

**R/V *Tioga* Cruise #729
Cruise Report**

January 29th – 30th, 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*. We are grateful for the efforts of Ann Tarrant and Tim White who assisted with transferring the water collected on the first day to Woods Hole Oceanographic Institution's Environmental Systems Laboratory. 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 (CO₂), the ocean is taking up extra CO₂ 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 CO₂ 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 (Ω_A) to be close to a chemical and ecological threshold (i.e. $\Omega_A = 1$).

In conjunction with this spatial variability in CO₂ 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 CO₂ (~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 CO₂ fluctuations are influenced primarily via upwelling events and eutrophication, this large variability in CO₂ 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 CO₂ 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. This cruise part of a series designed to determine the physiological response of the local population of thecosome pteropod, *Limacina retroversa* to seasonal variability in environmental CO₂. These aragonite-shelled individuals are found throughout the year in the GoME and by exploring what conditions they experience *in situ*, and then bringing animals back to the lab for metabolic, gene-expression and calcification studies, we can determine whether there are seasonal sensitivities to CO₂ 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 document the abundance and vertical distribution of the pteropod species *Limacina retroversa*, to capture live individuals for experimentation, and to sample the carbonate chemistry profile of two sites 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. 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.

2. Test whether there are seasonal patterns of gene expression, shell quality and metabolic rate linked to seasonal exposure.
3. Determine how experimentally enhanced levels of CO₂ 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.
4. Maintain the seasonal carbonate chemistry sampling time-series associated with Tioga cruises 668, 700, 715, and 725. 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.

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 CO₂.
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), and 715 (October 2013) 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 Tuesday January 28th the R/V Tioga was packed at WHOI and left port around 15:00 to transit to Provincetown. The scientific crew followed by car and stayed at Provincetown Inn. On Wednesday January 29th the boat left port at ~7:30 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 conducted the full sampling regime (Acoustics, CTD, MOCNESS, Reeve) and collected water with the little pump for experiments. Upon return we were met at the dock by Ann Tarrant and transferred water to a 55 gallon barrel and to multiple trash cans aboard a truck that she returned to WHOI. The next day we left at 7:20 am and traveled to our shallow station (standard 3/consecutive station 2 in Fig. 1) where we did our CTD, pumped new water for animal handling and conducted 9 Reeve nets. We returned to port at ~18:22 where animals were transferred from the refrigerator into coolers and into Amy's car. The R/V Tioga began the transit back to WHOI. The boat was met at the dock at ~23:00 WHOI and the final trash cans with water and animals was craned off using the hand-cart as a handle. Some preliminary unloading was done that evening and the rest completed the next day (Friday January 31st). Full information about casts and stations can be found in the Event Log (Appendix 1).

6. Cruise Narrative

Day 1: Wednesday January 29th

Wednesday morning the scientific crew met the R/V *Tioga* at the Provincetown dock and left port at 7:30. We reached the first station (consecutive 2, station 1 in Figure 1) at ~10:00. This site was chosen as it has been sampled previously for carbonate chemistry in May and August of 2013. We did our full sampling regime starting with the CTD. Unlike previous cruises we did not attach the VPR during the cast to the CTD due to difficulties with spin in the water. While the CTD was being sampled on deck an acoustic system was deployed over the starboard forward rail to look for high densities of pteropods, and the pump was deployed off the aft to retrieve water for later experiments. There were some difficulties with the pump as water was frozen in one of the hoses. This hose was removed and pumping proceeded quite effectively, taking about ~45 minutes to fill 5 trash cans. Next, we deployed a Reeve net to 90-130 m that caught only a few pteropods (which were preserved in RNAlater). While the Reeve was being sorted

through the MOCNESS was deployed. Peter and Robert retrieved samples of *Meganyctiphanes norvegica* from net 4 (n=1) and net 0 (n=16) which were put in vials and preserved in liquid nitrogen. A few *Limacina retroversa* (n=6?) were removed from net 0 and preserved in RNAlater. We left station at around 16:45 as ice and cold had become a problem and steamed towards Provincetown which we reached by ~19:45. At the dock we were met by Ann Tarrant and transferred water off the boat via the small pump. Ann drove the water back to WHOI where she and Tim White transferred it into the tank for filtration. There were some difficulties with frozen water, but these were surmounted and the water was transferred into the carboys at 14:00 on the 30th for pre-bubbling.

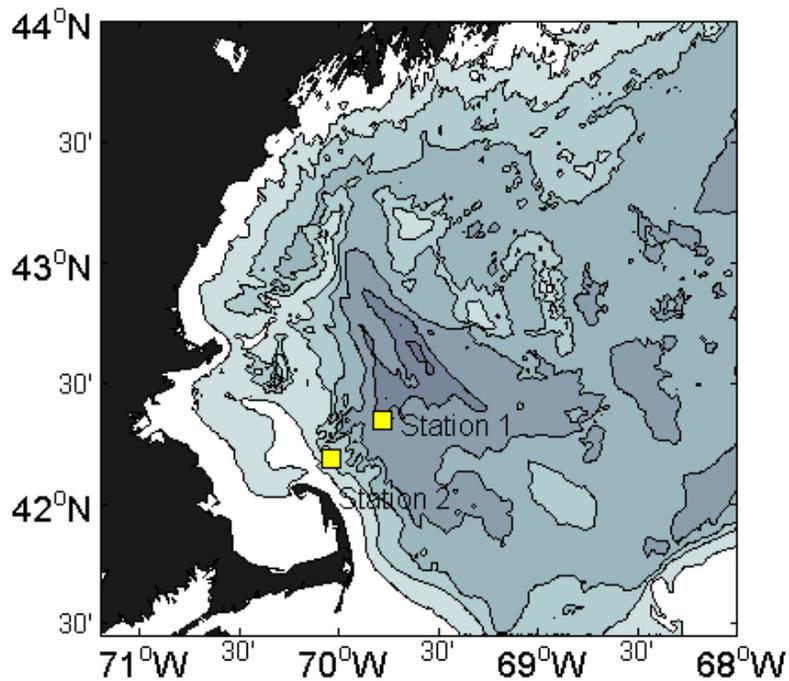


Figure1 – Gulf of Maine Map. We did full sampling, including a MOCNESS, Reeve, CTD and water pumping at Station 1 (standard station 2) on Day 1 (January 29th). On Day 2 we did a CTD, water pumping and multiple Reeve nets at Station 2 (standard station 3).

Day 2: Thursday January 30th

We left Provincetown at 7:18, and set out for the shallow station (standard 3, consecutive 2) and the CTD was deployed at 8:55. During CTD sampling the pump was put in the water and we filled the 1 L sampling jars which were kept in the refrigerator. Simultaneously three trash cans were filled. When the pump was done we started Reeve netting. Reeve nets were conducted at a number of depths to try to maximize catch and minimize wire time using a tow yow pattern. Animals seemed to be somewhat dispersed throughout the water column from 90 – 40 m. Shallower tows (40-20 m) based off of promising patches of acoustic backscatter were unsuccessful at catching large numbers of animals but did have a number of broken siphonophore pieces which may have contributed to the patch acoustic signal. It was difficult to tell from the acoustic display and with only the two frequencies whether the scattering was higher on the 120 or 200 kHz channel but overall they looked fairly similar, probably more consistent in hindsight with siphonophores than pteropods. Upon collection, thecosomes were sorted into medium/large and small ('pet') classifications, transferred into 1-L jars and stored in the refrigerator. Only ~300 large individuals, ~100 smallish individuals, and >150 very small animals were captured. We left station at 17:00 and started to transit back to Provincetown. During the transit Alex replaced the ethanol from the 1st days sampling. We returned to port at ~18:22 removed the pteropods and personal gear and Ian and Ken began the transit back to WHOI. The science crew had dinner at Sweet and

Savory, then drove back to WHOI. Amy dropped off everyone then headed to ESL with Ali where Leocadio Blanco Bercial was waiting to help deal with animals. Mike and Alex met up with the ship and got the trash can of water off the boat and moved up to ESL. Alex, Mike, Taylor and Robert then moved the big stuff into the staging room. Smaller things were organized aboard the boat for quick cleanup the following morning.

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 (Fig. 2A). The MOCNESS was tied down on the starboard aft, the CTD was positioned mid-ship. The VPR was port aft, although it ended up not being used. 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 (Fig. 2B). A standard refrigerator was strapped down forward of the winch and housed a number of glass jars for specimen collection (right of Fig. 2C). 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 (Fig. 2C). 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. On the second day a tarp was placed above the sampling station to prevent dripping of melting ice into the work area (Fig. 2D).

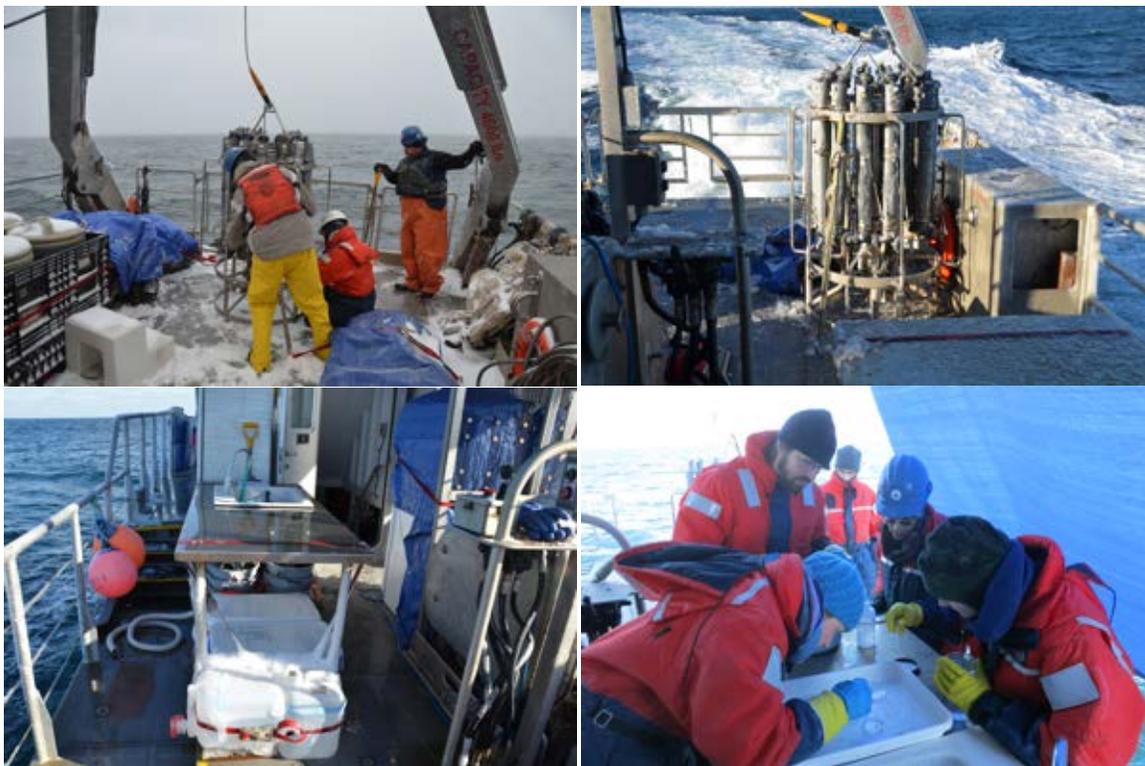


Figure 2 –A) Configuration of the back deck featuring our port side sampling station with sink. The refrigerator was wrapped in a blue tarp in the center of the ship to protect it from sea spray. B) Aft port was the CTD. C) Aft of the CTD was the VPR (not used) and the Reeve net which was strapped down to the port rail. Starboard aft was the ¼ m MOCNESS (under the blue tarp) which was just forward of the collapsible plastic crate with the water transportation trash cans. D) On the second day the processing table was plagued by melting ice and we rigged a cover using a spare tarp. Photos by Peter Wiebe

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, chemistry sampling equipment, a portable plug-in freezer and the BioSonics acoustic setup (Fig. 3A). On the floor was more MOCNESS backup/sampling equipment which continued into midship (Fig. 3B). 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. A small dry shipper with liquid nitrogen was strapped down to the starboard side of the stairs heading down into the lab.



Figure 3 –A) midship storage of supplies and prepping of the chemistry sampling bottles B) The BioSonics setup (center) and the mini-freezer (right) were strapped down on the starboard counter.

8. Hydrography: CTD

8.1.1. Introduction

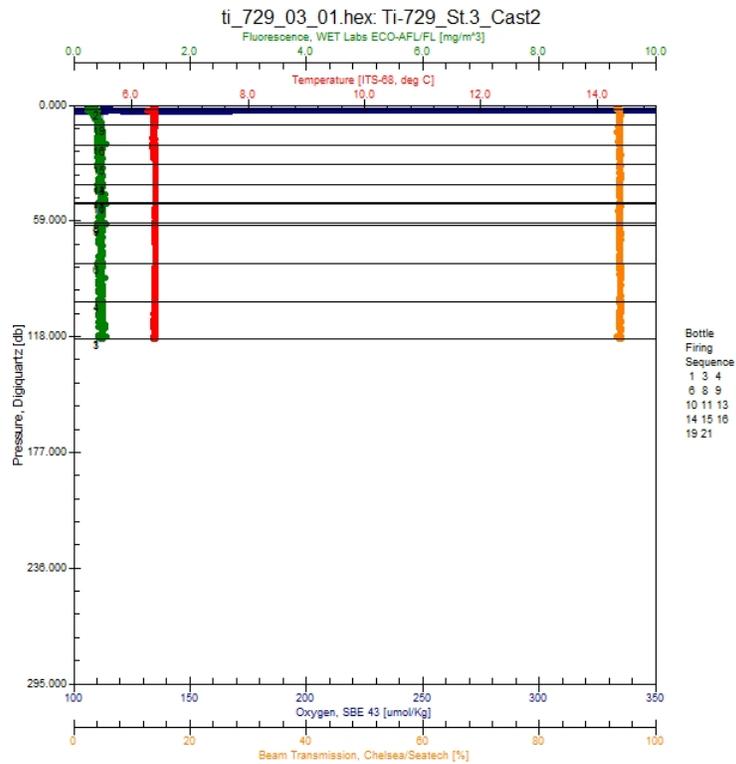
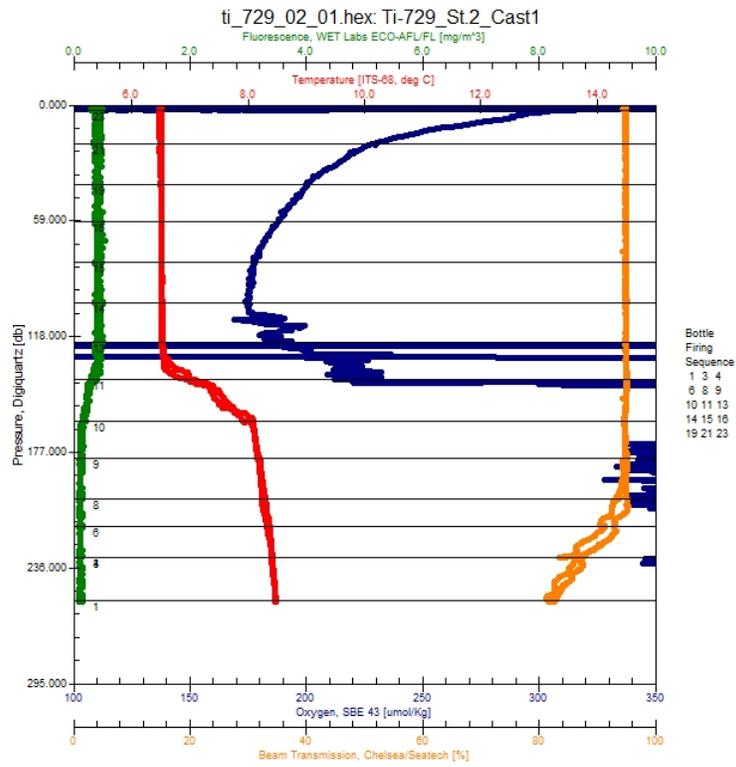
CTD rosette casts were used to get both a profile of the water column and to allow Niskin bottle sampling for the carbonate chemistry analysis. These CTD measurements of environmental conditions will provide key correlates of the distribution and abundance of pteropods with hydrographic features. Depths 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 16 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 O₂ sensor broke during the 1st deployment, but all other sensors were fully functional. It has now been confirmed that the O₂ sensor cannot be left overnight on deck during freezing conditions.



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

10.1.1. Introduction

A standard 1/4-m² 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. Net contents 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 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). All samples had the ethanol replaced with fresh 70% ethanol ~24 hours after sampling.

10.1.3. Animal sampling

During the MOCNESS animals were sampled from the nets for gene expression analysis.

Meganyciphanes norvegica individuals were handpicked from net 4 (n=1) and net 0 (n=16), put in cryovials and then preserved in liquid nitrogen. ~6 large pteropods were picked from net 0 and preserved in RNAlater.

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 150-um mesh net was deployed via the A-frame. The book-clamp to attach the net was borrowed from Carin Asjian's lab. Ship speed during tows was ~1-1.5 knots. The depth and duration of deployment varied widely. We occasionally focused on very deep (90-130) or very shallow (40-20) layers based on acoustic evidence, but had our greatest success with a tow-yo pattern that focused on depth ranges between 40-90 m (See Table 1 and Appendix 4 for 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 concentrated, again taking advantage of the pteropod tendency to sink, and then sorted to create two size classes – the “large” (big enough for respiration experiments) and the “small”. Animals 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).

10.2.3. Preliminary Findings

The first Reeve net (at standard station 2) had only a few *Limacina retroversa*. These were put into RNAlater. All subsequent Reeve nets were all at standard station 3. At the end of day 2 we had 41

individuals in RNAlater (R1 and R5), as well as 12 1-L jars with > 30 large individuals, 4 1-L jars with marginal individuals (possibly useful for respiration runs) and 7 1-L jars of small animals for culturing studies (see Table 1 for details). The most efficient tow, by duration and catch was R6 which sampled between 40-85 m.

Table 1: Success of Reeve net capture.

	Botes	Individuals for experiments			Sampling depth (m)	Duration (min)
		Big	Marginal	Small (pets)		
R1	11 - RNAlater				90-130	50
R2		30			60-90, 60-90	50
R3	scattering layer	25			0-40	15
R4	scattering layer			some	20-40	30
R5	30 - RNAlater	27		some	40-60 m	30
R6		86		some	40-85, 40-60	45
R7		43		some	40-60, 40-60	35
R8		30	55	some	40-90	30
R9		60	30	some	40-90, rest 90 and 60	35
R10		30	20	some	60-90	30

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 or strapped to the port rail.

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 (~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 hose was at depth (we aimed for 30 m) the pump was attached. One of the hoses had frozen water in it, preventing it from being useful, so the system had to be retrieved and the hose sent to ~15 m depth. Since the water column was well mixed throughout the top 120 m, this did not change the experimental design overly much. The pump was a "Little Giant" subpump with a watertight spliced extension cord. 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.

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. We filled up a 55 gallon drum and four trash bins on the truck.

The following day the pump was used again, filling three trash cans. While this was occurring we filled 45 1-L glass jars with filtered water. These jars were placed in the refrigerator to maintain as close to ambient seawater temperature as possible for pteropod transportation.

12. Acoustics

12.1. Introduction

In an effort to improve our ability to localize the pteropods in the water column in terms of vertical position, as well as to help identify regions of high pteropods abundance, on this cruise we brought along the WHOI Biology Department Biosonics DT-X echosounder. The system has two single-beam transducers, operating at 120 and 200 kHz, from a single deck transceiver. The system is very user-friendly and required minimal extra effort. The transducers could be deployed using the port-side boom that Jay Sisson developed for Rocky Geyer, which Andone Lavery has used previously with the Edgetech system, but for this cruise we decided to keep things simple and just hung the transducers bolted to a plate over the starboard side, tied off to the rail. The transceiver and a control laptop were housed in the main lab.

The transducers were deployed on multiple occasions, mostly at times when other operations were underway and so no time was being lost. They were also left in for many of the Reeve net tows, which worked fine although they tended more towards the stern when the vessel was underway and so the transducers were angled slightly away from horizontal, making it a little difficult to estimate the depth of acoustic features based on the range shown in the display. We put a second line on them and ran it forward, which helped a little in keeping the transducers from tending too far aft.

Relatively little scattering was evident over much of the water column. On the first cruise day there was a patchy layer evident at depths of 0-40 m and strongest at 20-40 m that initially gave us hope as it was of a size and at depths that seemed consistent with pteropods. The frequency response of the scattering was less clear and it was hard to discern whether the scattering was stronger on the 120 or 200 kHz channel, or perhaps more likely quite similar at the two frequencies. Scattering that was stronger at 200 kHz would have been consistent with pteropods. We spent two Reeve tows trying to fish right through one of these patches and succeeded in getting very close to a patch if not in it, but didn't come up with a huge haul of pteropods, making us think the scattering was due to something else, perhaps siphonophores.

Irrespective, given its ease of deployment and the fact that it didn't take any time away from other operations, the use of the system was overall a success and we will plan to use it again, in the hopes that we might some day see large patches of pteropods acoustically.

13. 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
5	Peter Wiebe	Scientist	WHOI	Biology
6	Mike Lowe	Postdoc	WHOI	Biology
7	Robert Levine	Guest Student	Cornell/WHOI	Biology
8	Ali Thabet	Guest Student	Assiut University/ WHOI	Biology

Officers and Crew

1	Ken Houtler	Captain
2	Ian Hanley	Mate

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