ATLANTIC MERIDINIONAL TRANSECT CRUISE 24: ZOOPLANKTON ECOLOGY

Erica Goetze, Michelle Jungbluth
Department of Oceanography, University of Hawaii at Manoa, Honolulu, HI 96822, USA. egoetze@hawaii.edu, mjjungb@hawaii.edu

Alice Burridge
Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam and Naturalis Biodiversity Center, Leiden, Netherlands.
akburridge@yahoo.co.uk

Sara Cregeen, Mike Zubkov
National Oceanography Centre Southampton, Southampton, UK

This document was prepared as part of the Final Cruise Report for the Atlantic Meridional Transect Cruise 24, which transited from Immingham, United Kingdom to Punta Arenas, Chile between Sept 21, 2014 – Nov 6, 2014. The final cruise report, including all science components, can be found online at the Atlantic Meridional Transect webpage (here: http://www.amt-uk.org/Cruises), or through the British Oceanographic Data Centre (BODC) (here: http://www.bodc.ac.uk/projects/uk/amt/).

Several teams on board were collaborating on research in zooplankton ecology, with three distinct projects ongoing. We include information on all related projects here.

(1) Evolutionary Ecology: Introduction and Objectives. [Goetze, Burridge]

A few of the primary goals of the AMT programme are to provide a means to assess biodiversity trends in relation to environmental change, improve our understanding of the structure and functioning of marine ecosystems, and understand the impact of climate change on the ocean. Our research is related to these efforts in that 1) we aim to assess biodiversity (both at specific and genetic levels) of several important components of pelagic foodwebs (copepods, chaetognaths and pteropods), and 2) the ability of zooplankton to respond to future changes in the ocean depends on the genetic structure of populations over space and time.

Our primary research objectives on this cruise were to obtain material for studies in:

1. Population genetic structure and gene flow at basin spatial scales in key zooplankton taxa,
2. Assess temporal stability in spatial genetic patterns by repeat transect sampling on AMT,
3. Test hypotheses about mechanisms that underlie genetic breaks known to occur across the equatorial upwelling zone in a number of key zooplankton species,
4. Assess species boundaries and adaptive potential of pteropods, with regards to ocean acidification
5. Determine if there are community-wide patterns in the spatial distribution of genetic variation in the holozooplankton, through comparative community-wide genetic studies.

We also collected material for community metagenetic sequencing of the adult mesozooplankton community.

(2) Copepod Gut Microflora Analysis: Introduction and Objectives. [Cregeen, Zubkov]

Microbial populations in the pelagic environment are pivotal to global biogeochemical cycling as major producers and degraders of organic matter in the oceans. Copepods modify organic matter in the water column through feeding, which makes it more accessible to other organisms. Additionally, they are pronounced vertical migrators and can thus aid in the transfer of organic matter from the surface to deeper waters. The usually nutrient-poor, open ocean pelagic environments present an interesting study site for microbial communities associated with nutrient rich habitats such as those found in copepod digestive tracts.

(3) Nauplii Diversity, Distribution, and Community Structure: Introduction and Objectives. [Jungbluth]

Copepods are often the most abundant zooplanktonic organisms in marine ecosystems around the world. Copepod nauplii alone contribute 30-70% of copepod abundance, while contributing up to 10% of biomass. Despite their low contribution to biomass, nauplii can be important components of pelagic food webs as both grazers and prey. To obtain better understanding of the role of copepod nauplii in

oceanic ecosystems, we aim to determine 1) whether copepod nauplii have higher abundance in resource-rich features of the water column, which would increase the localized grazing impact of the naupliar community, 2) if certain species dominate in these regions of higher abundance, resulting in some species having a higher contribution to total grazing impact, and 3) how naupliar species composition, abundance, and depth distribution differ across a range of ocean ecosystems. To date, no work has been published describing both the species composition and depth distribution of nauplii in a diverse open ocean environment.

Methods

Sample collection. Mesozooplankton samples were collected with 0.71m- diameter bongo nets (200 µm), and with an RMT1 midwater trawl (333 µm) that has a nominal mouth area of $1m^2$. A total of 47 mesozooplankton tows were conducted along the cruise leg (Table 1), with 45 tows conducted using the bongo and 2 samples collected with the RMT net. The bongo tows were oblique tows that sampled from between 401 to 70 m depth and the surface: The average maximum depth of tow for quantitative bongo tows was 305 m (CalBOBL). The bongo samples will be used for quantitative estimates of animal abundance along the cruise leg (target species only); tows were conducted with a time-depth-recorder attached and a General Oceanics flowmeter mounted in the mouth of one net. The RMT tows also were oblique tows that sampled to between 1218 m maximum depth and the surface. All tows were conducted at night, in order to efficiently sample the migratory community.

Samples of naupliar abundance and vertical distribution were taken with 20 L CTD Niskin bottles at each noon station. The sampling targeted features in the water column including the deep chlorophyll maximum (DCM), the mixed layer (ML), points in between (e.g., base of the mixed layer), and plankton thin layers observed on the SimRad EK60 in real time. These samples will be used for estimates of abundance, stage distribution, and diversity of nauplii in each depth sampled. DNA barcoding of individual nauplii will be used to determine species composition of the community across depth and latitude. Every other day, additional samples were collected from the ML and DCM for autotrophic and heterotrophic nano- and microplankton, ciliate abundance, and picoplankton abundance. Nauplii also were collected by net tows at the noon stations with a 60 µm mesh, 1 m diameter plankton net (5m length) with a filtering cod end (60 µm mesh). Vertical tows sampled from 200 m to the surface, and a General Oceanics flowmeter also was mounted in the mouth of this net.

Sample handling and preservation.
Evolutionary Ecology. Zooplankton from one net was preserved immediately in 100% ethyl alcohol for use in molecular studies (quantitative split, 2 x 50%), including DNA sequencing and microsatellite genotyping (and possibly RAD tag sequencing), in addition to estimates of abundance of target species. A quantitative split of the second net material was size-fractionated at 0.2 mm, 0.5 mm, 1 mm, 2 mm, and > 5 mm and preserved in RNALater for community metagenetic or metagenomic sequencing. Another quantitative fraction of net 2 material was sorted live immediately following collection, and animals were individually identified, and preserved in RNALater or cryopreserved (copepods, pteropods, heteropods, chaetognaths). These animals will be used for molecular, genomic and transcriptomic analyses. In total, over 20,000 animals from > 40 target species were individually sorted and preserved. Following live sorting, the remaining plankton was preserved either in 5% buffered formalin or 100% ethyl alcohol for morphological studies. Alice Burridge also sorted all ETOH preserved material for pteropods while onboard, and leaves the ship with a complete dataset of pteropod species abundance and distribution across the entire AMT24 transect.

Copepod Gut Microflora. Copepods from the genus *Pleuromamma* and *Euchaeta* were picked from the bulk zooplankton net tow samples and sorted by species. Samples were then fixed either for Fluorescence *in* situ hybridisation (FISH) or stable isotope analysis. For FISH samples for fixed in 2% paraformaldehyde in filtered seawater either overnight at 4 °C or for 6h at room temperature. They were then washed twice in filtered seawater water for 1h, once in 30% ethanol for 1h and finally transferred into 50% ethanol. The samples are stored at 4 °C until further processing. For stable isotope analysis samples were frozen at -80 °C and stored there until further analysis. Samples will be further analyzed at the National Oceanography Centre, Southampton, within the next 12 months.

Naupliar Community Structure. Nauplii were recovered from CTD seawater samples by filtering onto a 20 µm Nitex mesh sieve, with preservation in ETOH and storage at -80 °C. These samples will be analyzed for total abundance, stage distribution, and DNA barcoding to assess species composition across depth. Plankton recovered from tows of the 60 µm net was size-fractionated to remove > 200

µm organisms. This larger material was preserved in ETOH or quantitatively split and preserved in ETOH and RNALater (50% splits). The 60-200 µm fraction was quantitatively split, with 50% preserved in RNALater for metagenetic or metagenomic analysis, a 25% split also preserved in RNALater, and a 25% split analyzed by FlowCAM for naupliar abundance and biomass (Zubkov group instrument and protocols).

Experiments. Eleven egg production experiments were conducted on target copepod species *Haloptilus longicornis*, *Pleuromamma xiphias*, and *Pleuromamma abdominalis*. These experiments were conducted in 6-well sterile plates, with or without towers attached to extend seawater volume, depending on adult female body size. All dishes were checked for new eggs at 12 and/or 24-hours: At the end of each experiment, all females and nauplii were preserved for use in studies of mating dynamics in these species. Experiments also were conducted on pteropods to assess calcification rates. Different species of pteropods, mainly *Heliconoides inflata* and *Limacina helicina*, were used for shell growth experiments by culturing them under natural conditions in calcein-stained water for 26 – 48 hrs. Additional animals were preserved in RNALater for transcriptomic studies.

Science outreach. The zooplankton team ran an online blog about zooplankton ecology and the AMT24 cruise (www.atlanticplankton.wordpress.com). Erica, Michelle and Alice contributed posts about their work and experience, and Katja Peijnenburg and Pia Drent contributed as collaborators with illustrations and by posting for us while at sea (Katja was the administrator). The blog was posted on the Journal of Plankton Research facebook page (thanks to R. Harris), as well as the SOEST facebook page. One of Michelle's posts was also hosted at the SOEST graduate student blog at http://earthscigradblog.wordpress.com. Alice also wrote a blog (in Dutch) about her scientific activities and general experiences at the JCR (http://www.alicesadventuresunderwater.wordpress.com). In early 2015, she will give an interactive science talk at Naturalis Biodiversity Center, targeting a general audience including schoolchildren.

Table 1. List of all bongo and RMT tows conducted during AMT24. Time is local time, CalBOBL indicates bongo oblique tows (quantitative tows), and RMT1 tows used the midwater trawl. Live bongo tows were non-quantative tows used to collect animals for experiments and live sorting for other purposes (individual preservation). Latitude and longitude were recorded at the start of each tow.

	Station	Latitude	Longitude	Date	Tow Type	Start Time	End Time
1	ZPT_JR303_001	46° 22.683 N	10° 57.801 W	9/28/14	CALBOBL	2:58	3:54
2	ZPT_JR303_002	44° 05.228 N	14° 53.916 W	9/29/14	CALBOBL	3:00	3:53
3	ZPT_JR303_003	41° 46.1273 N	18° 44.2327 W	9/30/14	CALBOBL	2:56	3:45
4	ZPT_JR303_004	39° 24.6196 N	22° 28.6135 W	10/1/14	CALBOBL	2:55	3:43
5	ZPT_JR303_005	34° 44.991 N	26° 37.0633 W	10/3/14	CALBOBL	2:55	3:44
6	ZPT_JR303_006A	31° 52.2808 N	27° 33.1582 W	10/3/14	live bongo	22:58	23:22
7	ZPT_JR303_006	31° 17.998 N	27° 43.657 W	10/4/14	CALBOBL	2:56	3:44
8	ZPT_JR303_007	27° 29.951 N	28° 53.333 W	10/5/14	CALBOBL	2:58	3:46
9	ZPT_JR303_008A	24° 37.307 N	29° 44.774 W	10/6/14	live bongo	22:45	23:19
10	ZPT_JR303_008	24° 03.390 N	29° 54.489 W	10/6/14	CALBOBL	2:59	3:48
11	ZPT_JR303_009	20° 26.730 N	29° 16.250 W	10/7/14	CALBOBL	2:55	3:45
12	ZPT_JR303_010A	18° 19.88 N	28° 48.465 W	10/7/14	live bongo	23:07	23:32
13	ZPT_JR303_010	17° 49.3530 N	28° 41.9846 W	10/8/14	CALBOBL	2:59	3:48
14	ZPT_JR303_011	14° 12.3774 N	27° 55.7259 W	10/9/14	CALBOBL	2:59	3:48
15	ZPT_JR303_012A	11° 22.186 N	27° 19.942 W	10/9/14	live bongo	22:59	23:16
16	ZPT_JR303_012	10° 46.8399 N	27° 12.3512 W	10/10/14	CALBOBL	3:03	3:52
17	ZPT_JR303_013	7° 17.1336 N	26° 29.5001 W	10/11/14	CALBOBL	2:59	3:49
18	ZPT_JR303_014A	4° 24.6678 N	25° 54.2126 W	10/11/14	live bongo	22:58	23:17
19	ZPT_JR303_014	3° 48.233 N	25° 46.639 W	10/12/14	CALBOBL	2:58	3:48
20	ZPT_JR303_015	0° 04.8941 N	25° 00.9156 W	10/13/14	CALBOBL	2:57	3:47
21	ZPT_JR303_016	3° 53.199 S	25° 01.53 W	10/14/14	CALBOBL	3:00	3:50
22	ZPT_JR303_017A	6° 51.342 S	25° 01.6719 W	10/14/14	live bongo	22:56	23:17
23	ZPT_JR303_017	7° 28.2627 S	25° 07.2117 W	10/15/14	CALBOBL	2:55	3:45
24	ZPT_JR303_018A	10° 26.3 S	25° 02.836 W	10/15/14	live bongo	22:59	23:15
25	ZPT_JR303_018	11° 02.3303 S	25° 02.7162 W	10/16/14	CALBOBL	2:57	3:47
26	ZPT_JR303_019A	14° 02.5005 S	25° 04.0980 W	10/16/14	live bongo	22:57	23:19
27	ZPT_JR303_019	14° 39.544 S	25° 04.448 W	10/17/14	CALBOBL	2:56	3:46
28	ZPT_JR303_020A	17° 41.31 S	25° 05.523 W	10/17/14	live bongo	22:56	23:17
29	ZPT_JR303_020	18° 18.9835 S	25° 05.4257 W	10/18/14	CALBOBL	3:02	3:51
30	ZPT_JR303_021	20° 51.313 S	25° 04.677 W	10/20/14	CALBOBL	2:57	3:46
31	ZPT_JR303_022A	23° 51.240 S	25° 02.586 W	10/20/14	CALBOBL	22:55	n.r.
32	ZPT_JR303_022	24° 27.4443 S	25° 02.5063 W	10/21/14	CALBOBL	2:59	3:49
33	ZPT_JR303_023A	27° 08.0134 S	25° 00.0557 W	10/21/14	live bongo	22:59	23:21
34	ZPT_JR303_023	27° 45.660 S	25° 00.625 W	10/22/14	CALBOBL	3:01	3:51
35	ZPT_JR303_024A	30° 35.9 S	25° 48.311 W	10/22/14	live bongo	22:59	23:21
36	ZPT_JR303_024	31° 20.4147 S	26° 06.0235 W	10/23/14	CALBOBL	2:58	3:47
37	ZPT_JR303_025A	34° 11.0843 S	27° 12.4312 W	10/23/14	live bongo	22:58	23:20
38	ZPT_JR303_025	34° 10.857 S	27° 13.126 W	10/24/14	CALBOBL	2:57	3:32
39	ZPT_JR303_026A	37° 19.5333 S	28° 28.980 W	10/24/14	live bongo	23:02	n.r.
40	ZPT_JR303_026	37° 53.5626 S	28° 44.2004 W	10/25/14	CALBOBL	3:04	3:54
41	ZPT_JR303_027	40° 07.0035 S	30° 54.8200 W	10/26/14	CALBOBL	3:03	3:52
42	RMT1_JR303_01	41° 24.551 S	33° 38.292 W	10/26/14	RMT	23:04	1:38
43	ZPT_JR303_028	41° 28.648 S	33° 51.519 W	10/27/14	CALBOBL	2:59	3:48
44	ZPT_JR303_029	43° 01.199 S	37° 08.204 W	10/28/14	CALBOBL	3:00	3:49
45	ZPT_JR303_030	44° 37.427 S	40° 41.519 W	10/29/14	CALBOBL	3:00	3:49
46	RMT1_JR303_02	46° 05.413 S	44° 02.858 W	10/29/14	RMT	22:59	1:49
47	ZPT_JR303_031	46° 05.339 S	44° 11.762 W	10/30/14	CALBOBL	2:57	3:46

Table 2. List of all naupliar net tows conducted during AMT24. Time is local time, and latitude and longitude were recorded at the start of each tow.

	Station	Latitude	Longitude	Date	Start Time	End Time
1	NAU-JR303_001	47° 11.182 N	8° 49.53 W	9/26/14	12:06	12:39
2	NAU-JR303_002	47° 34.0311 N	8° 30.2926 W	9/27/14	13:10	13:30
3	NAU-JR303_003	45° 30.124 N	12° 29.371 W	9/28/14	13:24	13:50
4	NAU-JR303_004	43° 10.2156 N	16° 25.6105 W	9/29/14	13:15	13:40
5	NAU-JR303_005	40° 50.5337 N	20° 12.8915 W	9/30/14	13:17	13:36
6	NAU-JR303_006	38° 29.7579 N	23° 53.5054 W	10/1/14	13:14	13:35
7	NAU-JR303_007	36° 57.0277 N	25° 54.6441 W	10/2/14	13:10	13:31
8	NAU-JR303_008	33° 21.2313 N	27° 05.0728 W	10/3/14	13:14	13:35
9	NAU-JR303_009	29° 53.9194 N	28° 09.9120 W	10/4/14	13:17	13:33
10	NAU-JR303_010	26° 06.3498 N	29° 18.3141 W	10/5/14	13:17	13:38
11	NAU-JR303_011	22° 39.5260 N	29° 51.9854 W	10/6/14	13:15	13:35
12	NAU-JR303_012	19° 51.3242 N	29° 08.4371 W	10/7/14	13:16	13:37
13	NAU-JR303_013	16° 26.1649 N	28° 24.2905 W	10/8/14	13:12	13:33
14	NAU-JR303_014	12° 50.6365 N	27° 38.8267 W	10/9/14	13:15	13:36
15	NAU-JR303_015	9° 22.1016 N	26° 55.2588 W	10/10/14	13:20	13:40
16	NAU-JR303_016	5° 53.9474 N	26° 12.2774 W	10/11/14	13:22	13:46
17	NAU-JR303_017	2° 22.5491 N	25° 29.1733 W	10/12/14	13:32	13:53
18	NAU-JR303_018	5° 19.3411 S	25° 01.5730 W	10/14/14	13:14	13:37
19	NAU-JR303_019	8° 54.6725 S	25° 02.5586 W	10/15/14	13:21	13:43
20	NAU-JR303_020	12° 29.6856 S	25° 03.9180 W	10/16/14	13:14	13:35
21	NAU-JR303_021	16° 07.8922 S	25° 05.0685 W	10/17/14	13:10	13:31
22	NAU-JR303_022	22° 18.9844 S	25° 03.6114 W	10/20/14	13:13	13:34
23	NAU-JR303_023	25° 35.6842 S	25° 02.1444 W	10/21/14	13:12	13:34
24	NAU-JR303_024	29° 09.2390 S	25° 16.5373 W	10/22/14	13:10	13:33
25	NAU-JR303_025	32° 42.9935 S	26° 37.9111 W	10/23/14	13:11	13:32
26	NAU-JR303_026	35° 47.798 S	27° 52.496 W	10/24/14	13:12	Aborted
27	NAU-JR303_027	40° 41.126 S	32° 06.198 W	10/26/14	13:15	13:36
28	NAU-JR303_028	43° 35.233 S	38° 23.348 W	10/28/14	13:13	13:31
29	NAU-JR303_029	45° 17.248 S	42° 11.902 W	10/29/14	13:11	13:37

Table 3. List of all CTD casts that were sampled for nauplii during AMT24. Time is reported as local time; CTD cast depth is reported in meters.

Cast ID	Date (mm/dd/yy)	CTD time	Latitude	Longitude	Cast Depth	# depths sampled
CTDJR303_003	9/26/14	13:07	47° 11.181' N	8° 49.53' W	500	4
CTDJR303_004	9/27/14	13:04	47° 34.32' N	8° 30.292' W	500	5
CTDJR303_006	9/28/14	13:02	45° 30.123' N	12° 29.370' W	500	5
CTDJR303_008	9/29/14	13:05	43° 10.217' N	16° 25.610' W	500	5
CTDJR303_010	9/30/14	13:04	40° 50.533' N	20° 12.890' W	500	5
CTDJR303_012	10/1/14	13:04	38° 29.757' N	23° 53.505' W	500	4
CTDJR303_013	10/2/14	13:04	36° 57.28' N	25° 54.646' W	500	4
CTDJR303_014	10/3/14	13:04	33° 21.233' N	27° 05.76' W	500	4
CTDJR303_017	10/4/14	13:03	29° 53.919' N	28° 09.912' W	500	4
CTDJR303_019	10/5/14	13:03	26° 06.351' N	29° 18.314' W	500	4
CTDJR303_021	10/6/14	13:03	22° 39.527' N	29° 51.985' W	500	4
CTDJR303_024	10/7/14	13:06	19° 51.325' N	29° 08.437' W	500	4
CTDJR303_026	10/8/14	13:02	16° 26.125' N	28° 24.290' W	500	4
CTDJR303_028	10/9/14	13:02	12° 50.637' N	27° 38.826' W	500	4
CTDJR303_030	10/10/14	13:04	9° 22.101' N	26° 55.259' W	500	4
CTDJR303_032	10/11/14	13:04	5° 53.947' N	26° 12.277' W	500	4
CTDJR303_034	10/12/14	13:05	2° 22.550' N	25° 29.174' W	500	4
CTDJR303_037	10/14/14	13:07	5° 19.340' S	25° 01.573' W	500	4
CTDJR303_039	10/15/14	13:04	8° 54.672' S	25° 2.560' W	500	4
CTDJR303_041	10/16/14	13:03	12° 29.685' S	25° 3.919' W	500	4
CTDJR303_043	10/17/14	13:03	16° 7.89' S	25° 5.069' W	500	4
CTDJR303_046	10/18/14	14:40	18° 33.35' S	25° 4.269' W	300	4
CTDJR303_048	10/20/14	13:04	22° 18.985' S	25° 3.612' W	500	4
CTDJR303_050	10/21/14	13:02	25° 35.684' S	25° 02.148' W	500	4
CTDJR303_052	10/22/14	13:02	28° 09.238' S	25° 16.536' W	500	4
CTDJR303_054	10/23/14	13:01	32° 42.992' S	26° 37.912' W	500	4
CTDJR303_056	10/24/14	13:02	35° 47.733' S	27° 52.498' W	500	4
CTDJR303_058	10/25/14	13:06	38° 57.675' S	29° 10.45' W	500	4
CTDJR303_060	10/26/14	13:06	40° 41.126' S	32° 6.197' W	500	4
CTDJR303_062	10/27/14	13:30	42° 4.835' S	35° 6.113' W	500	6
CTDJR303_064	10/28/14	13:03	43° 35.232' S	38° 23.35' W	500	4
CTDJR303_066	10/29/14	13:05	45° 17.248' S	42° 11.902' W	500	6
CTDJR303_068	10/31/14	13:13	48° 14.707' S	50° 20.450' W	500	6