.2. 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. For the first tow, the downcast was done at ca. 5 m/min to 60 mwo and the upcast at 5 m/min. For the second and third tows, the downcast was at 10 m/min to 20 mwo and then 5 m/min below that to 50 mwo.

In the wet lab, the cod end was promptly divided among a number of buckets. These buckets were individually poured into a white plastic tray for sorting. Since pteropods tend to sink, the bottom buckets were examined first.

10.3.3. Preliminary Findings

The first Reeve net had a few some small spiraled individuals, as did the second. Examination of RNAlater preserved specimen revealed some very small *L. retroversa* and a number of gastropod veligers of a brown color. Since all the Reeves were similarly unsuccessful at capturing large and numerous thecosomes, we ended up concentrating on gymnosome pteropods, probably Clione limacina, as they were high in abundance and serve well as a good test surrogate because of their similar size and phylogenetic relationship. Collection by Taylor, Sophie and Amy resulted in two very densely packed jars which were subsequently split into ~4 1L glass jars with ~50 individuals per jar.

11. Pumps

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, four "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 zip tie was 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" subpump borrowed from Bruce Keafer with a watertight spliced extension cord. Another hose, borrowed from the salt water sink, 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.

When we got to port we transferred water from two of the four to a 55 gallon drum which had been driven to Provincetown by Ann Tarrant in a WHOI truck. The truck pre-positioned on the dock and then we dropped the pump directly into the trash bins and extended the 100 feet of hose via the outflow to the truck. The following day we re-filled the empty barrels with new seawater.

11.1.3. Preliminary Findings

It takes between 9-11 minutes to fill a 30 gallon trash can. It takes about two trash cans to fill the 55 gallon drum. The system seemed to work quite well; although having the pump on deck meant that it got oil on it which ended up in the water when the pump was submerged in the barrels.

11.2. *Gas-powered diaphragm zooplankton pump (aka the BIG Pump)*

11.2.1. Introduction

The objective of the large pump was to try to quickly pump animals from depth in a non-harmful way when they are concentrated in a patch at depth.

11.2.2. Methods and Approach

This zooplankton pump system consists of a gas engine pump attached by a short length of fire hose to an equilibrator (aka the Framus) that is attached via another short length of fire hose to a holding tank. A long length of large gauge hosing is then affixed to the pump and sent down to depth with the wire and a weight. Once the wire gets to an appropriate depth, the pump is started and animals can be captured with a small plankton net positioned in the holding tank.

11.2.3. Preliminary Findings

Due to a failure of the winch block we were unable to test the large pump at depth. Deploying the hose over the side, however, verified that it pumps a copious amount of water in a very short period of time. Whether this would leave animals (i.e. delicate thecosomes) in good enough condition to do physiological experiments with remains to be tested.

12. Cruise Participants

Science Party

1	Zhaohui 'Aleck' Wang	Chief Scientist	WHOI	Chemistry
2	Gareth Lawson	Scientist	WHOI	Biology
3	Amy Maas	Postdoc	WHOI	Biology
4	Mike Lowe	Postdoc	WHOI	Biology
5	Sophie Chu	Graduate Student	WHOI	Chemistry
6	Taylor Crawford	Research Assistant	WHOI	Biology
7	Peter Wiebe	Scientist	WHOI	Biology

Officers and Crew

1	Ken Houtler	Captain
2	Ian Hanley	Mate

13. References

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