Phytoplankton Of Warming Ocean Waters (POWOW#1)

Cruise Report

29 February– 11 March 2012

Honolulu, Hawaii to
San Diego, California

R/V Thomas G Thompson

Zackary Johnson (Duke University), Chief Scientist
Erik Zinser (University of Tennessee)
Steve Wilhelm (University of Tennessee)

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Abstract

The POWOW#1 cruise was a trip of opportunity to sample along temperature gradients and test out new protocols. The primary goal of this cruise was to measure the abundance, diversity and activity of *Prochlorococcus* and associated bacterial and viral communities across temperature (and other environmental) gradients to understand how climate change may impact ocean ecology and biogeochemistry. There are many additional scientific and broader impact goals including characterizing oxidative stress and investigating nitrogen uptake/utilization molecular diversity. The official title of the project is "Collaborative Research: Seasonal and decadal changes in temperature drive *Prochlorococcus* ecotype distribution patterns" and it is part of NSF #1031064 (Duke) and 1030518 (UTK). The abstract from the grant is:

The two numerically-dominant ecotypes of the marine cyanobacterium *Prochlorococcus* partition the surface ocean niche latitudinally, with ecotype eMIT9312 dominant in the 30°N-30°S region and eMED4 dominant at higher latitudes. These ecotypes may account for 25-50% of primary production in open ocean ecosystems, but this percentage is dependent on which ecotype dominates. The relative abundance of the two ecotypes follows a log-linear relationship with temperature, with the transition from eMIT9312 to eMED4 occurring at ~18 °C. From these descriptive data, it has been hypothesized that temperature is the primary driver of relative abundance. Their contribution to net primary production, however, appears to be independent of temperature, suggesting temperature regulates ecotype dominance through photosynthesis-independent mechanisms. To test these hypotheses, the PIs are undertaking a series of field and lab studies to investigate the effect of temperature change on the distribution of these ecotypes. Two cruises in the North Pacific will trace the transitions from eMIT9312- to eMED4-dominated regions, with one cruise during the winter and the other during summer. They have hypothesized that the ratio of ecotype abundance will move latitudinally with the seasonal shift in temperature gradient: migration of the 18° C isotherm northward in the summer will be matched by a similar migration of the 1:1 ecotype transition point. Multiple crossings of the 18° C isotherm are proposed, and the summer cruise will also follow the isotherm to the Western US coast to gain insight on physical and geochemical influences. Environmental variables such as nutrient concentrations, light/mixing depths, and virus /grazing based mortality, which may impinge on the relationship between temperature and ecotype ratio, will be assessed through a series of multivariate analyses of the collected suite of physical, chemical and biological data. Seasonal comparisons will be complemented with on-deck incubations and lab competition assays (using existing and new isolates) that will establish, for the first time, how fitness coefficients of these ecotypes relate to temperature. As latitudinal shifts in temperature gradient and migration of ecotypes during seasonal warming likely share common features with high latitude warming as a consequence of climate change, the investigator's analyses will contribute important biological parameters (e.g., abundances, production rates, temperature change coefficients) for modeling biological and biogeochemical responses to climate change. This research will be integrated with that of committed collaborators, generating data sufficient for ecosystem-scale characterizations of the contributions of temperature (relative to other forcing factors) in constraining the range and seasonal migration of these numerically dominant marine phototrophs.

Disclaimer

All data and findings in this report are to be considered preliminary and subject to change without notice following instrument calibration and other data quality checks. Please contact the PIs for the most recent version of the data referenced in this report.

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Participants

Scientists (Total = 19)

Name	Group	Role
Dr. Zackary Johnson	Duke-PI	Chief Scientist
Emily Dick	Duke	FIRe profiles
Katrina Gazsi	Duke	NH_4 / frozen nutrients
Allie Geiger	Duke	Experimental (temperature shift / flow cytometry)
Caroline Howes	Duke	flow cytometry (FACSCalibur)
Erin Lineberger	Duke	Experimental (temperature shift / FIRe)
Michael Shaughnessy	Duke	DIC/pH
Heather Winterhalter	Duke	Size fractionated chlorophyll
Alyse Larkin	Duke	qPCR/RNA/diel sampling
Dr. Thais Bittar	Duke	Flow cytometry lead / CDOM / oxidative stress protocol development
Dr. Erik Zinser	UTK-PI	PI, Chief Mate Scientist / *Prochlorococcus* redox
Jeremy Chandler	UTK	*Prochlorococcus* diversity
Alise Ponsero	UTK	Virus, Het. Bacteria
Tiana Pimental	UTK	Virus, Het. Bacteria
Jackson Gainer	UTK	Virus, Het. Bacteria
Dr. Bettina Voelker	CSM-PI	PI, Go-Flo sampling, superoxide analyses
Robin Schneider	CSM	H_2O_2 incubations
Ryan Marsico	CSM	^{18}O-labeled H_2O_2 incubations
Dr. Anton Post	MBL-PI	PI, Urea, metagenomics/transcriptomics

Marine Technicians (University of Washington)

Name	Group	Role
Brandi Murphy	UW	Lead Marine Technician
Patrick A'Hearn	UW	2nd Marine Technician
Tony Burke	UW	3rd Marine Technician

Friends of the Cruise (non-cruise participants)

Name	Group	Role
Dr. Steve Wilhelm	UTK-PI	Virus, Het. Bacteria
Rick Rupan	NOAA/PMEL	ARGO
Dr. Elizabeth Steffen	NOAA/PMEL	ARGO
Dr. Dana Swift	UW	ARGO
Dr. Barbara Block	Stanford	Apex Predators / Shark Cafe
Jules Hummon	University of Hawaii	ADCP

Figure 1: Group Photo

Contact Information

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Post Lab
The Josephine Bay Paul Center for Comparative Molecular Biology and Evolution,
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Woods Hole, MA

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Colorado School of Mines
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Golden, CO 80401

Research Objectives

Johnson Lab - Duke (chief scientist, co-PI)

1. *Prochlorococcus* abundance, diversity and activity and environmental variables
2. DNA, RNA
3. nutrients (NO_2, NO_3, $SiOH_4$, PO_4, NH_4)
4. ISUS NO_3
5. flow cytometry (FASCalibur on board, Influx on land) – het. Bacteria, *Prochlorococcus*, Synechococcus, photosynthetic picoeukaryotes
6. size fractionated chlorophyll
7. pH (spectrophotometric), DIC
8. *Prochlorococcus* isolations

Zinser Lab – UTK (co-PI)

1. Depth profiles of the *Prochlorococcus* ecotypes at each station along the transect
2. Isolation of new *Prochlorococcus* strains, especially of the eMED4 ecotype, using liquid and semisolid media enrichments (live and DMSO frozen)
3. Metabolomic profiles of whole community- and Prochlorococcus-specific-carbon flux, with 13C bicarbonate substrate additions (deck incubations)
4. Impacts of HOOH on Prochlorococcus and the rest of the microbial community (deck incubations)

Wilhelm Lab – UTK (co-PI)

1. To determine the distribution and productivity rates of cyanomyoviridae along the available temperature gradient and within the water column
2. To determine the distribution of N4-like roseoviridae along the transect
3. To test out our Turner Designs Phytoflash on both profiles and in underway mode
4. To collect samples for DNA analyses of microbial community structure.

Post Lab – MBL

1. Illumina metagenomics/transcriptomics focusing on N regulation
2. qPCR of the same
3. urea

Voelker Lab – CSM

1. oxidative chemistry

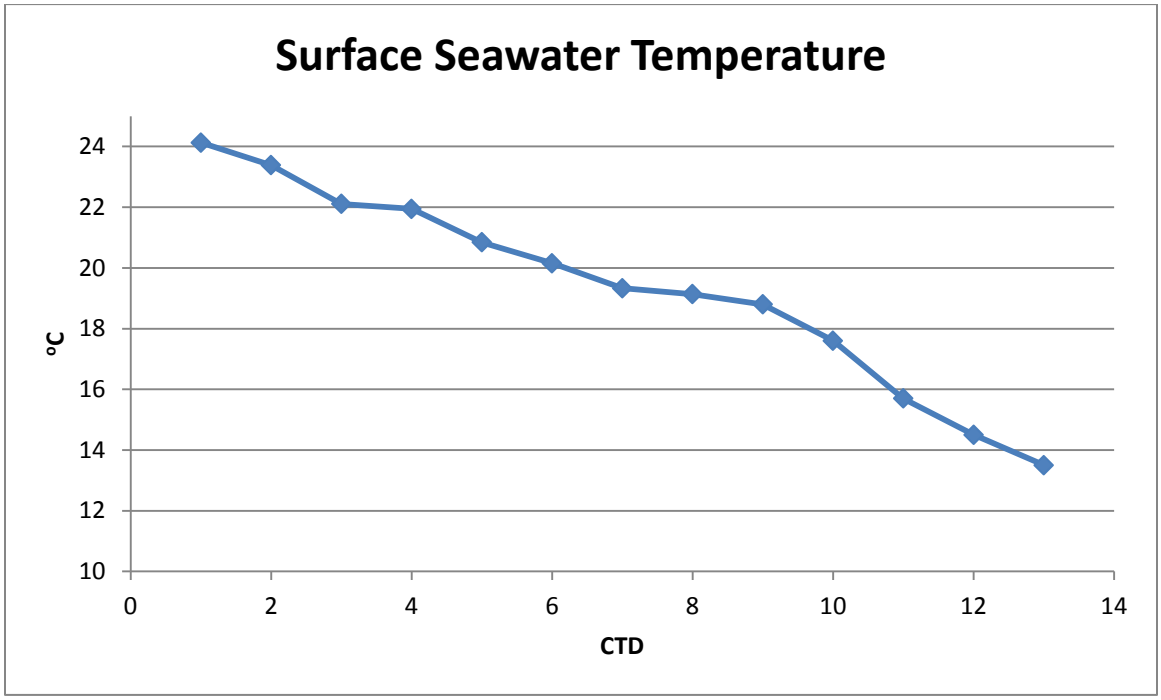
Swift, Steffen, Rupan & Johnson Groups - UW/NOAA

1. ARGO floats

Outcomes

Johnson Lab

The Johnson group analyzed samples for numerous physical, chemical and biological variables along the zonal transect across the sea surface temperature gradient. Many of the physical and chemical variables are plotted below as part of the cruise hydrography.



Specifically we ran samples to investigate oxidative stress in natural populations of *Prochlorococcus* and other phytoplankton using the flow cytometer and two dyes, the (DCF) and (DHR). The goal is to be able to quantify H_2O_2 inside the cells. Samples stained with DCF yielded very high fluorescence overall and a lot of noise and phytoplankton populations were no longer discernible in DCF stained samples. This suggests that the dye combined mainly with other particulates in the sample. Samples stained with DHR yielded some fluorescence at 530 nm that could be related to the amount of H_2O_2 in the cells. However, fluorescence intensity at 530 nm in DHR stained samples was not dramatically higher than the background fluorescence (i.e., fluorescence intensity at 530 nm in unstained samples). We ran a quick experiment where samples with spiked with H_2O_2 (from Zinser's group) to see how high the fluorescence at 530 nm in the DHR stained sample would be. It was not distinctively higher than the 530 nm fluorescence in the stained sample that was not spiked with H_2O_2. We collected samples for later analysis of phytoplankton community composition in the lab using flow cytometry. These samples were collected from the CTD, preserved and frozen. We also collected samples for later analysis of dissolved organic matter concentration and characterization. These samples were filtered sterilized and frozen.

On this cruise we also collected RNA and DNA filtration samples from 10 depths on 11 different CTD casts, resulting in over 300 RNA samples and 350 DNA samples for molecular characterization of *Prochlorococcus*. Additionally, we collected 14 flow cytometry samples per day from the shipboard flow-through and 4 total Sterivex filter samples, which will be used for nucleic acid extraction tests.

Zinser Lab

The Zinser research team collected samples to characterize the genetic structure of the *Prochlorococcus* populations along the temperature gradient provided by the Hawaii to San Diego transect. We also began enrichment cultures in attempts to isolate new *Prochlorococcus* strains along the transect. These live cultivation attempts were complemented with cryopreservation of cells for later thawing and cultivation on shore. In addition, we examined the depth profile of hydrogen peroxide at several stations along the transect, and noted that there was a distinct maximum at the surface, with a significant dropoff to undetectable levels below the mixed layer. We also performed on-deck incubation studies with dilutions of the microbial community, challenged with hydrogen peroxide at several concentrations. As predicted, more dilute concentrations of the microbial community were less able to degrade peroxide. Finally, we collected samples of the microbial community to assay their intracellular pools of small metabolites, comparing those at unchanged versus those with elevated nitrogen concentrations in the incubation bottles. These metabolite pools will be quantified on shore.

Wilhelm Lab

Our main objective during POWOW #1 is to study how temperature and other environmental gradients affect the abundance and diversity of cyanophages in the Pacific Ocean. Most of the samples that were collected over the duration of this cruise will be processed on shore through flow cytometry (to count all bacterial cells), epifluorescense microscopy (to get a total virus count), quantitative PCR (to quantify cyanophages through amplification of target genes), and metagenomic analysis (to ascertain total community structure). Viral production assays were performed on board the Thompson with surface samples collected throughout the cruise. Time point samples taken every three hours during these assays will be examined on shore through epifluorescence microscopy and qPCR to quantify viral production.

Post Lab

The main objective of our study was to establish whether the temperature gradient from the Central North Pacific Gyre into the California Current creates different nitrogen niches for the marine picocyanobacteria *Synechococcus* and *Prochlorococcus*. In the absence of opportunities for in situ or on deck incubations we aimed to correlate distributions of the various nitrogen sources with the genetic potential to acquire them. To meet this goal we carried out the following samplings:

1. Duplicate 12 ml samples for urea and cyanate analyses were taken along daily depth profiles. Together with the ammonium, nitrate and nitrite determinations performed by the Johnson team these analyses will provide a detailed picture of nitrogen distribution along said temperature gradient. Samples were passed over a 0.2 µm filter and stored at -80 °C prior to shipping. Urea analyses by standard colorimetric assay will be carried out

in the Post lab while cyanate will be determined by an HPLC method that was recently developed in the lab of our collaborator Dr. Mulholland at Old Dominion University.

2. Environmental samples (4 L) for metagenome analysis were taken from surface (0 m) and deep (DCM at 110-160 m) layers, filtered onto 0.45 µm Sterivex filters and flash frozen in LN_2. DNA will be extracted with standard protocols and 500-800 bp DNA fragments will be sequenced at the MBL following random shearing and size selection. The sequencing strategy will be a hybrid of pyrosequencing and Illumina HiSeq to obtain reliable identification of nitrogen assimilatory genes and sufficient sequencing depth to quantitatively establish abundances of these genes in the metagenome.
3. Environmental samples (4 L) for the determination of microbial community structures were taken from surface waters via the on board continuous sampling system, filtered onto 0.2 µm Sterivex filters and flash frozen in LN_2. DNA will be extracted with standard protocols and 540 bp amplicons libraries will be sequenced at the MBL following amplification with universal primer pairs that amplify the V4-V6 hypervariable region of 16S ribosomal RNA. These samples will further serve as back-up samples for qPCR applications downstream from the metagenome analysis under 2.
4. In addition to nutrients and temperature mortality (including that caused by viral infection) is a main factor in controlling microbial population dynamics. We have complemented viral production assays by the Wilhelm group with strain isolation of predatory bacteria. Samples (50 ml) from surface and from the DCM were filtered onto 0.2 µm filters and layered upon a streak of prey bacteria (*Ruegeria, Erythrobacter, Vibrio* and *Shewanella*) on marine agar plates lacking in the carbon sources that would support heterotrophic growth. Isolation were started on board and further purification and characterization will be carried out by Post et al at MBL. Microbial diversity assessment under 3. will be used to identify further, putative predatory species.

Voelker Lab

The Voelker group's objective was to measure biological production and decay rates of hydrogen peroxide and superoxide in oligotrophic water samples. We observed slow decay of superoxide, consistent with our expectation that the water samples would be low in concentrations of superoxide-reactive metals and organic compounds. Slow biological decay of hydrogen peroxide was also observed. Biological production of hydrogen peroxide and superoxide was undetectable.

ARGO Deployment

Six Argo floats were deployed on the cruise, including 4 from UW and 2 from PMEL. All are successfully transmitting and details of the deployment are listed below.

Data

Station Locations & Times

Station	UTC-YD	UTC-Date	UTC-Time	Bottom Depth	Latitude	Longitude	CTD	Go-Flo
1	61	03/01/2012	06:43		21.7488	-158.3048	1	
2	61	03/01/2012	12:46	4837	22.7500	-158.0000	2	
3	61	03/01/2012			22.9533	-157.2104		1
4	62	03/02/2012	15:11	5035	23.9059	-153.4245	3	2
5	63	03/03/2012	15:12	5323	25.0231	-148.8906	4	3
6	64	03/04/2012	14:02	5045	26.1459	-144.4109	5	4
7	65	03/05/2012	14:02	4560	25.7662	-139.6017	6	5
8	66	03/06/2012	14:06	4260	24.2661	-134.8874	7	
9	67	03/07/2012	01:33	4980	23.5828	-132.7552	8	
10	67	03/07/2012	13:03	4569	24.1275	-131.8529	9	6
11	68	03/08/2012	13:05	4815	26.5498	-127.8058	10	7
12	69	03/09/2012	13:03	4335	29.6116	-123.7789	11	8
13	69	03/09/2012	19:28	3948	30.2635	-122.9865	12	9
14	70	03/10/2012					13	10

ARGO deployment

Float #	WMO ID#	Latitude	Longitude	Deployment UTC Date	Deployment UTC Time
7107 (UW)	5903738	23.9047	-153.4217	2012/03/02	16:25
7118 (UW)	5903737	25.0228	-148.8873	2012/03/03	16:23
7112 (UW)	5903740	26.1440	-144.4064	2012/03/04	15:13
7074 (UW)	5903739	25.7652	-139.5988	2012/03/05	15:23
5835 (PMEL)	4901492	23.5848	-132.7527	2012/03/07	01:25
5827 (PMEL)	4901444	29.6159	-123.7784	2012/03/09	14:28

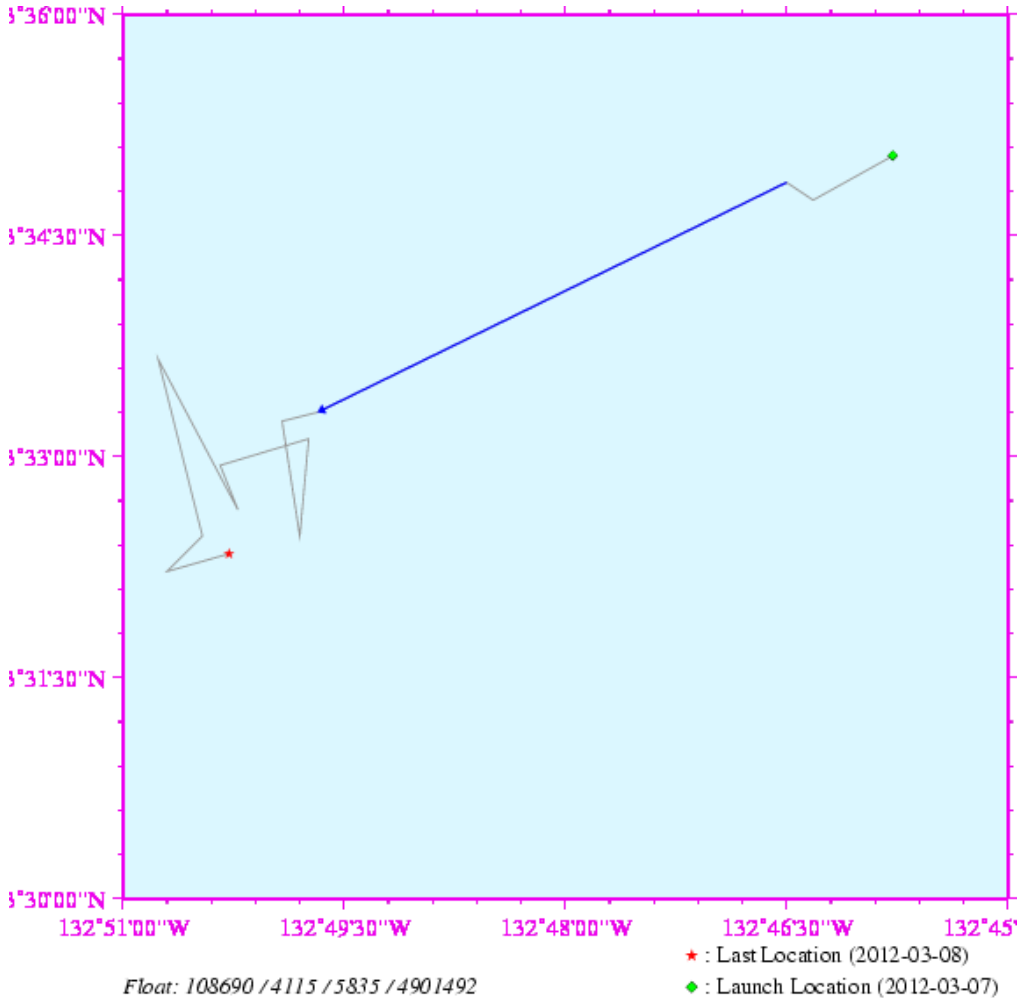


Figure 2: Sample Float Trajectory: WMO ID#4901492

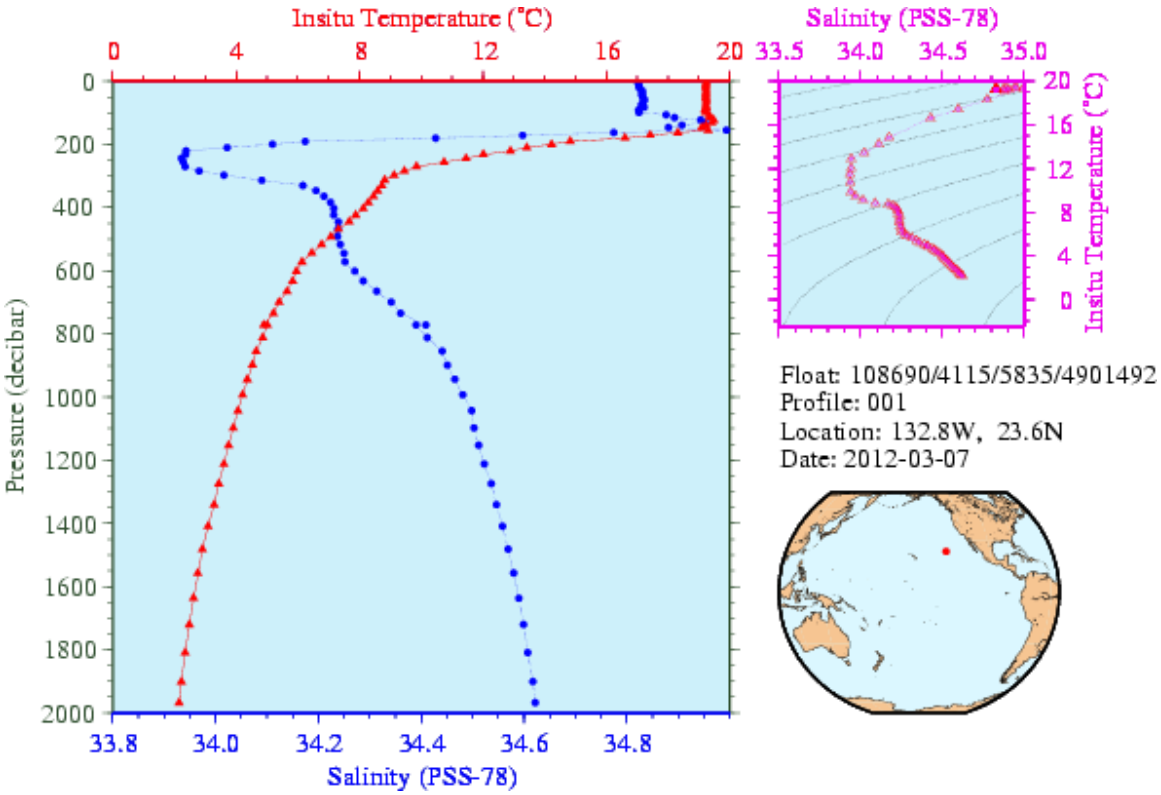


Figure 3: Sample profile for ARGO float (WMO ID#4901492)

Maps

Satellite Images

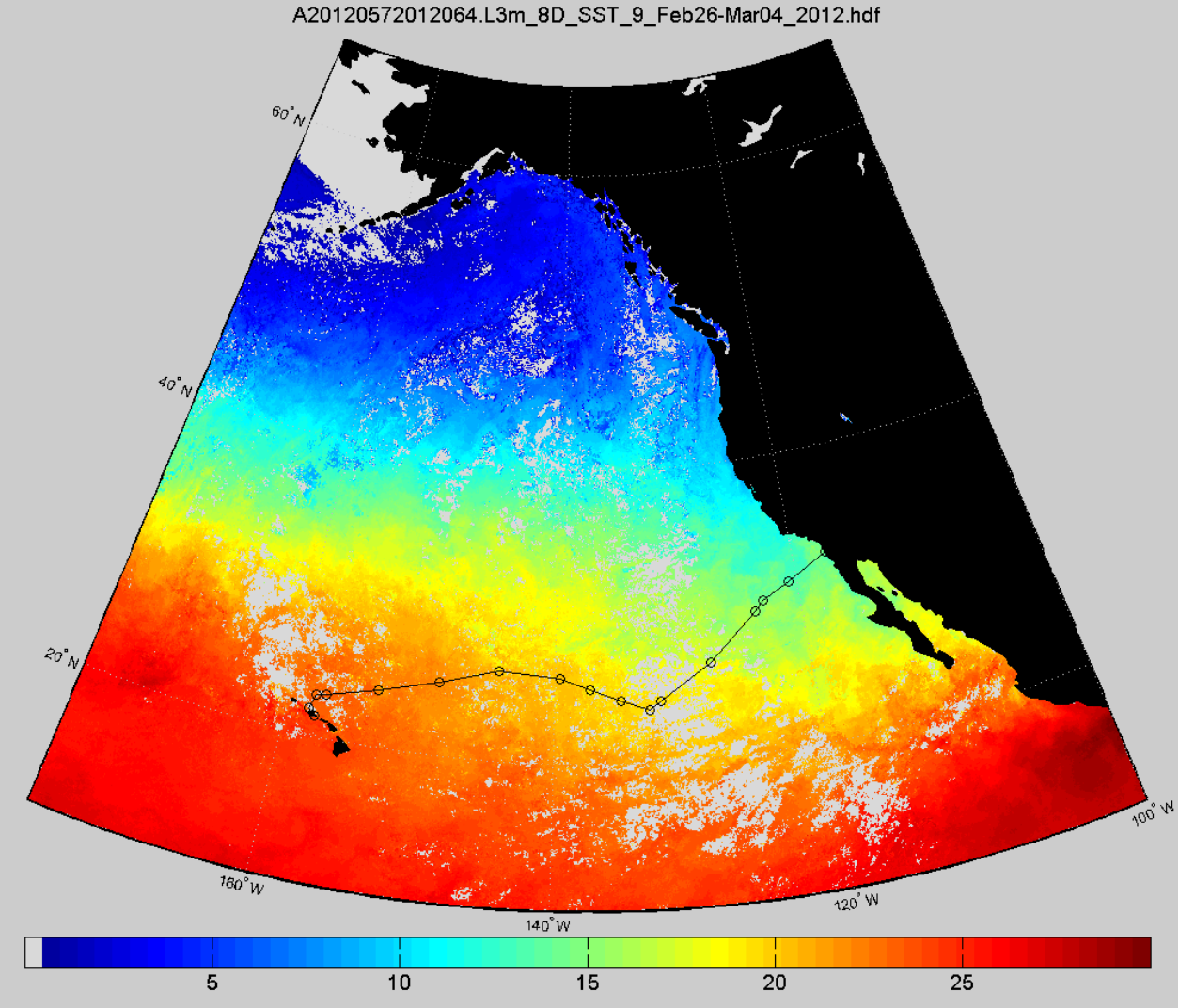


Figure 4: NASA Aqua MODIS estimated day time (11μ) sea surface temperature (Feb 26 – Mar 04, 2012) with overlaid cruise track and stations

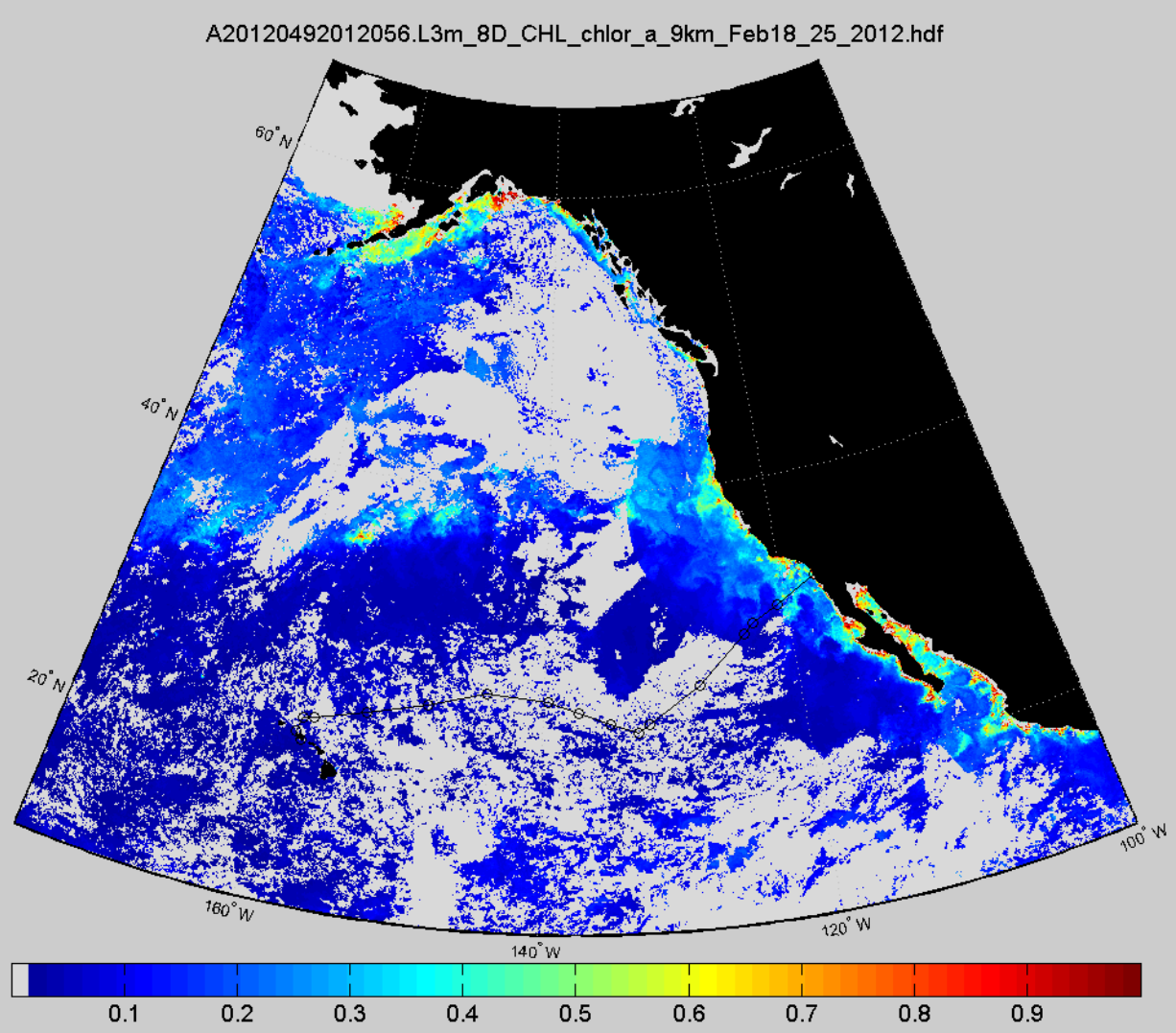
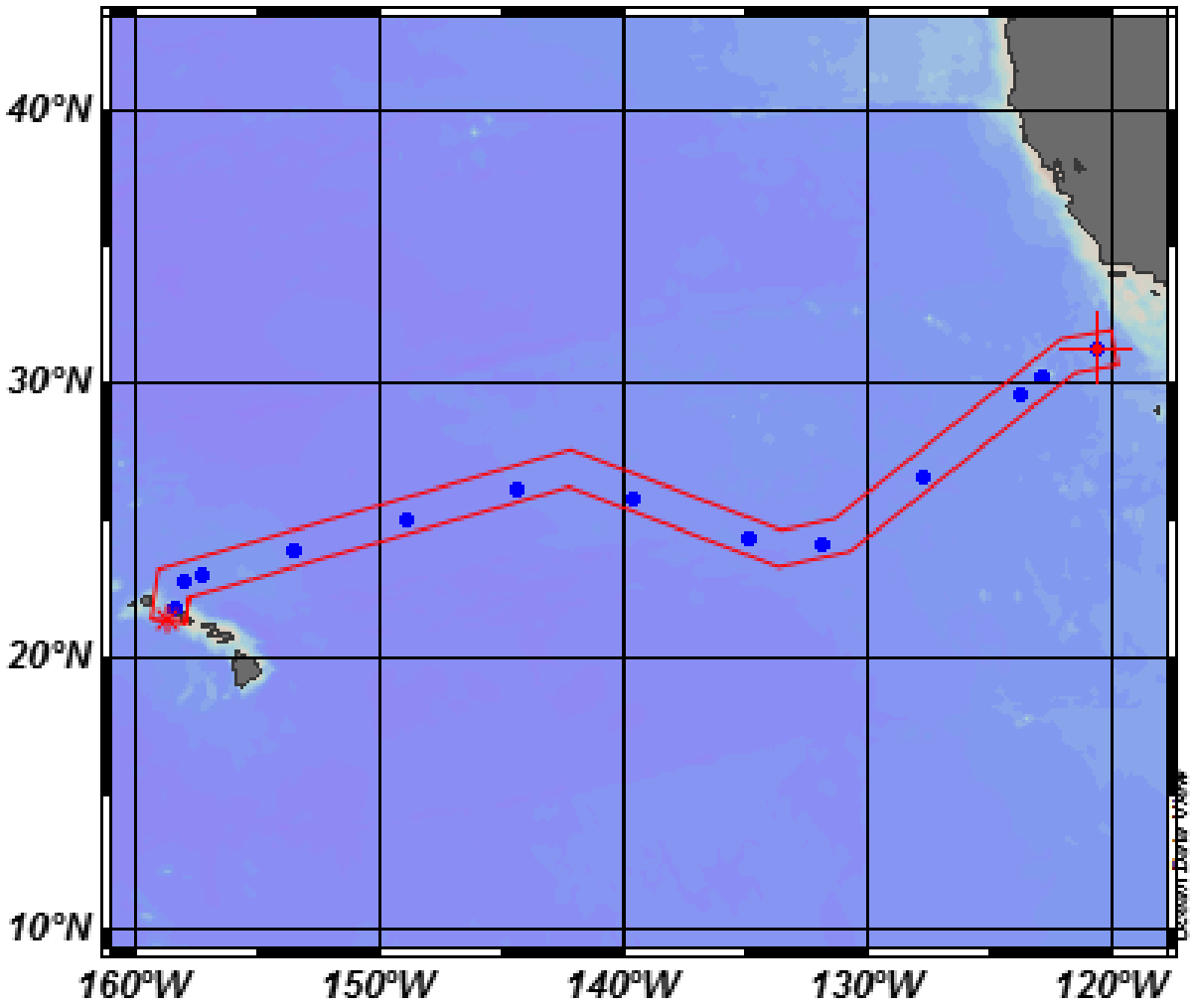
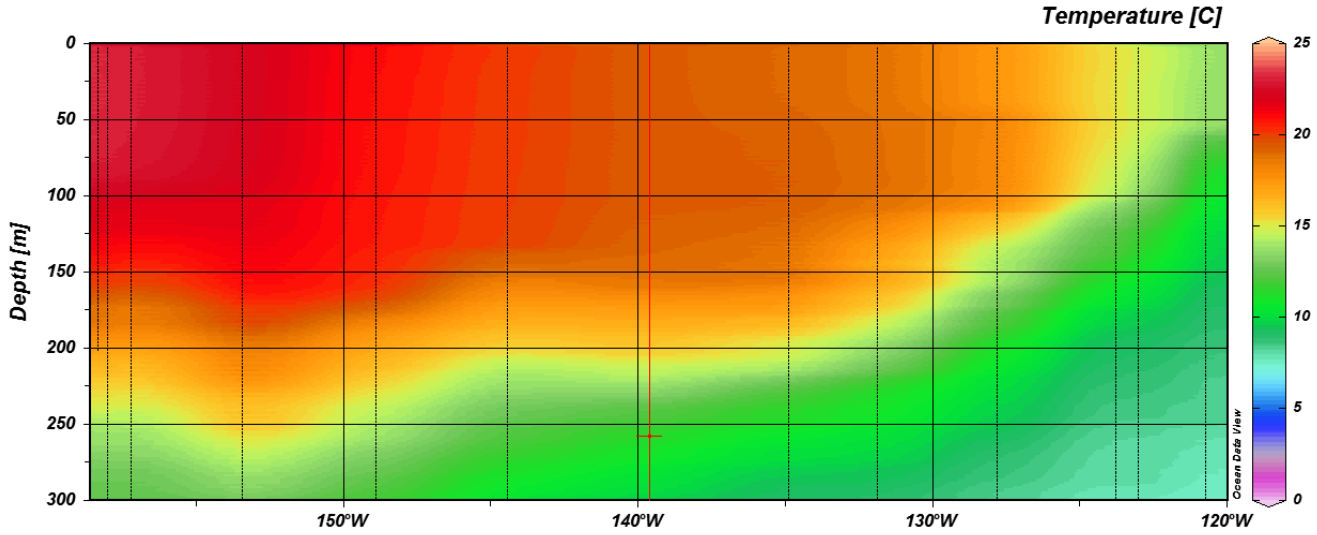
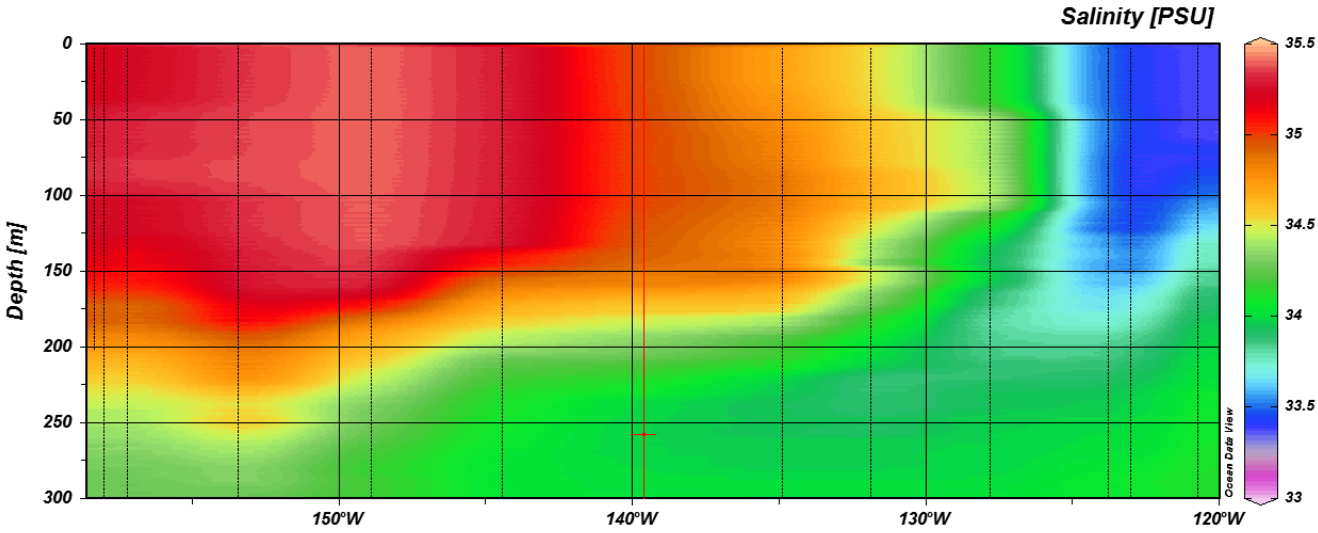


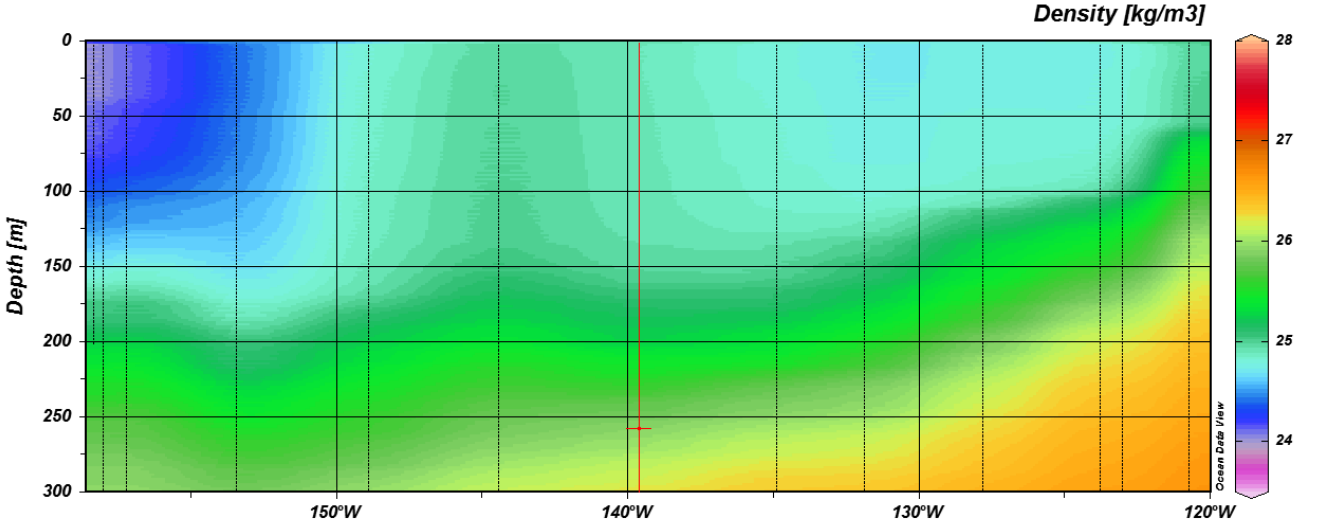
Figure 5: NASA Aqua MODIS estimated chlorophyll concentrations with overlaid cruise track and stations

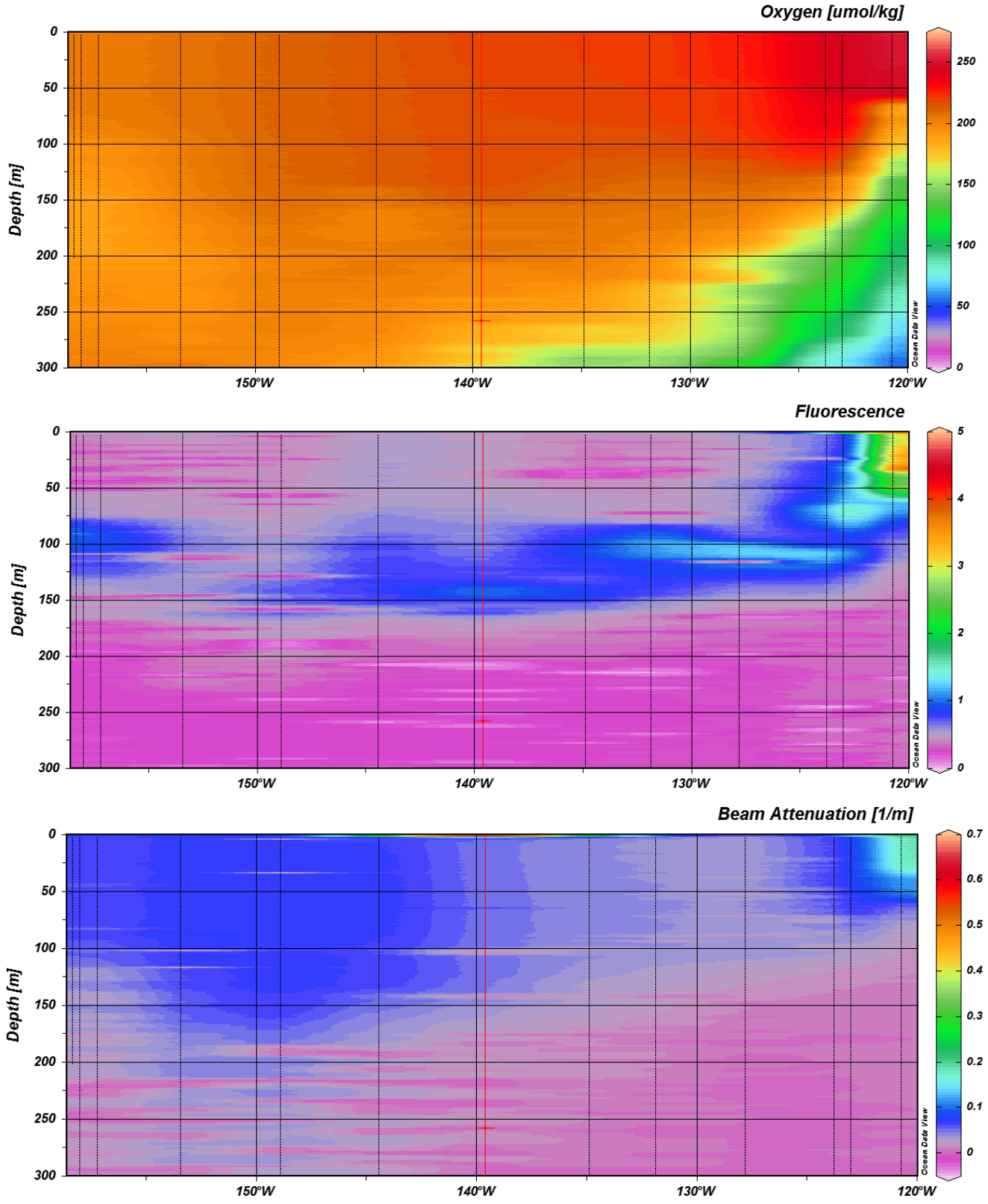
Hydrography: Sectional Data

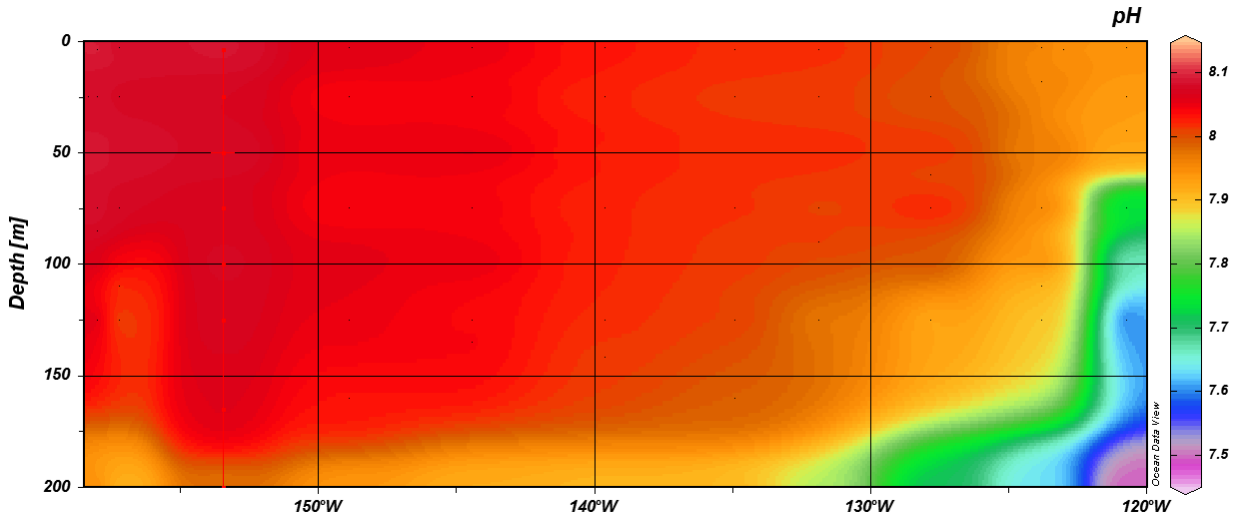


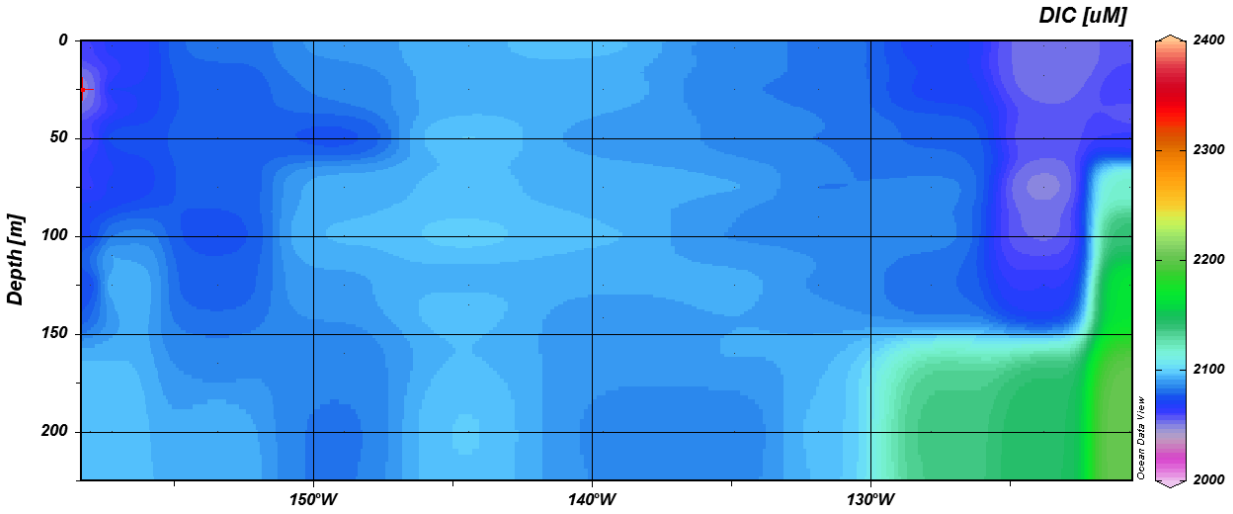

Temperature [C]
Depth [m]
0
50
100
150
200
250
300
150°W
140°W
130°W
120°W
25
20
15
10
5
0
Ocean Data View

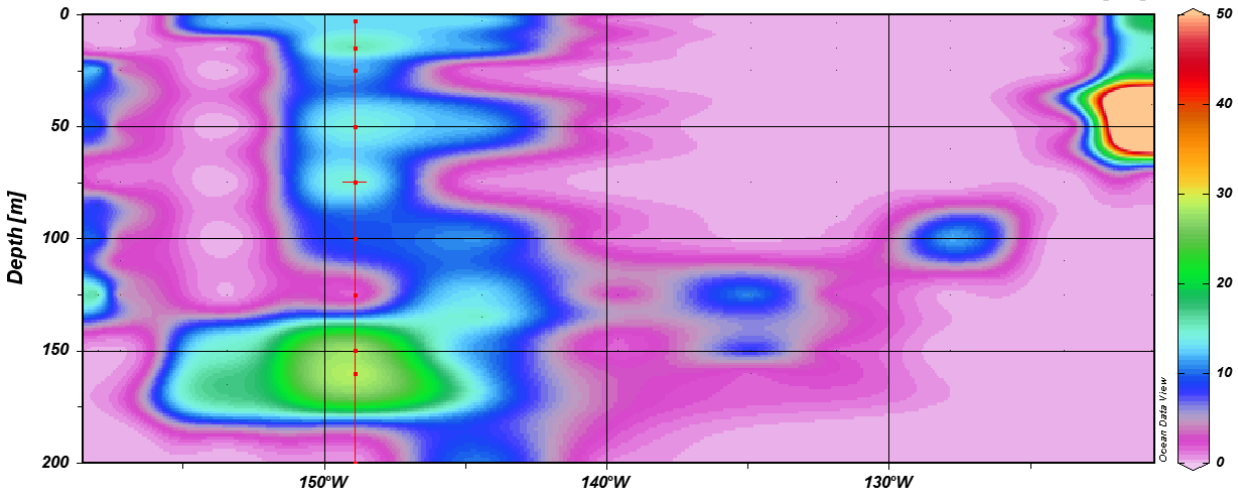

Salinity [PSU]
Depth [m]
0
50
100
150
200
250
300
150°W
140°W
130°W
120°W
35.5
35
34.5
34
33.5
33
Ocean Data View

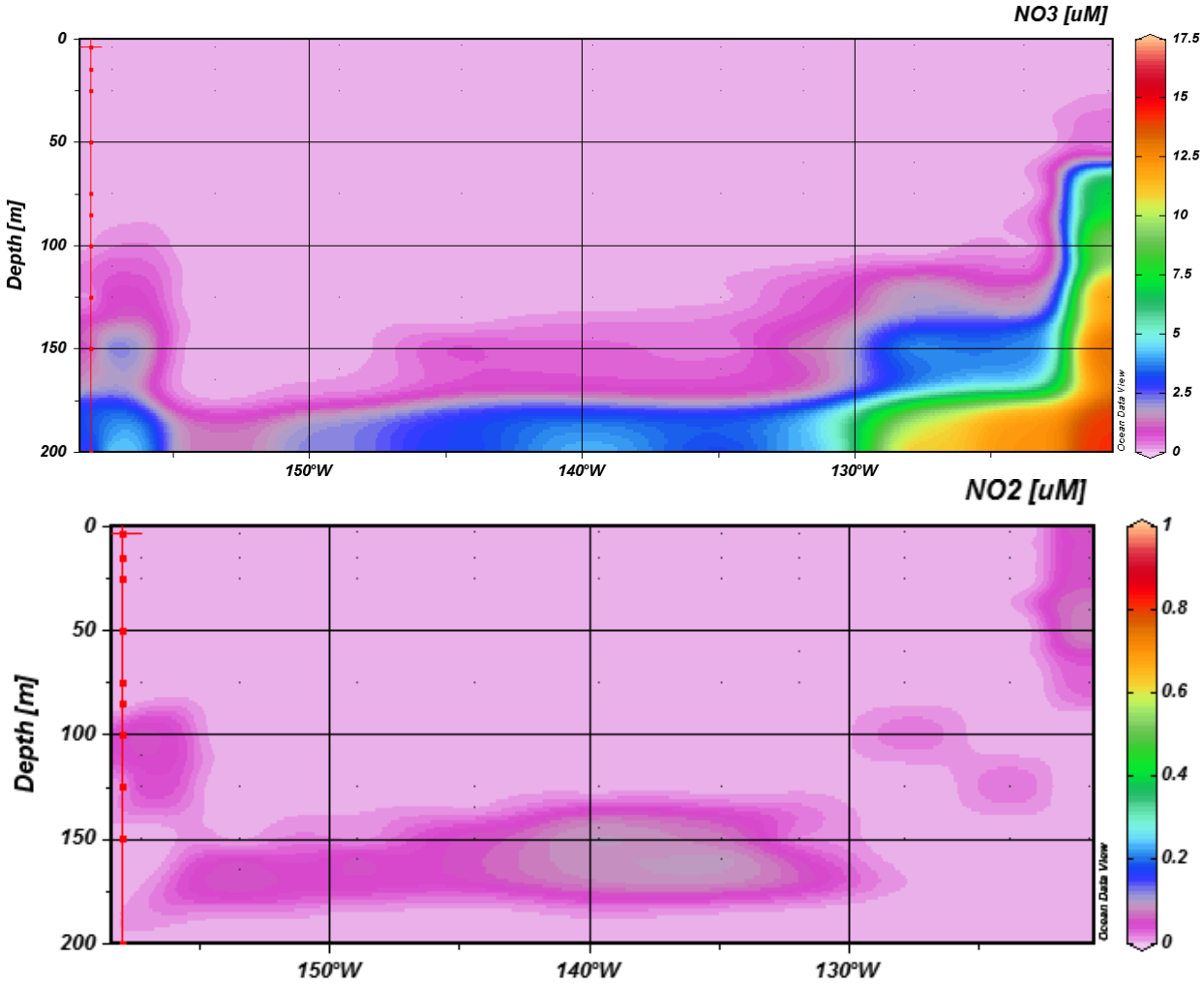

Density [kg/m3]
Depth [m]
0
50
100
150
200
250
300
150°W
140°W
130°W
120°W
28
27
26
25
24
Ocean Data View

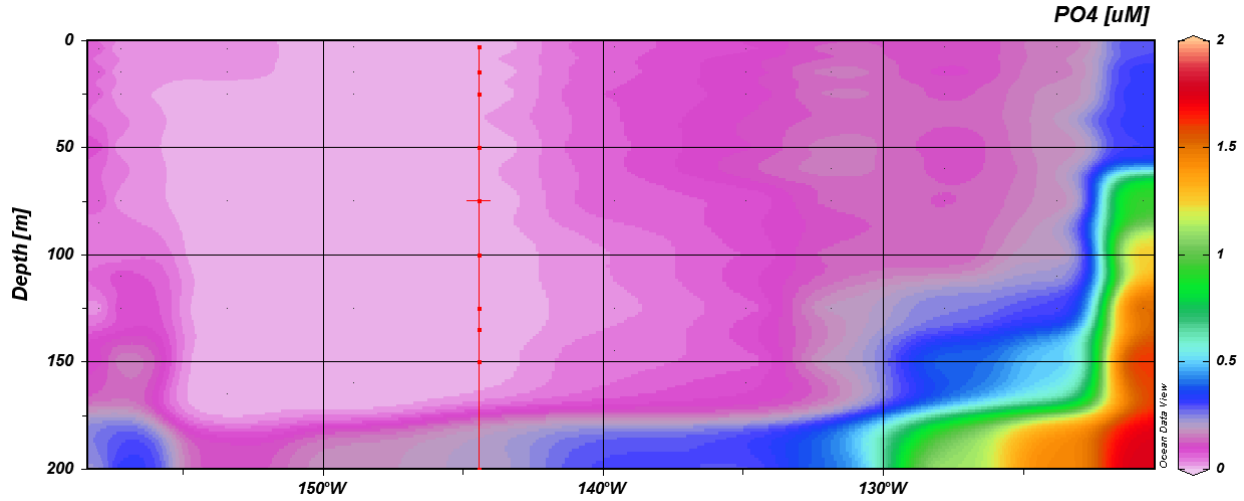

Oxygen [umol/kg]
Depth [m]
0
50
100
150
200
250
300
150°W
140°W
130°W
120°W
250
200
150
100
50
0
Ocean Data View
Fluorescence
Depth [m]
5
4
3
2
1
0
Beam Attenuation [1/m]
Depth [m]
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0

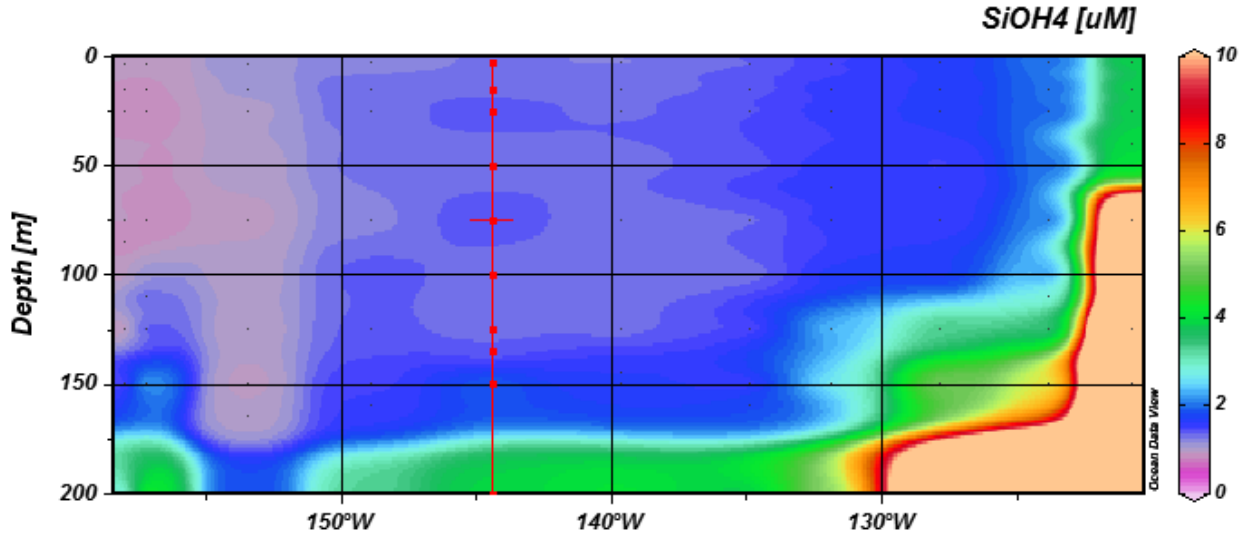

pH
Depth [m]
0
50
100
150
200
150°W
140°W
130°W
120°W
8.1
8
7.9
7.8
7.7
7.6
7.5
Ocean Data View


DIC [uM]
Depth [m]
0
50
100
150
200
150°W
140°W
130°W
2400
2300
2200
2100
2000
Ocean Data View

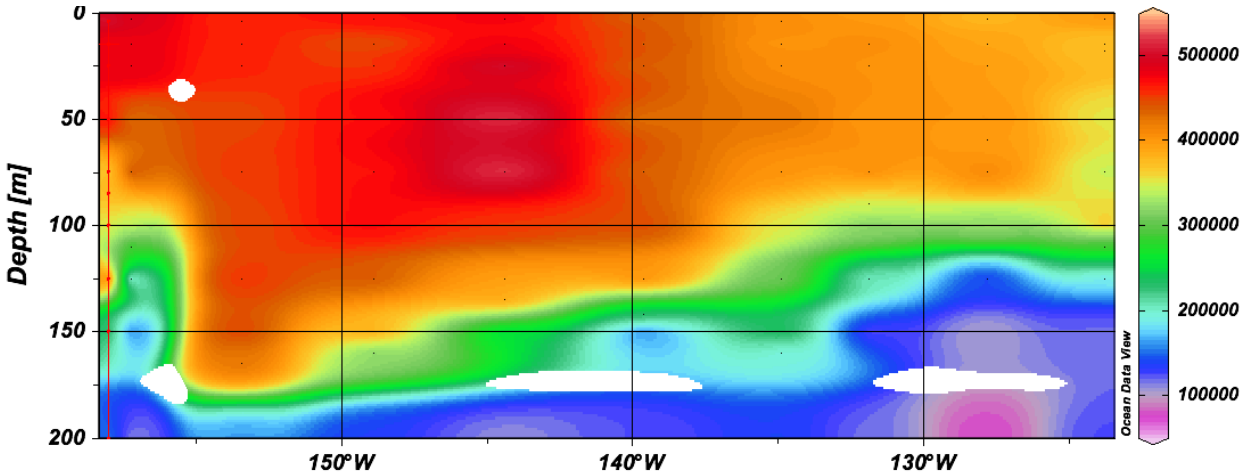

NH4 [nM]
Depth [m]
0
50
100
150
200
150°W
140°W
130°W
50
40
30
20
10
0
Ocean Data View


NO3 [uM]
Depth [m]
0
50
100
150
200
150°W
140°W
130°W
17.5
15
12.5
10
7.5
5
2.5
0
Ocean Data View
NO2 [uM]
Depth [m]
0
50
100
150
200
150°W
140°W
130°W
1
0.8
0.6
0.4
0.2
0
Ocean Data View

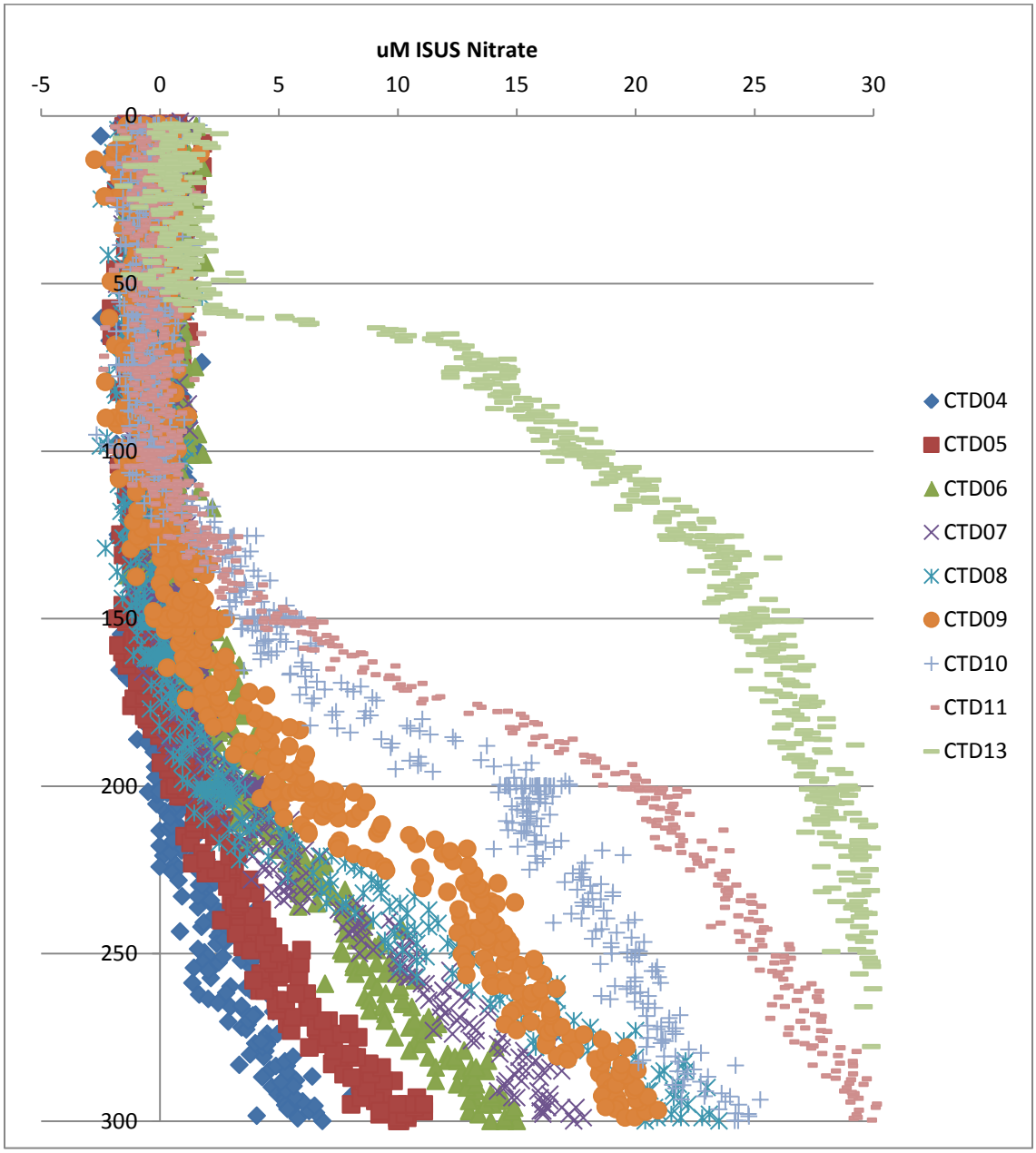


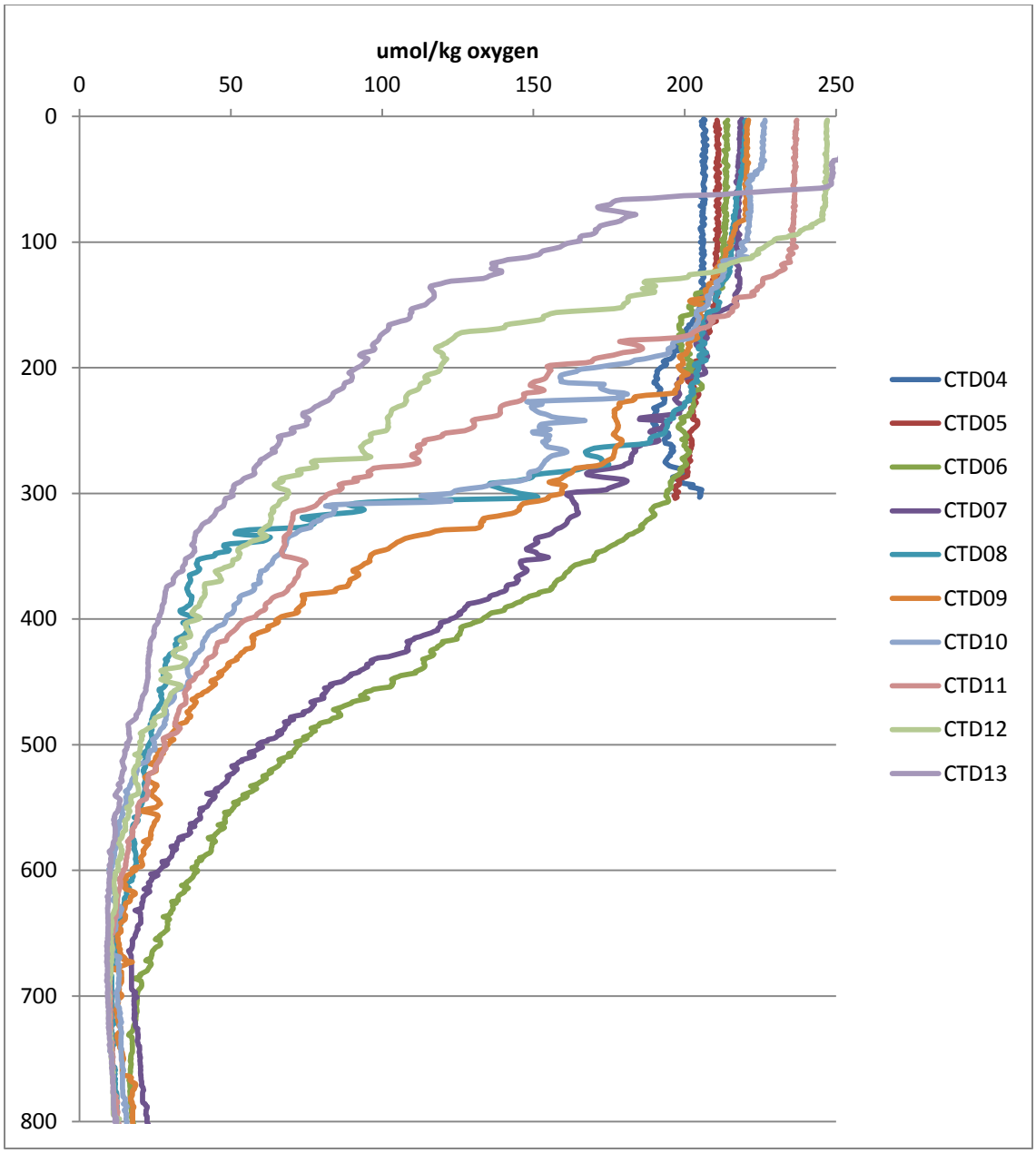


total bacteria / mL (including photosynthetic)



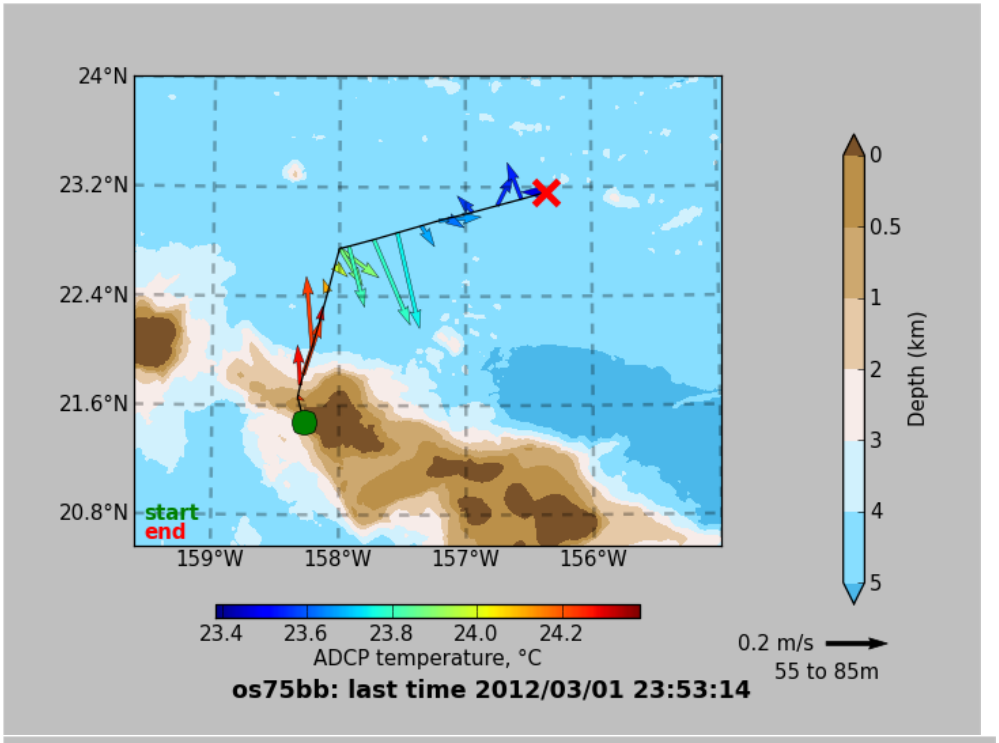
Hydrography: vertical station plots

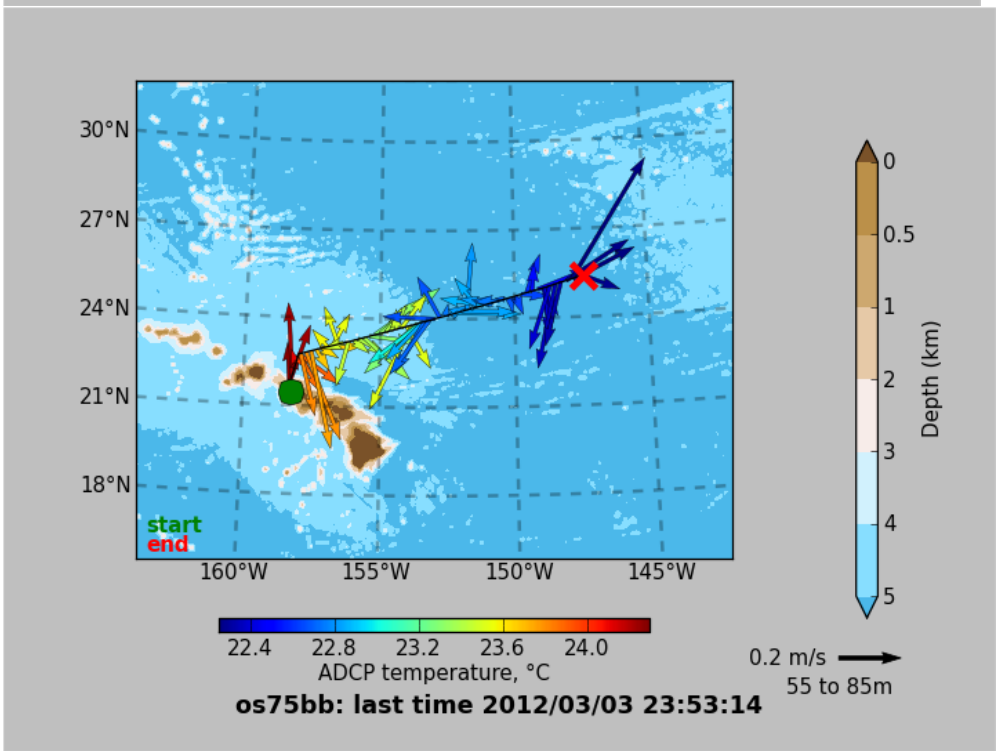


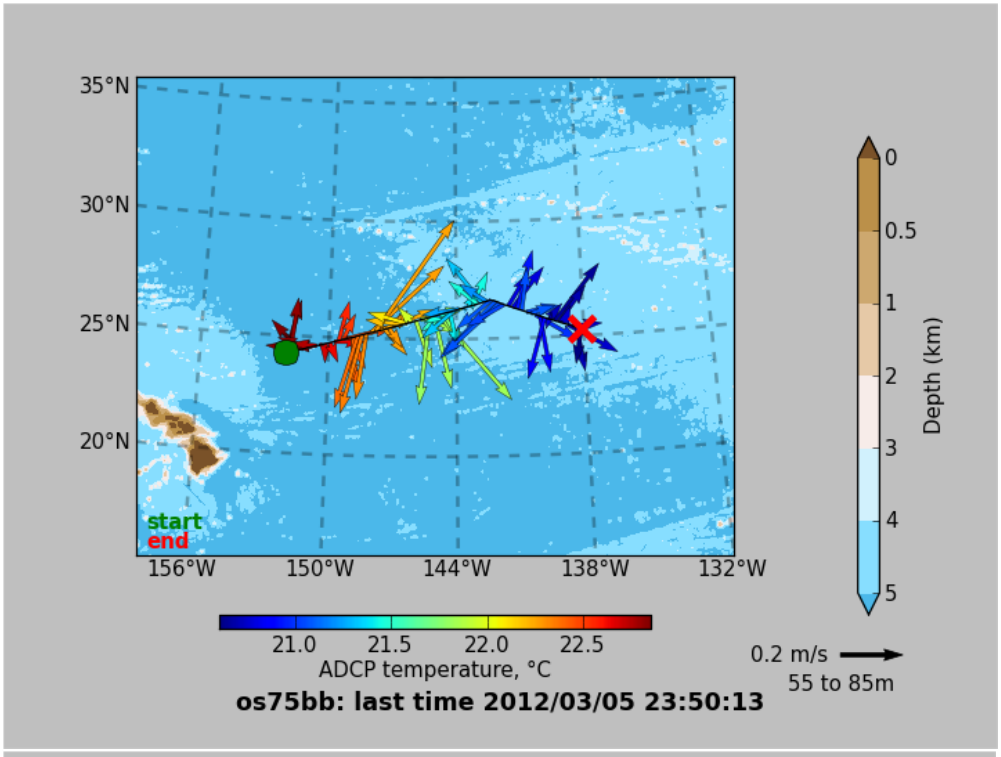

umol/kg oxygen
0
50
100
150
200
250
0
100
200
300
400
500
600
700
800
CTD04
CTD05
CTD06
CTD07
CTD08
CTD09
CTD10
CTD11
CTD12
CTD13

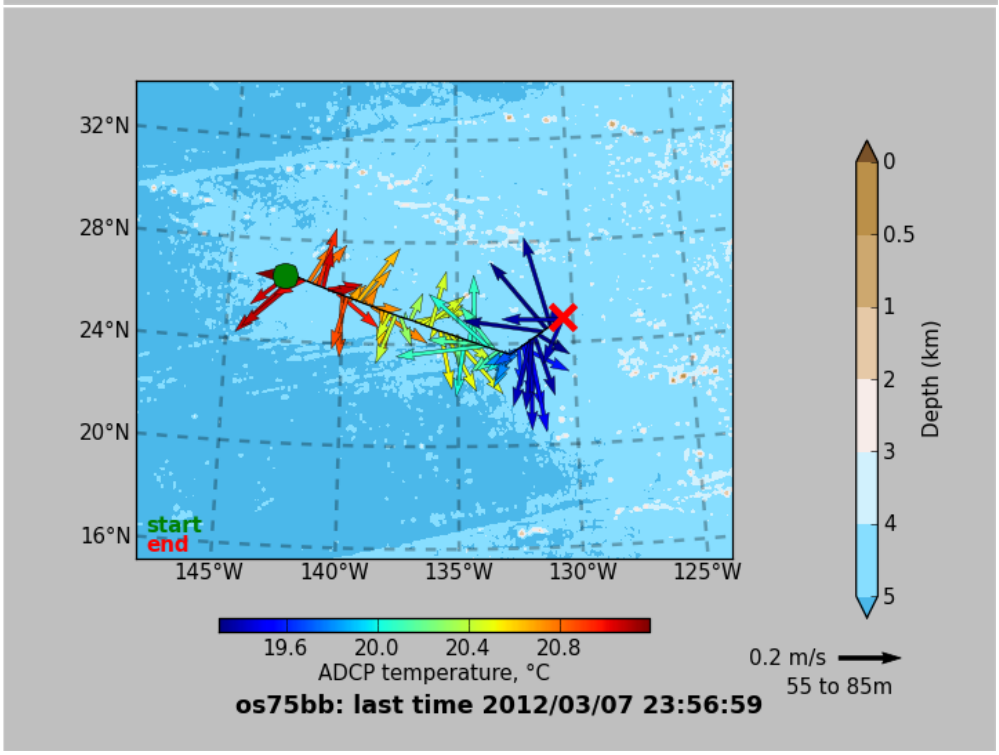
ADCP (OS75BB):

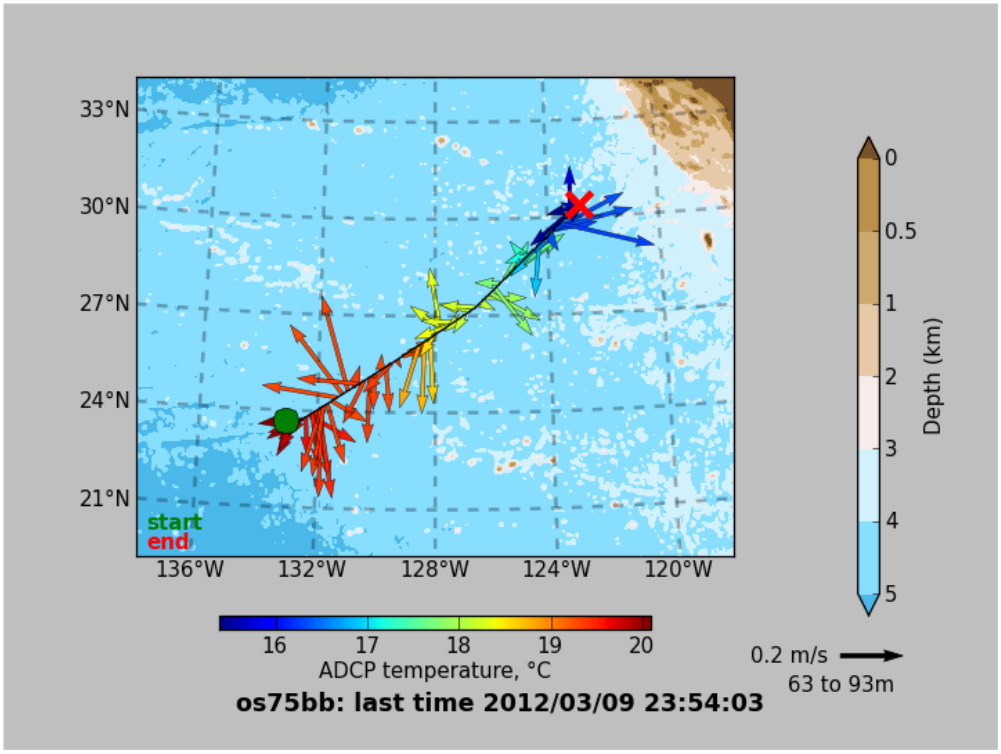
Surface Vectors



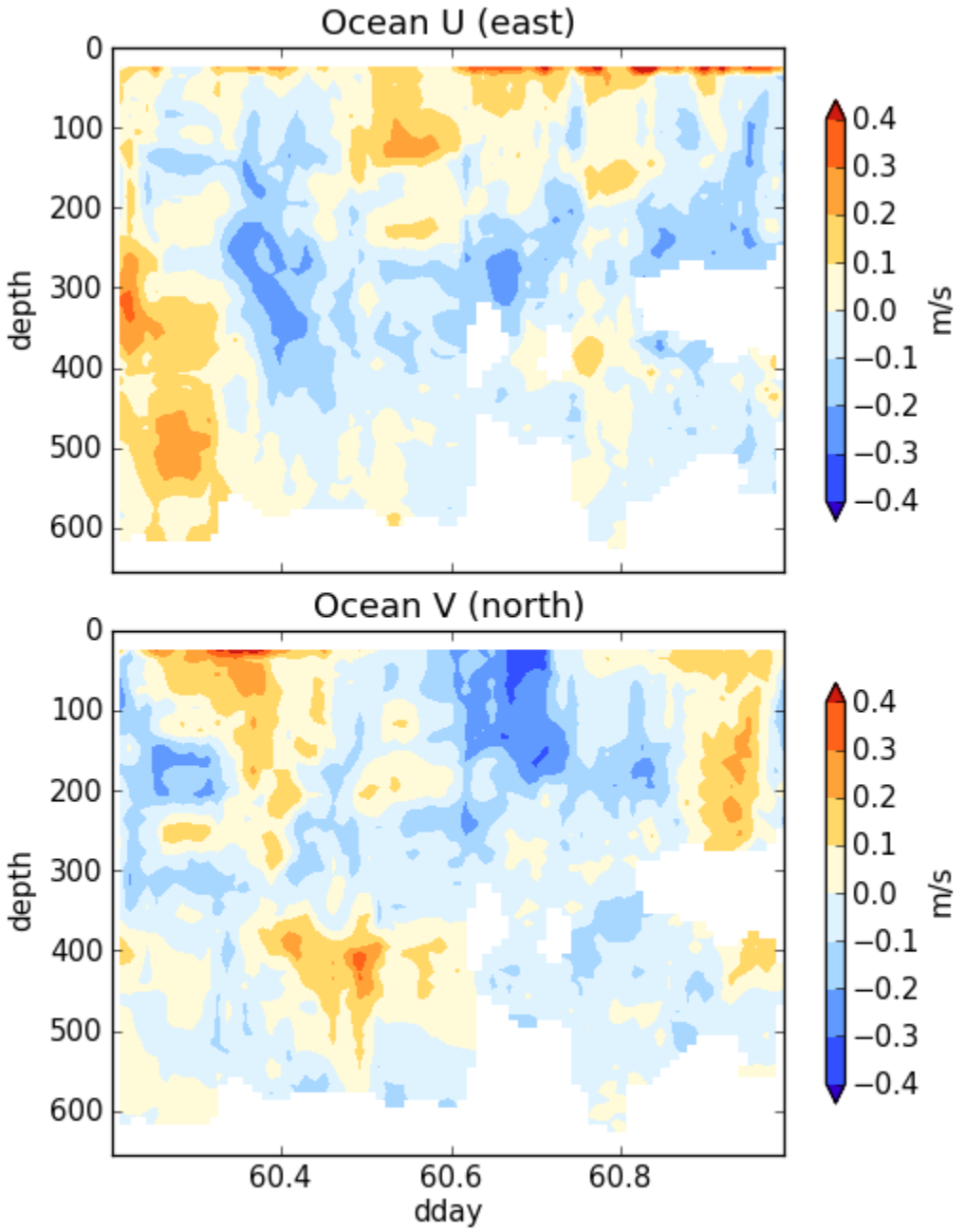



35°N
30°N
25°N
20°N
start
end
156°W
150°W
144°W
138°W
132°W
21.0
21.5
22.0
22.5
ADCP temperature, °C
0
0.5
1
2
3
4
5
Depth (km)
0.2 m/s
55 to 85m
os75bb: last time 2012/03/05 23:50:13

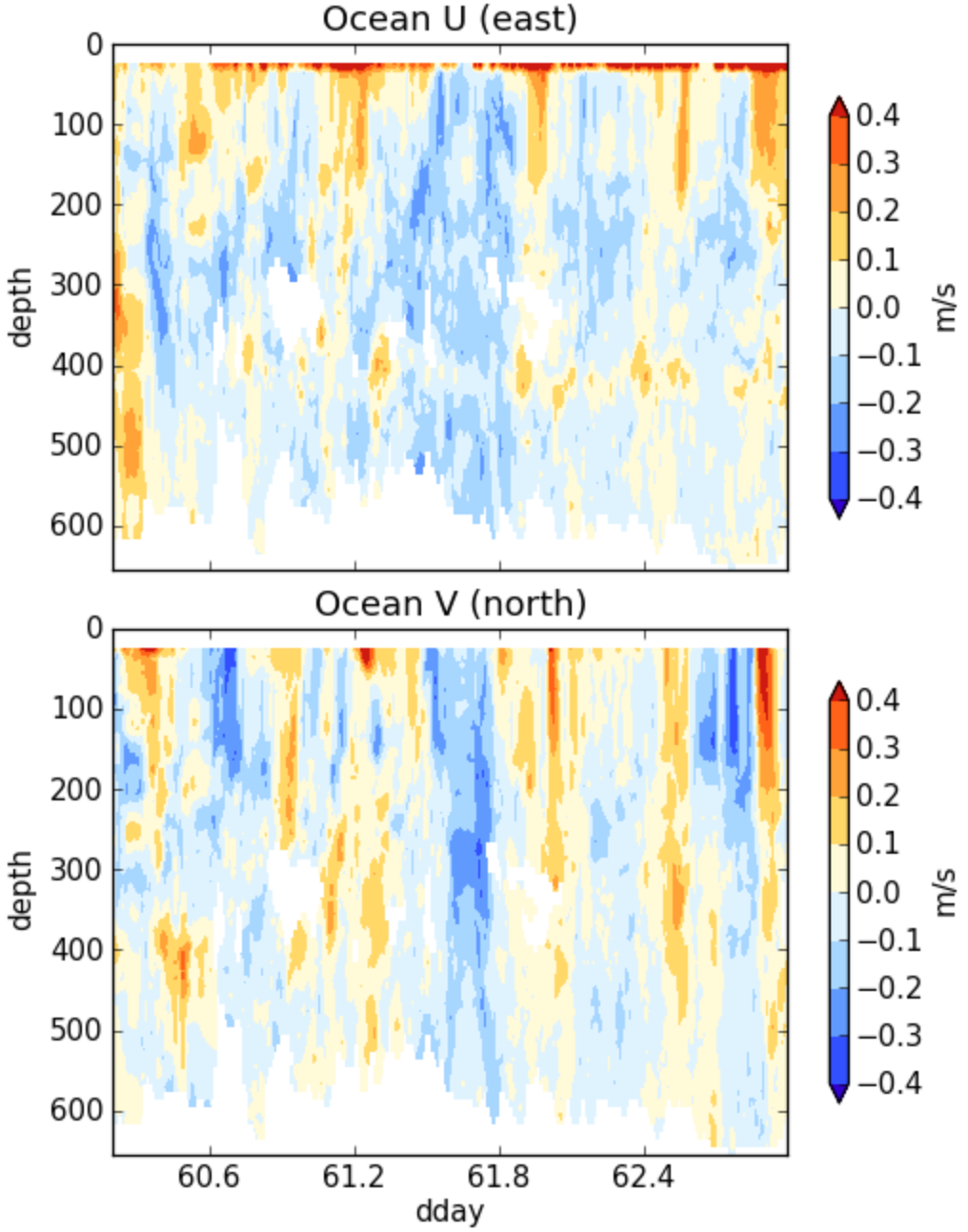

32°N
28°N
24°N
20°N
16°N
start
end
145°W
140°W
135°W
130°W
125°W
19.6
20.0
20.4
20.8
ADCP temperature, °C
0
0.5
1
2
3
4
5
Depth (km)
0.2 m/s
55 to 85m
os75bb: last time 2012/03/07 23:56:59


33°N
30°N
27°N
24°N
21°N
start
end
136°W
132°W
128°W
124°W
120°W
16
17
18
19
20
ADCP temperature, °C
0
0.5
1
2
3
4
5
Depth (km)
0.2 m/s
63 to 93m
os75bb: last time 2012/03/09 23:54:03

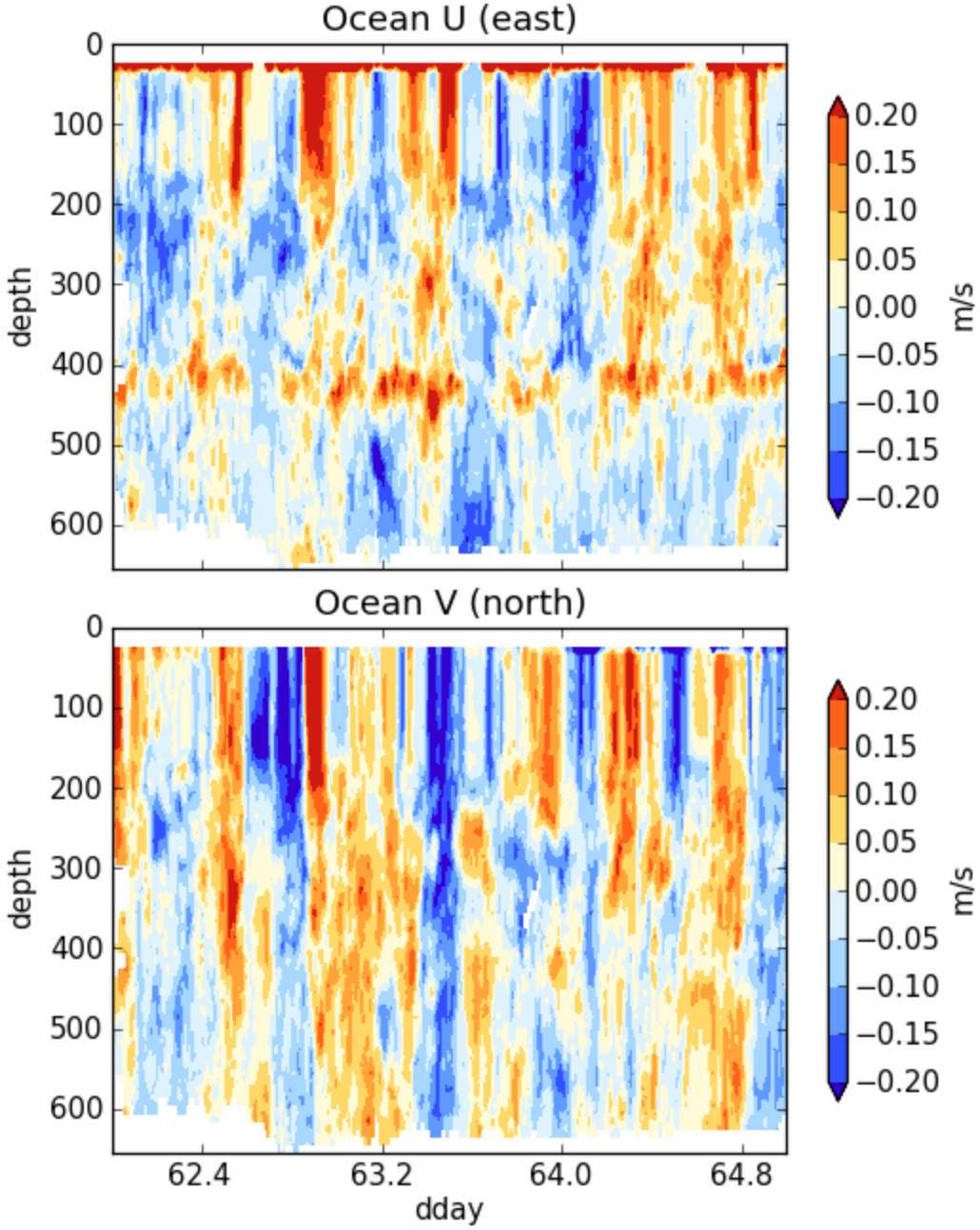
Depth Vectors (decimal day)



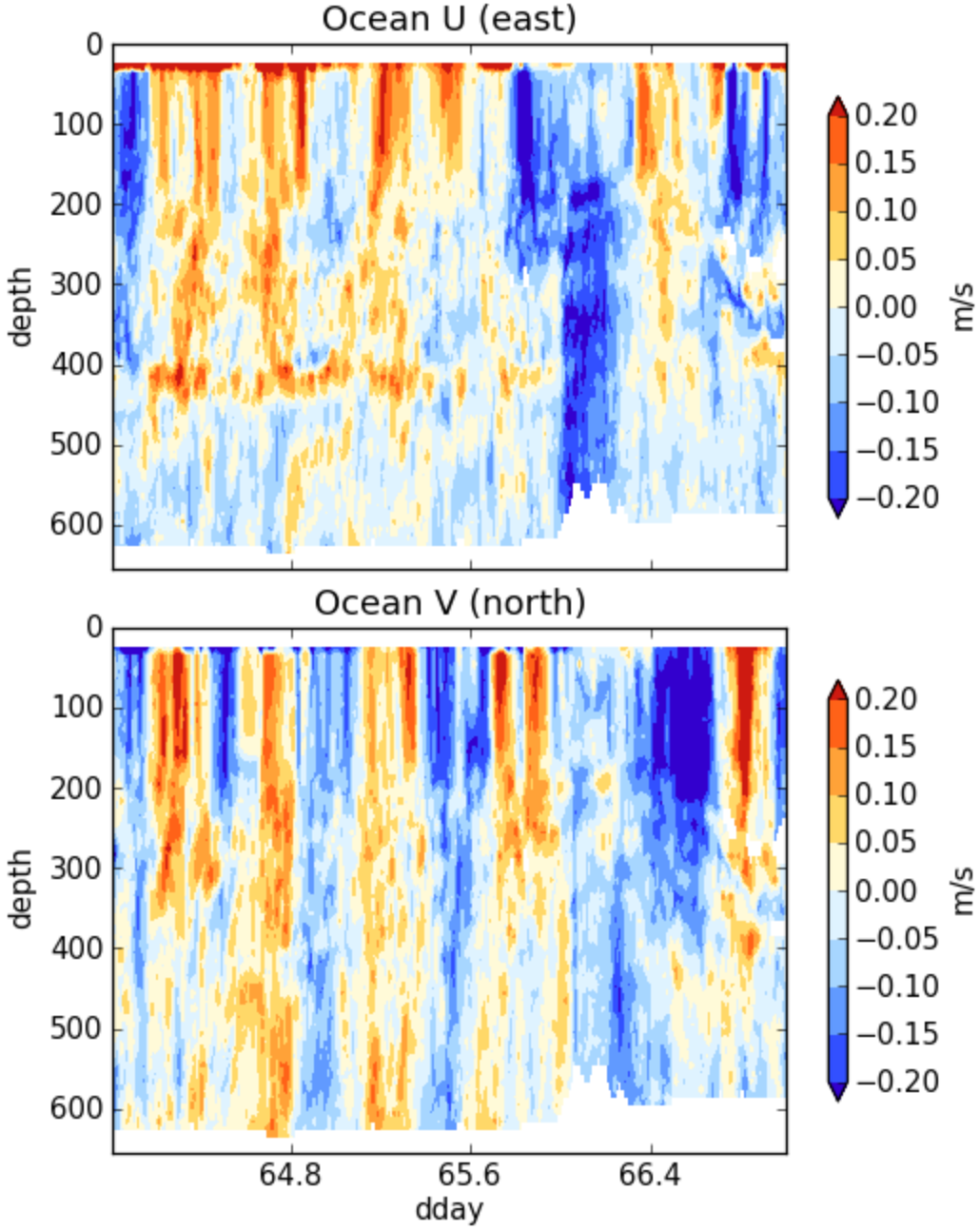
os75bb: last time 2012/03/01 23:48:13



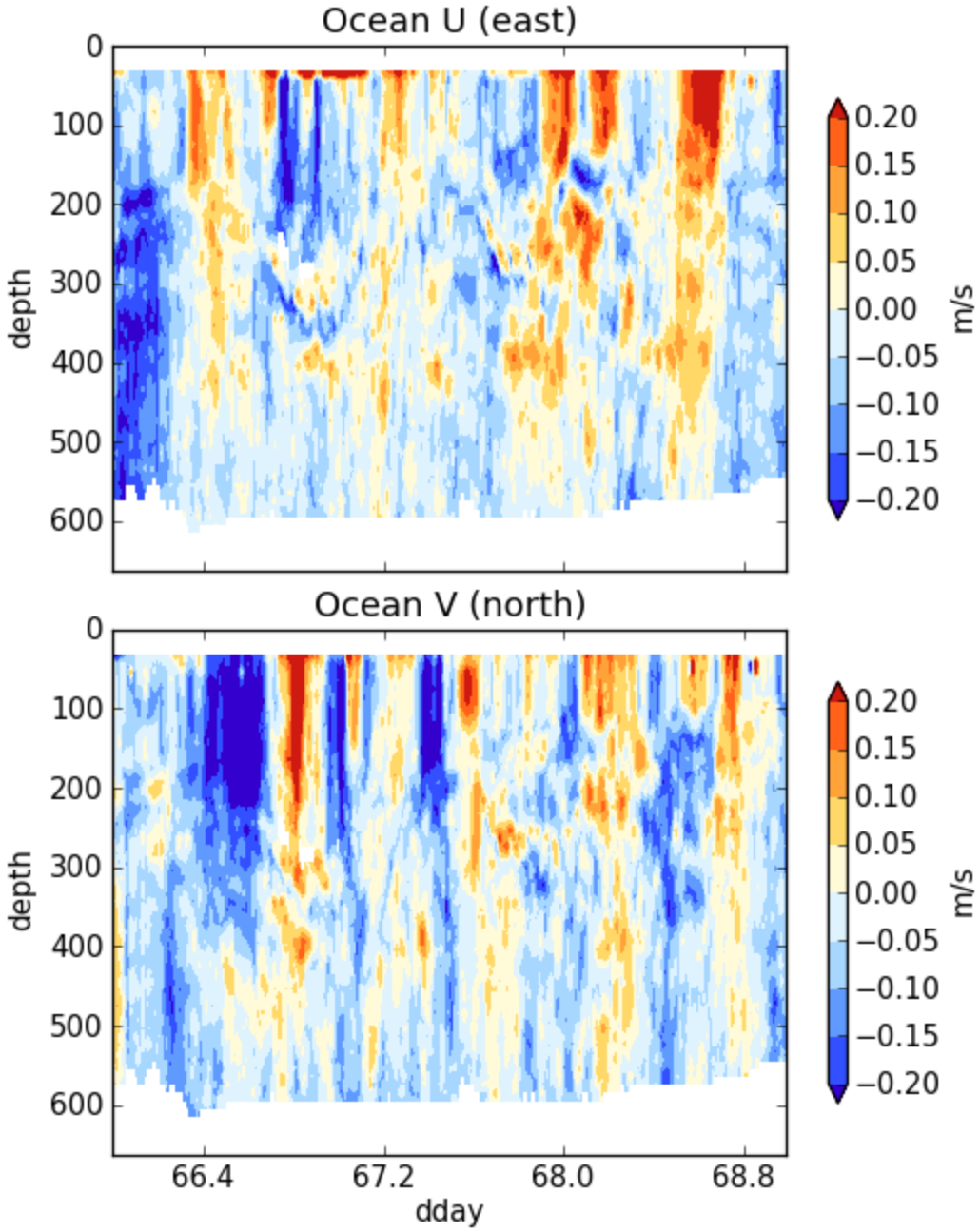
os75bb: last time 2012/03/03 23:48:13



os75bb: last time 2012/03/05 23:53:13

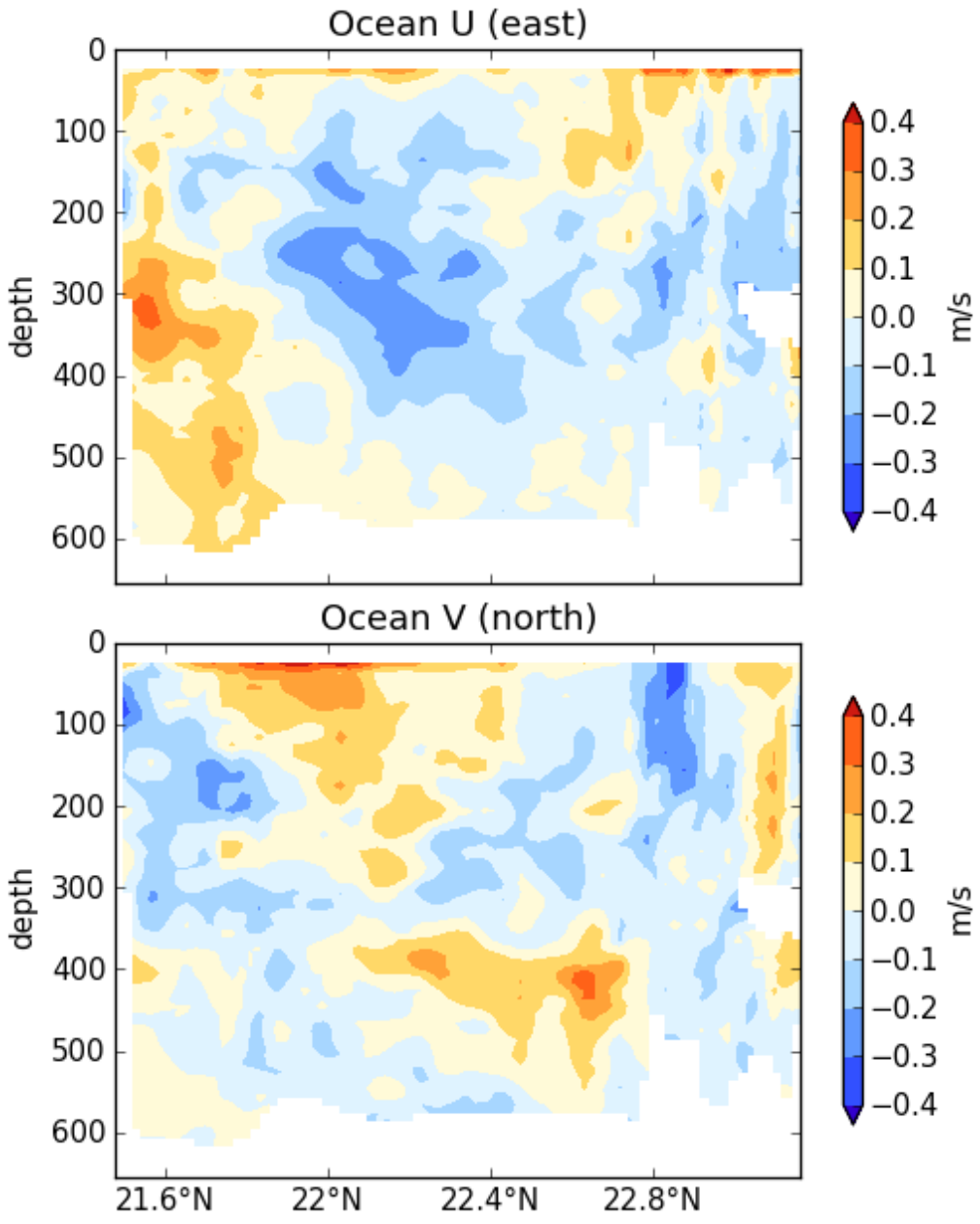


os75bb: last time 2012/03/07 23:58:13



os75bb: last time 2012/03/09 23:40:14

Depth Vectors (Latitude)



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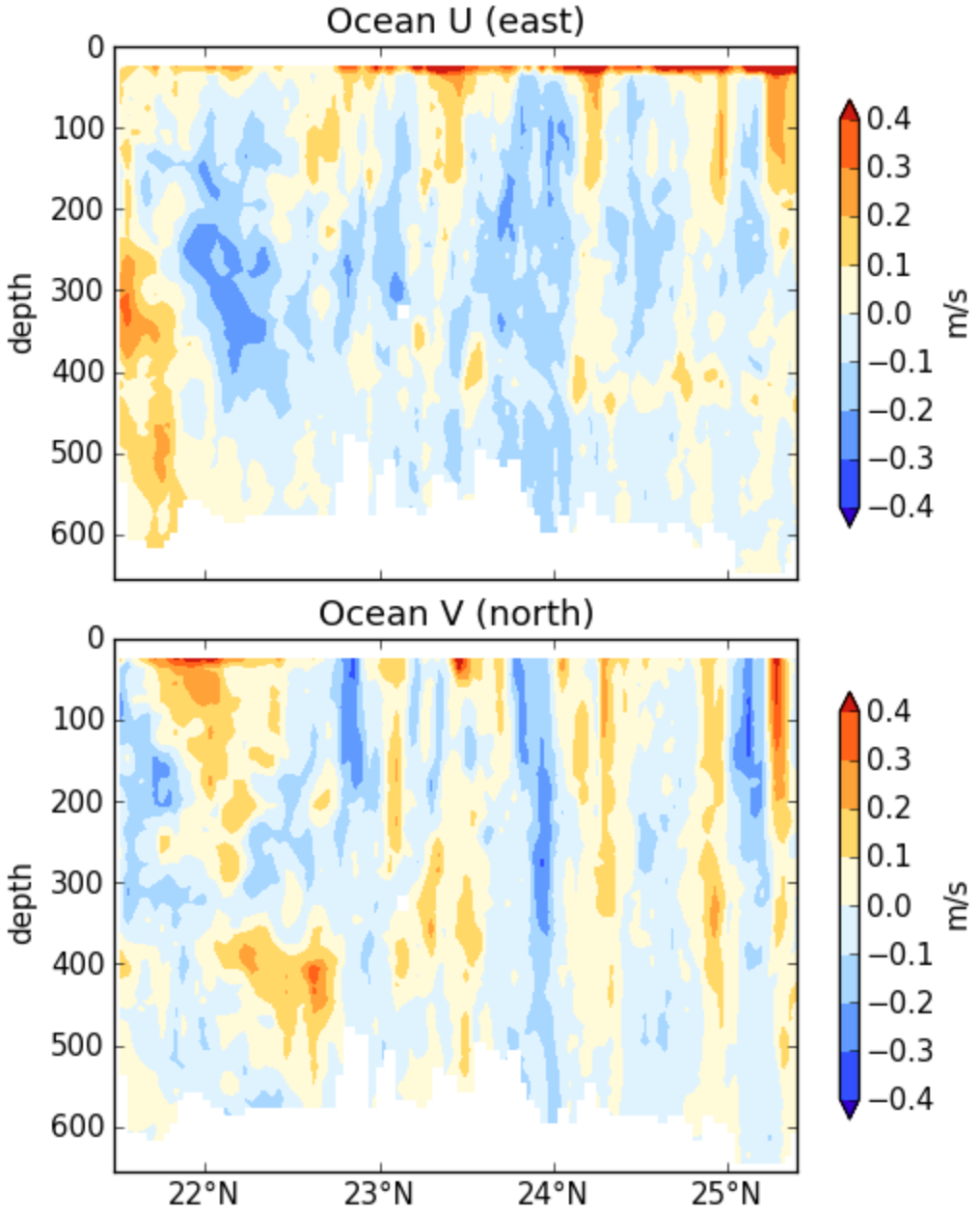
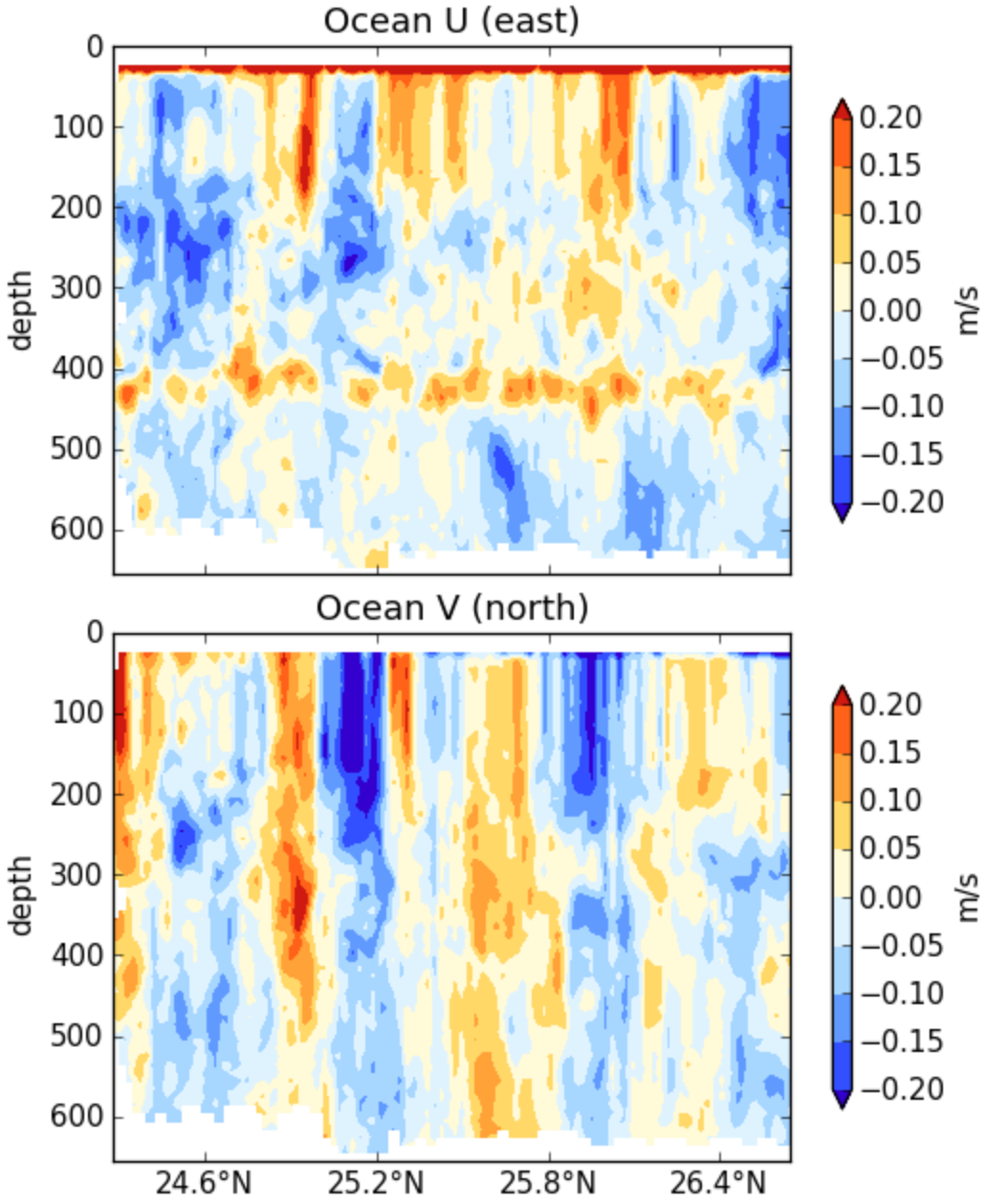


os75bb: last time 2012/03/03 23:48:13



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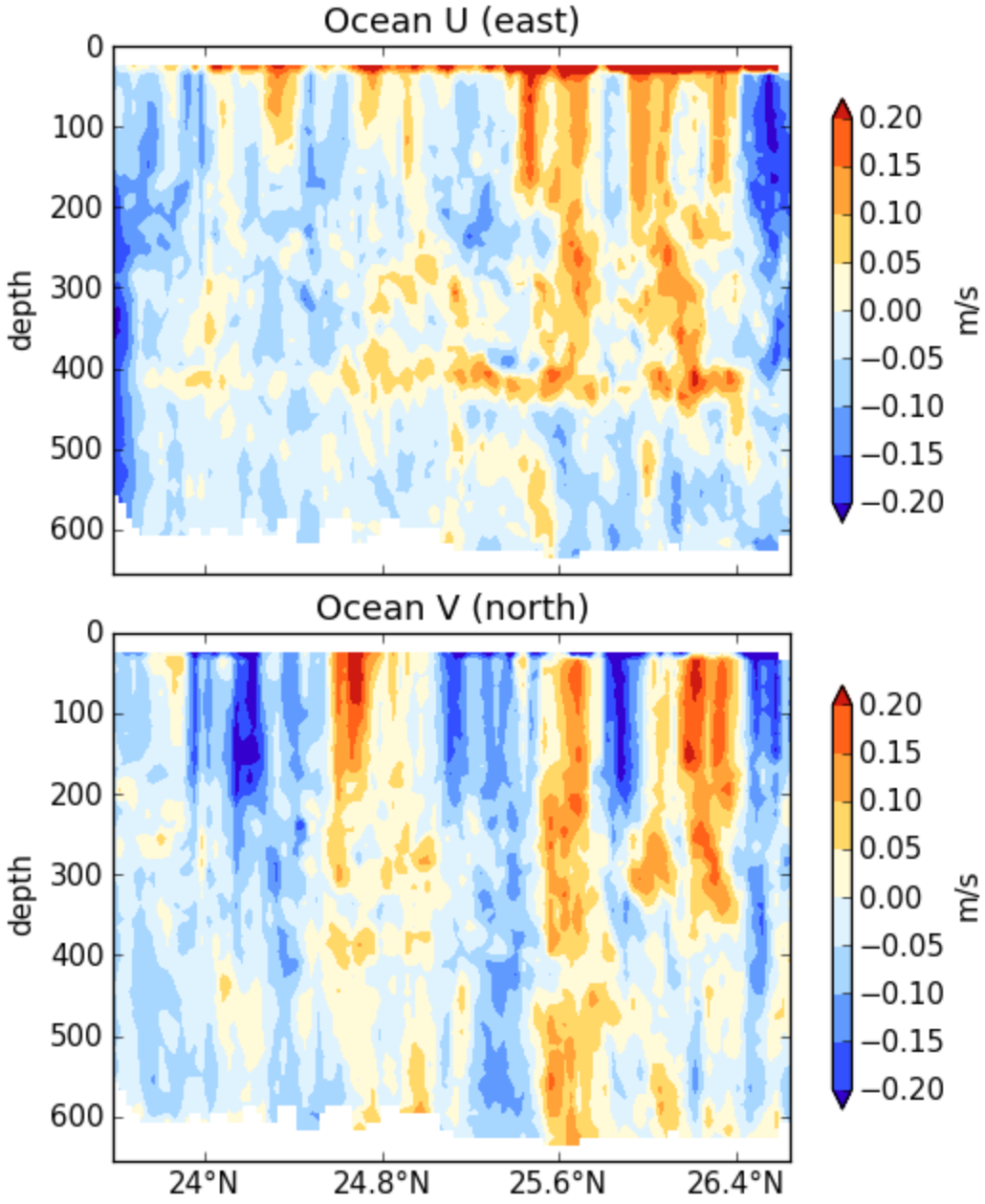
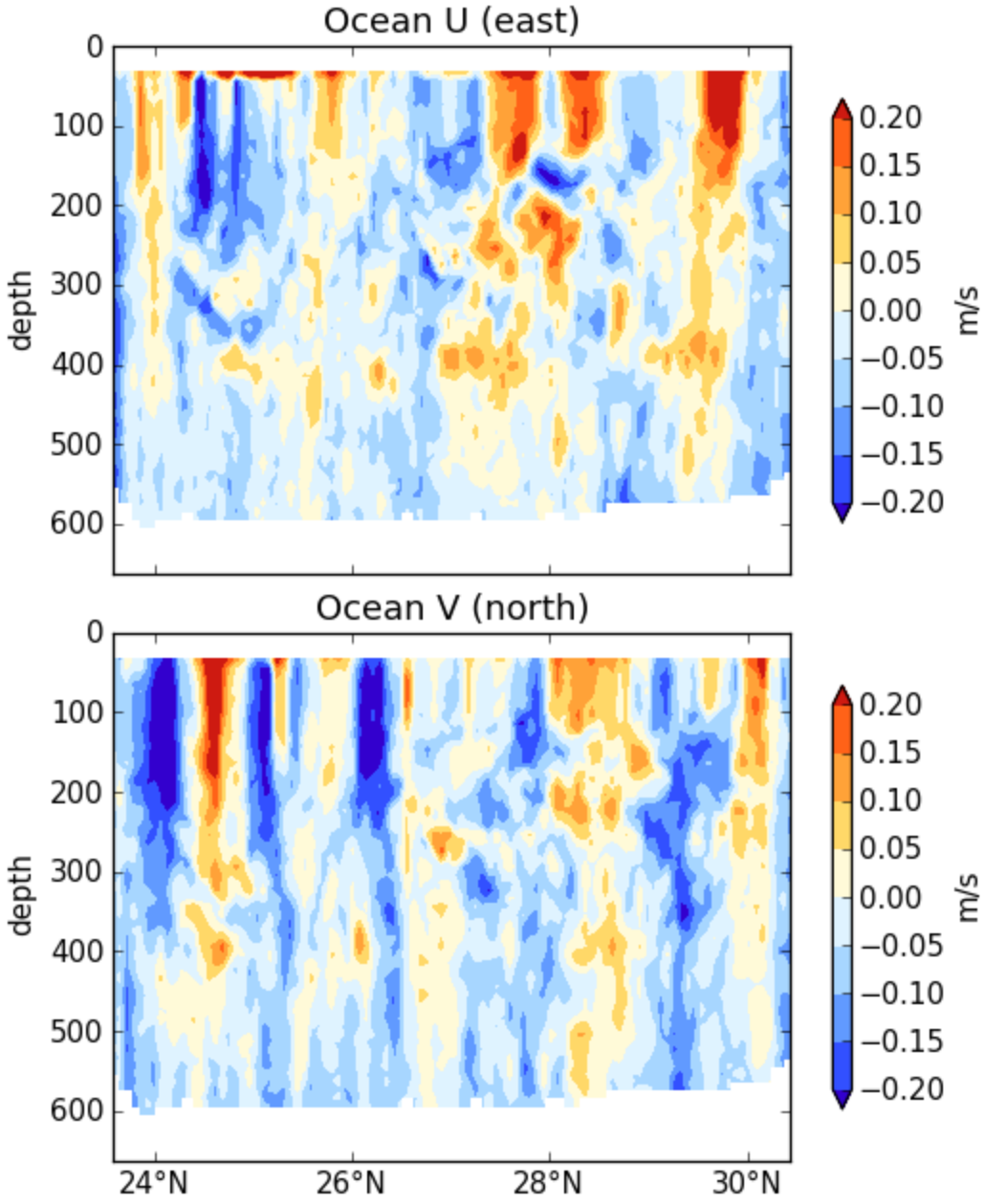


os75bb: last time 2012/03/07 23:58:13



os75bb: last time 2012/03/09 23:55:13

References

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http://oceanography.ml.duke.edu/johnson/research/powow

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http://www.nicholas.duke.edu/insider/trips/researchatsea

Data Archive
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Major Funding Agency:
National Science Foundation
4201 Wilson Boulevard
Arlington, VA 22230
http://www.nsf.gov

University of Washington Marine Center (R/V Thomas G. Thompson)
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