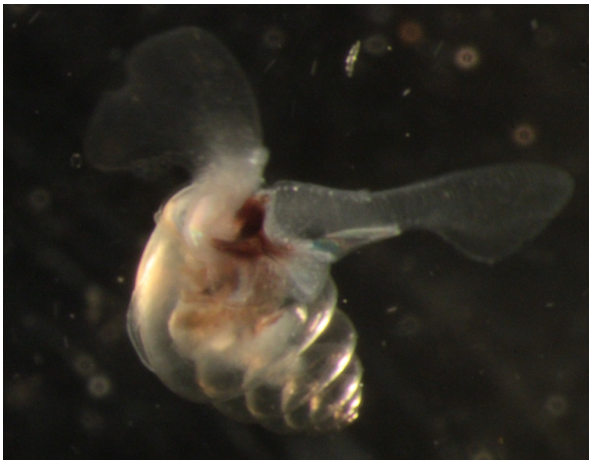
R/V *Tioga* Cruise #787
Cruise Report

November 4th – 6th, 2014



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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

This cruise would not have been possible without the hard work, resourcefulness and dedication Captain Ken Houtler and mate Ian Hanley of the *R/V Tioga*. We are particularly thankful for the fast thinking that allowed the donation of one work vest zipper to a higher cause – fixing the Reeve net. 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 already cause aragonite saturation states (ΩA) to be close to a chemical and ecological threshold (i.e. $\Omega A = 1$).

In conjunction with this spatial variability in CO_2 is a documented seasonal variability in the surface carbonate chemistry of the GoME. The spring season in the GoM corresponds to the timing of the lowest levels of documented CO_2 (~250 ppm) in contrast to highs of ~550 ppm evident in early winter (Fig. 3; Irish et al. 2010; Vandemark et al. 2011). Unlike other regions, where natural CO_2 fluctuations are influenced primarily via upwelling events and eutrophication, this large variability in CO_2 is driven primarily by riverine outputs, seasonal cycles in primary production, and sea-surface temperature changes (Previdi et al. 2009; Salisbury et al. 2009). This pattern continues to be monitored at UNH by a NOAA CO_2 buoy moored at 43° N 70° W in the GoME: http://www.pmel.noaa.gov/co2/story/GOM

The implications of this spatial and temporal variability in carbonate chemistry are that organisms in this system may already be exposed to conditions of undersaturation during certain times of year. This cruise is part of a series designed to determine the physiological response of the local population of thecosome pteropod, *Limacina retroversa* to seasonal variability in environmental CO_2. These aragonite-shelled individuals are found throughout the year in the GoME and by exploring what conditions they experience *in situ*, and then brining animals back to the lab for metabolic, gene-expression and calcification studies, we can determine whether there are seasonal sensitivities to CO_2 exposure on time-scales relevant to acclimation responses. Paired with this primary objective is the opportunity to continue an ongoing seasonal time series designed to more fully understand the vertical and spatial variability in the carbonate chemistry of the GoME.

4. Cruise Objectives

The central goal of this cruise was to capture live individuals of *Limacina retroversa* for experimentation, and to sample the carbonate chemistry profile of two concurrent sites of animal capture in the GoME. The long-term goal of this research is to understand variability in the carbonate chemistry of the Gulf of Maine and to understand how these changes in the natural environment impact the local planktonic calcifiers, specifically the thecosome pteropods. The specific goals are to:

1. Test whether there are seasonal patterns of gene expression, shell quality and metabolic rate linked to seasonal exposure.

2. 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.
3. Maintain the seasonal carbonate chemistry sampling time-series associated with Tioga cruises 668, 700, 715, 729, 746, and 777. This dataset will allow us to test the hypotheses that deep waters of the GoME are already seasonally under-saturated with respect to aragonite saturation state.
4. Obtain egg masses of *Limacina retroversa* to test the effects of CO_2 on early life stages.

The specific goals of this particular cruise were to:

1. Catch *L. retroversa* with a vertically stratified net system to quantify their size class, abundance and vertical distribution in the context of the carbonate chemistry and season.
2. Collect *L. retroversa* to preserve in ethanol (70%) for shell studies and in RNAlater for gene expression studies to explore the seasonal response to CO_2.
3. Collect surface water and *L. retroversa* for live animal laboratory experiments.
4. Measure the carbonate chemistry of the water column at multiple sites in the Gulf of Maine, targeting the sites which were sampled during Tioga cruises 668 (May 2013), 700 (August 2013), 715 (October 2013), 729 (January 2014), 746 (April 2014), and 777 (August 2014) to provide a seasonal contrast in the measurements.
5. Measure the carbonate chemistry in the nepheloid layer to provide a seasonal contrast in the measurements.

5. Survey Design

On Monday November 3rd the R/V Tioga was packed at WHOI. That evening the scientific staff pre-deployed to Provincetown, staying at the Provincetown Inn. Due to inclement weather Monday evening the ship remained at WHOI and left port early on Tuesday November 4th to transit to Provincetown where the scientific crew met the boat, leaving port at ~7:00 am. An initial Reeve net on the way out allowed for verification of pteropod presence and then the ship transited to Murray Basin, an offshoot of western Wilkinson Basin (standard station 2/consecutive station 1 in Fig. 1; ca. 260 m) where we deployed the CTD, the MOCNESS, and collected water with the pump for experiments. We then transited to standard station 3/consecutive station 2 (Fig 1; ca 135) where we deployed one Reeve net. On the way back into Provincetown we put a Reeve net in the water at the site which had been most successful during the previous trip (now considered standard station 4). The boat reached port by ~17:30 and the whole party went out and had dinner. Phil Alatalo arrived with the truck at ~ 19:00 and the water was transferred water to a 55 gallon barrel and to multiple trash cans. The truck was driven back to WHOI by Leocadio Blanco Bercial and Ann Tarrant along with a cooler full of krill.

Wednesday November 5th the remaining science crew met the boat and departed at ~7:00 am, transiting to Race Point, where favorable winds and megafauna suggested easy animal capture. Reeve nets were deployed for much of the day at this station and standard station 4. Mid-day the pumps were deployed to refill the remaining trash cans and the boat returned to port by ~ 18:00. The following day, Thursday November 6th, was similarly spend conducting Reeve nets. Mid-day the CTD was deployed to characterize the water column near Race Point. As a consequence of ill weather, the boat headed for port at ~15:00, landing at 16:00 in Provincetown. Two coolers of jars containing pteropods were offloaded and the science party departed for dinner and WHOI. The boat began its transit back to WHOI, reaching the dock at ~20:00 where the rest of the water and animals were unloaded onto a truck and brought to ESL. The rest of demobilization occurred the following day, Friday November 7th. Full information about casts and stations can be found in the Event Log (Appendix 1).

6. Cruise Narrative

Day 1: Tuesday November 4th

Tuesday morning the scientific crew met the R/V *Tioga* at the Provincetown dock and left port at ~7:00. On our way to standard station 1 we chose to stop at standard station 3 and put in a Reeve net. This site has consistently been a location of high pteropod density and we thus hoped to gain insight into the numbers and sizes of individuals available during this trip. This net caught a few decent sized individuals which were preserved in RNAlater, the remainder where rather small. We left the station at ~8:00 and continued our transit to station 1, reaching the site at 9:49. This site was chosen as it has been sampled previously for carbonate chemistry multiple times in 2013 and 2014 and for zooplankton distribution in 2011, 2013 and 2014. Here we conducted a CTD which had both an oxygen sensor and a transmissometer, although it had a reduced number of bottles (12 on the rosette). The bottle 2nd from the bottom failed to trigger, but another deployment was not made. During the CTD the MOCNESS net system was readied and was put in the water at 10:50. A new strobe fuse and setup had been applied during prior to this cruise and there were no difficulties with the unit this cast. We were, however, not receiving net trigger responses, so all flow counts are estimates. When the net came to the surface it was unclear how many nets had triggered (we appeared to not have triggered from 25-0 m) and we reviewed our documentation and realized that we had planned for too many depth intervals. As a consequence all intervals were properly samples except for the 25-0m portion of the water column. There were a few large *Limacina retroversa* in the net, but these remained in the main ethanol preserved sample. Individuals of *Meganyctiphanes norvegica* were recovered from multiple nets and placed in water drawn from the CTD then put in the fridge. While the MOC was being processed the two submersible pumps were deployed to obtain water from ~30 m depth for animal culture. In total time the pumping took ~ 1h and we filled six 40 gallon trash cans and over 48 1-L glass jars. After pumping the ship transited to standard station 2 where a Reeve net was deployed. There were many very small sized pteropods in this Reeve, but nothing of use for the experiment. After this cast the ship headed into port, arriving at 17:24. The crew all went for dinner at the Squealing Pig, finishing just as Phil Alatalo arrived with the truck for transport of water and krill back to ESL. The water transfer was made via the small pump and then Ann Tarrant and Leocadio Blanco Bercial drove the truck and samples back to WHOI. Gareth Lawson left for Boston and the remainder of the crew, which now included Phil Alatalo, stayed overnight in the hotel in Provincetown.

Day 2: Wednesday November 5th

The morning of the second day the ship left port ~7:00 and transited out to standard station 3. There was not a lot of macrofauna activity at the site so we shifted around the point of Cape Cod to Race Point where there were a number of marine mammals, seabirds and fish visible. We ran a few moderately successful Reeve nets and then re-filled the empty trash cans then continued Reeve net "fishing" between station 4 and station 3 for the remainder of the day. During the 13th cast the metal body of the zipper on the Reeve net completely broke off making the net unusable. With some quick thinking and ingenuity Mike Lowe and mate Ian Hanley realized that an old work-vest zipper was of the same size and swapped it onto the Reeve net saving the day. Although most casts contained a large number of individuals, only a few were of workable size. At the end of the day a little more than half of the required individuals has been placed in glass jars in the fridge. The boat reached port at 1745 and the science crew went out to dinner at Tin Pan Alley then returned to the Providence Inn.

Day 2: Thursday November 6th

The morning of the third day the ship left port ~6:48 and transited out to standard station 3. There appeared to be decent macrofauna activity at the site, and a few of Reeve nets were cast here before we transitioned to station 4 where we spent the remainder of the day. Towards the afternoon a CTD cast was made of station 4 to document the water column. We finished sampling at 1457 having reached the

desired target number of individuals and facing increasingly unpleasant seas and returned to Provincetown. At port (~16:00), two coolers of pteropods were transferred to Amy's car and the boat began its return to WHOI. The science crew met the Tioga at the WHOI dock at ~20:00 and the remaining individuals and trash cans of water were brought from the dock to ESL. Full information about casts and stations can be found in the Event Log (Appendix 1).

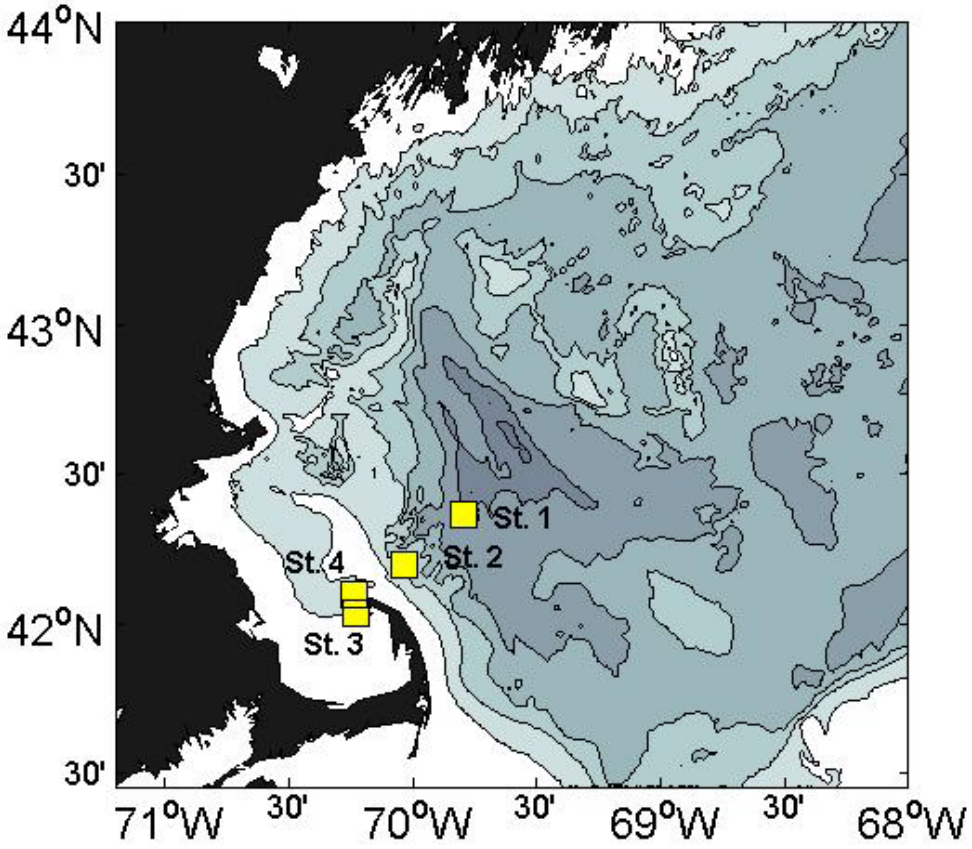


Figure1 – Gulf of Maine Map. On the 4th we stopped first at standard station 3 to conduct a Reeve net, then continued on to standard station 1, where we sampled with the CTD, pump, MOCNESS and Reeve net. We then transited to standard station 2 where we did one Reeve net. The following day we moved between standard station 3 and on to station 4 (Race Point), pumping fresh water at station 4 and conducting Reeve nets at both stations but having more sampling success at station 4. On the 6th we continued a similar sampling strategy as on the previous day, Reeve netting at stations 3 and 4. We also conducted a CTD at station 4.

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 MOCNESS was tied down on the starboard aft, the CTD was positioned mid-ship. The Reeve net was stowed on the port aft rail. We used the same cable for all deployments. When the back deck space was needed for other deployments the CTD was moved to the forward port side of the deck. A standard refrigerator was strapped down starboard of the winch and housed a number of glass jars for specimen collection. There was a table with a built in sink bolted down at the forward port portion of the back deck that was used for chemistry sampling and net processing. The ethanol for preservation 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. The VPR, although it was not used, was tied down on the bow of the ship.

7.2. *Lab configuration*

The main lab aft counter housed the laptop which was used for event logging and VPR processing. The starboard counter had MOCNESS sampling supplies and chemistry sampling equipment. On the floor was more MOCNESS backup/sampling equipment which continued into midship. The rest of the backup supplies, foul weather gear and personal belongings were stored in the port bunk space. The MOCNESS computer unit was housed in the wheelhouse.

8. Hydrography: CTD

8.1.1. Introduction

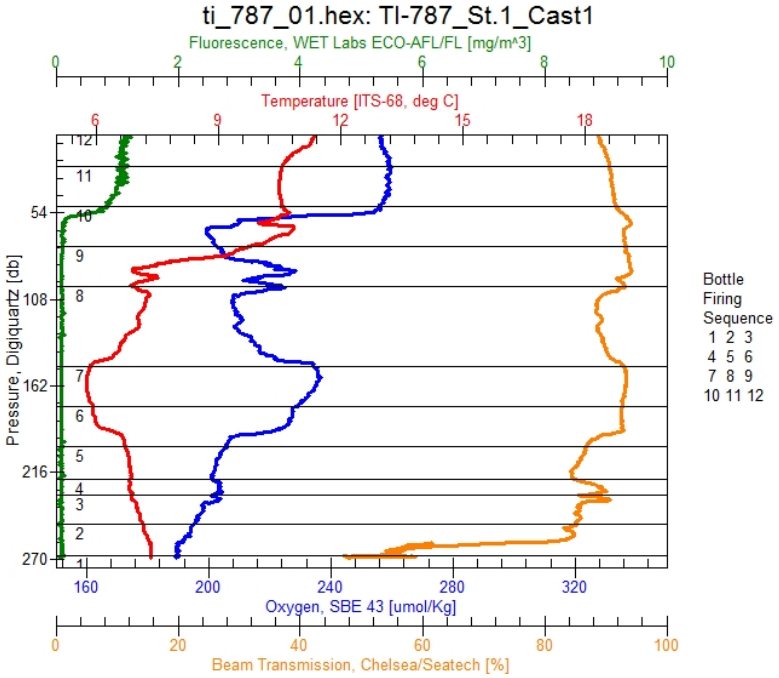
CTD rosette casts were used to get both a profile of the water column and to allow Niskin bottle sampling for the carbonate chemistry analysis. These CTD measurements of environmental conditions will provide key correlates of the distribution and abundance of pteropods with hydrographic features. Depths were chosen to characterize the bottom nepheloid layer (BNL) and then to continue at pre-designated intervals throughout the rest of the water column.

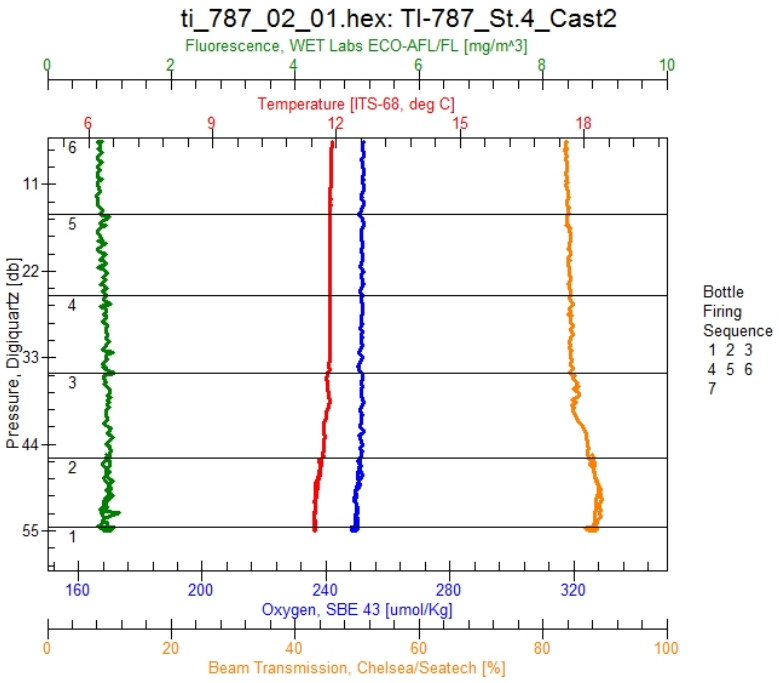
8.1.2. Methods

The R/V *Tioga* CTD rosette had a 12 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 the upper 100m at 10 m intervals, 100-200m at 20 m intervals, and less frequently below. At the shallow station (consecutive station 4) the water depth necessitated only the firing of 7 bottles, following a similar pattern established on previous cruises at station 3 (Appendix 2).

8.1.3. Preliminary Results

The O_2 sensor and transmissometer were present on this cruise allowing us to appropriately decide on bottles associated with the bottom nephloid layer. One bottles (#2) did not fire during the first cast.





9. Chemistry

9.1. *Introduction*

Carbonate chemistry samples are to be analyzed by Dr. Zhaohui Aleck Wang's group from the Department of Marine Chemistry and Geochemistry at WHOI. Aboard ship we 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*

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*

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.

Table 1. Individual counts of *M.*

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. This system is particularly useful for capturing mobile zooplankton like the krill *Meganyciphanes norvegica* which were being opportunistically sampled for a temperature experiment. To optimize the chance of catching these animals a longer time was spent dragging net 2, 3, and 4, where krill have previously and consistently been caught.

The MOCNESS was deployed from the aft winch and A-frame. Oblique casts with the MOCNESS were made to ca. 10 m off bottom (based on the Knudsen echosounder on the ship's depth estimate) with a ship speed nominally of 1.5-2.5 kts. Consistent with previous cruises, sampling for the top 5 nets was intended to be taken at 150-100, 100-75, 75-50, 50-25, and 25-0 m. For the bottom two nets the goal was to choose depths to cover the lower water column and ensure that the lowest net occurred exclusively in the nepheloid layer (see Appendix 3- 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). The resulting bottom nets were ~260-250, 250-220, 220-180 and 180-150 m. A confusion about the number of nets caused there to be too many depth strata chosen. As such there was no net for 0-25 m. Due to some mechanical or electrical failure the system also did not automatically sense the bar dropping, causing us to have to advance the net number in the computer manually, but all triggering seemed to happen at the appropriate depths.

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 that were suspected of containing krill were assessed first (see krill section 10.3). After desired animals were documented and removed, net contents were preserved in 70% ethanol. All samples had the ethanol replaced with fresh 70% ethanol ~24 hours after sampling.

10.1.3. Animal sampling

Meganyciphanes norvegica individuals were target during the MOCNESS sampling for use in an exploratory temperature experiment. During the MOCNESS tow, 1 L jars were filled up with the leftover water on the CTD bottles (# 4 to 7). Once the MOCNESS was recovered, krill from the different nets were picked and placed on jars, at an approximated density of 10 individuals per jar. Nets were processed starting at nets # 4, 3, 5, and 2. Higher densities were found in nets 4 and 5 (Table 1); 99 individuals were collected in total, from different life stages (or, at least, from a wide range of sizes). Individuals from each different net were kept separately. Post transport dead individuals were preserved in 70% ethanol for inclusion with the larger MOCNESS sample while live individuals were maintained in captivity for 3 days.

norvegica used for the experiment from each of the MOCNESS nets. High mortality was observed on individuals in jars from the Net 4 during transport.

Net	Live	Dead	**Total**
2	10	1	**11**
3	16	1	**17**
4	27	13	**40**
5	26	5	**31**
Total	**79**	**20**	**99**

Very few pteropods were observed in the MOCNESS sampling. All individuals were left in the 70% ethanol.

10.2. *Reeve Net*

10.2.1. 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.

10.2.2. Methods and Approach

A 1-m diameter Reeve net with a 333-um mesh net was deployed via the A-frame by being directly shackled to the weight at the bottom of the line. Ship speed during tows was ~1-1.5 knots. The depth and duration of deployment varied based on station. Once the net was onboard 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 concentrated, again taking advantage of the pteropod tendency to sink. Much of this finer work was done on the sink/table installed on the back deck, while some was done sitting on the starboard side of the winch housing.

A number of Reeve nets were deployed, predominantly at station 3 and 4, although one was also collected at standard station 2. All of these generally had high numbers of small individuals along with few to a reasonable number (40-160) of workable sized individuals. As most of the sampling was done at shallow stations it is difficult to determine where in the water column the individuals were densely packed, although it appeared that casts which included the whole water column (~ 50 m) were the most successful. Reeve net datasheets and a summary of the number of individuals collected per tow are included in Appendix 4.

11. "Little Giant" Pump

11.1. *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 or strapped to the port rail.

11.1. *Methods and Approach*

First, two separate 100 feet of heavy duty hose were lowered into the water attached to the winch line

(~30 m depth). 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 hoses were at depth a pump was attached to each hose. The pumps were "Little Giant" sub-pumps with a watertight spliced extension cord. Another hose was attached to the outflow of these units. The pumps were lowered until just below the surface using a safety line, lashed down and then turned on. The outflow hoses were strapped to the trash barrels using clamps and filtered through a 63 micron sieve. At this point we also filled all of the glass jars, most of which were placed in the refrigerator or in a number of coolers on deck

When we got to port we transferred water into a truck that had been driven to Provincetown by Phil Alatalo. The truck pre-positioned on the dock and then we dropped a pump directly into the trash bins and extended the one of the 100 ft hoses via the outflow to the truck. We filled up a 55 gallon drums and moved three of trash bins onto the truck for transport to ESL on the evening of Tuesday the 4th. To ensure that we had enough water for all the intended experiments, the two trash cans which had been emptied were re-filled on Wednesday the 5th.

12. Cruise Participants

Science Party

1	Amy Maas	Chief Scientist	WHOI	Biology
2	Gareth Lawson	Scientist	WHOI	Biology
3	Alex Bergan	Graduate Student	WHOI	Biology
4	Taylor Crawford	Research Assistant	WHOI	Biology
6	Ann Tarrant	Scientist	WHOI	Biology
7	Leocadio Blanco Bercial	Research Faculty	UConn/WHOI	Biology
8	Phil Alatalo	Research Associate	WHOI	Biology

Officers and Crew

1	Ken Houtler	Captain
2	Ian Hanley	Mate

13. References

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