R/V *Tioga* Cruise #777
Cruise Report

August 19th – 20th, 2014

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2. Acknowledgements

This cruise would not have been possible without the dedication, skill and flexibility of Captain Ken Houtler and mate Ian Hanley of the *R/V Tioga*. We also appreciate the onshore assistance and late night driving of Ann Tarrant. 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 GoME 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, and 746. 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), and 746 (April 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 Friday August 15th the R/V Tioga was partially packed at WHOI and left port to do other work over the weekend. On Monday August 18th they transited to Provincetown where the scientific crew met the boat to finish packing. That evening the scientific staff stayed at the Mainstay Motor Inn. On Tuesday August 19th the boat left port at ~7:00 am and traveled 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 little 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) where we had a profoundly productive tow. We then put in the CTD to take carbonate chemistry measurements and conducted a final Reeve. We returned to Provincetown dock by 20:30 where we were met at the dock by Ann Tarrant. We transferred water to a 55 gallon barrel and to multiple trash cans aboard a truck that she returned to WHOI. All coolers were filled with animals which were driven back to ESL for experimentation. The following day, Wednesday August 20th, a skeleton crew met the boat and filled two more trash cans full of water on the transit back to WHOI. The boat was met at the WHOI dock at ~10:55 and the remaining animals and the final trash cans with water were removed and transported to ESL via truck. The rest of the gear was unloaded and moved into the staging room and Lawson lab for cleanup and stowage. Full information about casts and stations can be found in the Event Log (Appendix 1).

6. Cruise Narrative

Day 1: Tuesday August 19th

Tuesday morning the scientific crew met the R/V *Tioga* at the Provincetown dock and left port at ~7:00. The skies were clear, the sea was flat and the temperature was lovely. Transit was thus quite rapid and we reached standard station 2 (consecutive station 1 in Figure 1) at 9:17. 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. Our CTD had both an oxygen sensor and a transmissometer during this trip, although it had a reduced number of bottles (12 on the rosette). During the initial cast two of the

surface bottles failed to trigger and were immediately re-deployed. During CTD water sampling the two pumps were deployed to obtain water from ~30 m depth for animal culture. Initial deployment was faulty due to a slippage of the hoses on the line, but was quickly fixed. In total time the pumping took ~ 1.5 h and we filled six 40 gallon trash cans and over 48 1-L glass jars. After pumping the MOCNESS was prepped for deployment. At this time a fuse blew in the underwater unit due to a fault in the strobe system. The MOC was thus deployed without a new fuse or the strobe unit. Despite this slight hiccup in operations the MOCNESS tow went smoothly, lasting 1.5 h in duration. While the MOC was being processed we began to transit to our next station. Samples of *Meganyctiphanes norvegica* were recovered from net 4 (n=2) and net 3 (n=1) and placed in GoME water in the fridge.

Once we reached standard station 3 (consecutive station 2 in Figure 1), a Reeve cast was made. Individuals from this cast were stored in RNAlater. A second Reeve net was cast at this station, and the individuals were preserved in 70% ethanol. Post-processing the ship began transit back towards Provincetown and stopped at standard station 4 (consecutive station 3 in Figure 1). At this site a Reeve net was cast. Based on its success (>3000 individuals) we put in a CTD, then another Reeve net (>3000 individuals) before returning to port at ~20:30. At the dock we were met by Ann Tarrant and transferred water off the boat via the small pump. The crew, excepting Gareth had lunch then Amy, Leo, Taylor, Ann and Ali returned to WHOI where they transferred the truck full of water and 3 coolers full of animals (~2000 individuals) into the cold room of ESL. The remainder of the crew stayed overnight in the hotel in Provincetown.

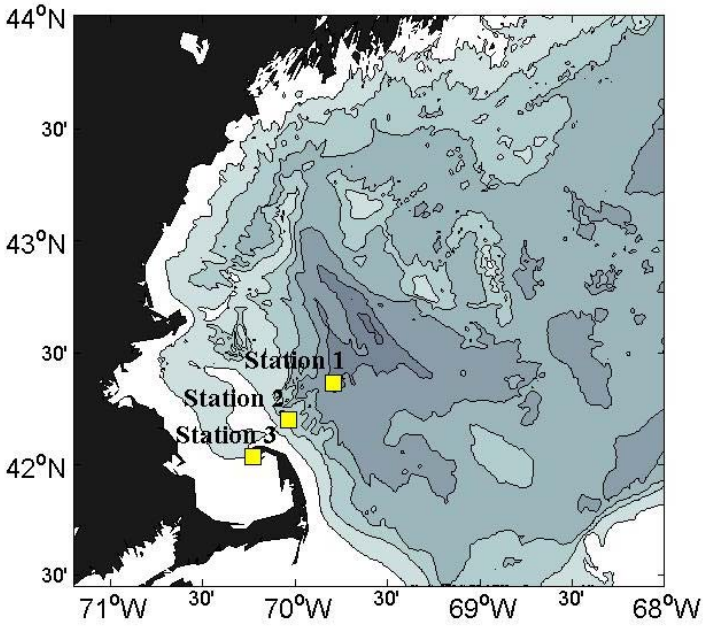


Figure1 – Gulf of Maine Map. We sampled with the CTD, pump, MOCNESS and Reeve net at Station 1 (standard station 2) on Day 1 (Aug. 19th). At Station 2 (standard station 3) on the 19th we did one Reeve net, while at Station 3 (standard station 4) we did two Reeve nets and a CTD. The following day, the 20th the pump was deployed at Station 3 prior to the ship returning to WHOI.

Day 1: Wednesday August 20th

Wednesday morning Sophie dropped Alex and Ian back at the boat and drove Alex's car back to WHOI. Ian and Alex left port at 7:07, reaching an offshore point for water collection by 7:25. Here they deployed the pump system, filling the remaining two trash cans with local water. The boat returned to port at WHOI at 10:55. Amy transferred the remaining animals to ESL in coolers immediately. Taylor, Ian and Alex moved the gear into the staging room and Lawson lab for cleanup and stowage. Ian and Alex moved the water onto a truck and transported them to ESL, experiencing a rear-end hit and run during the process. No-one was hurt although some water was lost and one trash can was damaged during the incident. Full information about casts and stations can be found in the Event Log (Appendix 1).

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.

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. *Introduction*

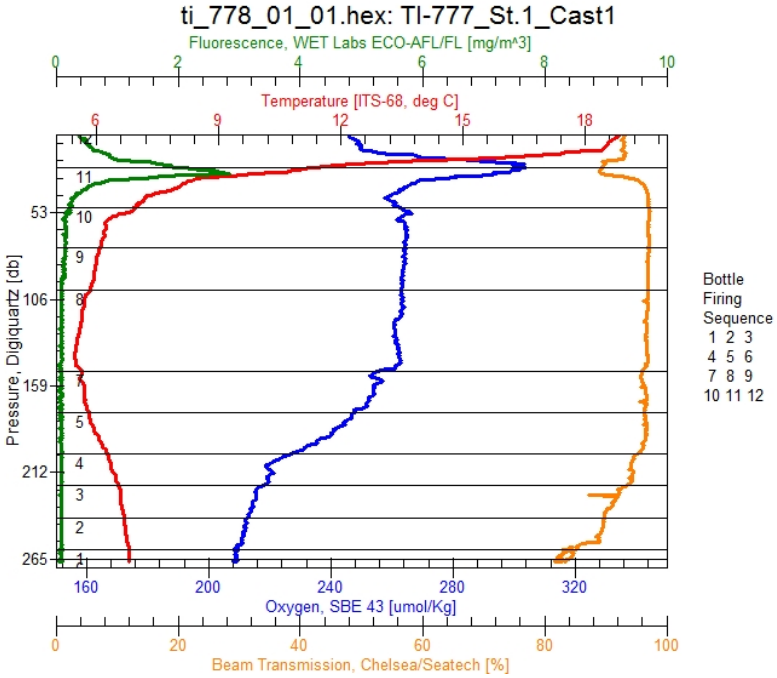
CTD rosette casts were used to get both a profile of the water column and to allow Niskin bottle sampling for the carbonate chemistry analysis. These CTD measurements of environmental conditions will provide key correlates of the distribution and abundance of pteropods with hydrographic features. Depths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

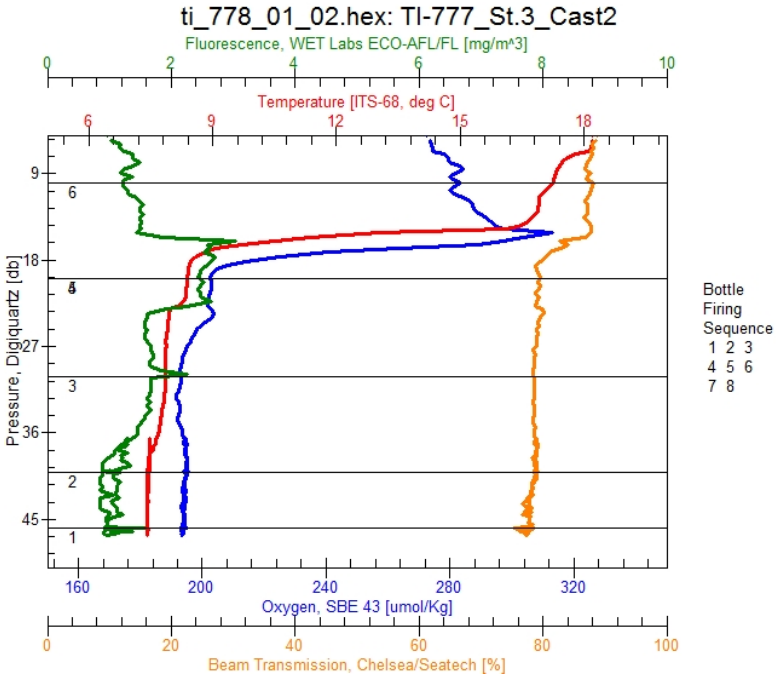
8.2. *Methods*

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 upper 100m at 10 m intervals, 100-200m at 20 m intervals, and less frequently below. At the shallow station (consecutive station 3, standard station 4) the water depth necessitated only the firing of 7 bottles, following the pattern established on previous cruises (Appendix 2).

8.3. *Preliminary Results*

The O_2 sensor and transmissometer had been replaced for this cruise allowing us to appropriately decide on bottles associated with the bottom nepheloid layer. Two bottles (10 and 11) did not fire during the first cast and the CTD was immediately re-deployed (same cast) to collect water from these depths.





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*

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. *MOCNESS*

9.3.2 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.

9.3.3 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. During the deck test of the MOCNESS unit the strobe lights blew a fuse on the underwater unit. The decision was made to continue the cast without the strobe light and without a new fuse (all replacement fuses were of the wrong resistance). This was unfortunate as there was a goal of collecting a large number of krill (*Meganyciphanes norvegica*) for a temperature experiment, and these animals are known to be more easily caught with a strobe equipped. To optimize the chance of catching these animals a long time was spent dragging net 2, 3, and 4, where krill have previously and consistently been caught. Fortunately, a newly made connecter between the Tioga conducting line and the MOCNESS system appears to have fixed the difficulties with unreliable communication which were experienced on the previous cruise causing the cast to go quite smoothly.

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. Maintaining this speed on the Tioga can be difficult. 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 ~255-240, 240-200 and 200-150 m.

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. Net contents were preserved in 70% ethanol. All samples had the ethanol replaced with fresh 70% ethanol ~24 hours after sampling.

9.3.4 Animal sampling

Meganyciphanes norvegica individuals were collected from net 3 (1, but with a lot of siphonophore debris) and net 4 (1 adult, 1 juvenile). These were kept in filtered chilled water in the fridge for later experimentation. Very few pteropods were observed in the MOCNESS sampling. All individuals were left in the 70% ethanol.

10.2. *Reeve Net*

9.3.5 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. The leftover samples from the Reeve net were preserved in 70% ethanol for Dr. Blanco Bercial.

9.3.6 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.

The Reeve at consecutive station 1 (standard station 2) was moderately successful. We filled 6 vials of RNAlater with 5 individuals each. The next cast at consecutive station 2 (standard station 3) was less successful and only 8 individuals were put in ethanol from this net. The Reeve at consecutive station 3 (standard station 4), however, was wildly successful with >3,000 animals collected in >40 minutes. We estimate the catch could have been as many as 6,000 individuals. The second tow at this station was similarly successful and, much to our surprise, with the animals from these two tows we were able to meet our cruise objectives within the first day. From this last tow (Reeve 4) another set of RNAlater samples were taken filling 7 vials with 5 individuals each. Since so many animals were available in the second two tows it was not necessary to collect any but the largest and most active animals which were put into seawater filled pre-cleaned glass jars that had been kept in the fridge before and after filling to maintain ambient temperature (see Pump section for details on filling the jars). The final count was ~4000 individuals.

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.

11.2. *Methods and Approach*

First two separate 100 feet of heavy duty hose was 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. There was a bit of difficulty with the hoses not staying attached to the bottom of the down-line, forcing us to retrieve the initial deployment of the pump. The slippage was fixed by Mate Ian Hanley using string and electrical tape and subsequent deployments were without difficulty.

When we got to port we transferred water a truck that had been driven to Provincetown by Ann Tarrant. The truck pre-positioned on the dock and then we dropped the pump directly into the trash bins and extended the two 100 ft hoses via the outflow to the truck. We filled up a 55 gallon drums and four trash bins on the truck, also filling all of the glass jars, most of which were placed in the refrigerator of a number of coolers on deck.

To ensure that we had enough water for all the intended experiments, the during transit back to WHOI on Wednesday the 20th the pump was used again, filling the remaining two trash cans with water from Cape Cod Bay.

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	Sophie Chu	Graduate Student	WHOI	Chemistry
7	Leocadio Blanco Bercial	Research Faculty	UConn/WHOI	Biology
8	Ali Thabet	Guest Student	Assiut University/WHOI	Biology
9	Ian Jones	Summer Fellow	WHOI	Biology

Officers and Crew

1	Ken Houtler	Captain
2	Ian Hanley	Mate

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