

Plankton Of Warming Ocean Waters (POWOW#3)

Cruise Report

July 1-28, 2013

Honolulu, Hawaii to San Diego, CA

R/V Kilo-Moana 1312

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Erik Zinser (University of Tennessee)
Steve Wilhelm (University of Tennessee)

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Abstract

The POWOW#3 cruise was the third in a series of cruises to study the influence of temperature and other environmental variables on *Prochlorococcus*, its viruses and other members of the microbial community in the Northern Pacific Ocean. 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

Name	Group	Role
Dr. Zackary Johnson	Duke-PI	Chief Scientist (of paper work), ISUS, PE response curves
Ming Leung	Duke	NH ₄ / frozen nutrients
Justin Cruz	Duke	Ap / FRe (profiles + incubations)
Molly Reichert	Duke	Size fractionated chlorophyll (profiles + T-shift)
Sarah Loftus	Duke	at sea flow cytometry (FACSCalibur)
Alyse Larkin	Duke	RNA + DNA; frozen fcm; Sterivex
Maria de Oca	Duke	Primary production / Flow cytometry lead
Jeff Carr	Duke/UTK	DIC/pH
Dr. Erik Zinser	UTK-PI	Chief Mate Scientist / <i>Prochlorococcus</i> Oxidative Stress
Jackson Gainer	UTK	Virus, Het. Bacteria
Megan Silbaugh	UTK	<i>Prochlorococcus</i> metabolic profiling
Helena Pound	UTK	Virus, Het. Bacteria
Lewis Walker	UTK	Confessionals / outreach
Nana Ankrah	UTK	Roseobacter

Friends of the Cruise (not participating onboard, but sending group, equipment, etc.)

Name	Group	Role
Dr. Steve Wilhelm	UTK	Co-PI UTK
Dr. Allison Buchan	UTK	Roseobacter
Dr. Elizabeth Steffen	NOAA/PMEL	ARGO floats
Dr. Ken Johnson	MBARI	APEX floats
Rick Rupan	UW	APEX floats
Jules Hummon	University of Hawaii	ADCP

Crew

Name	Group	Role
Rick Meyer	UH	Captain
Trevor Goodman	OTG/UH	Lead Marine Technician
David Hashisaka	OTG/UH	Marine Technician



Figure 1: Group Photo of cruise participants

Contact Information

Johnson Lab

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Wilhelm Lab

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Research Objectives / Data

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

1. *Prochlorococcus* abundance, diversity and activity and environmental variables
2. DNA, RNA for specific activity of microbial community including *Prochlorococcus*
3. nutrients (NO₂, NO₃, SiOH₄, PO₄, NH₄)
4. ISUS NO₃
5. flow cytometry – het. Bacteria, *Prochlorococcus*, *Synechococcus*, photosynthetic picoeukaryotes
6. size fractionated chlorophyll (0.22 and 0.8 µm)
7. pH (spectrophotometric), DIC
8. *Prochlorococcus* isolations
9. Temperature shift incubations
10. Size fractionated (0.22 and 0.8 µm) C-14 Primary production
11. Size fractionated (0.22 and 0.8 µm) C-14 photosynthesis-irradiance curves
12. Fluorescence induction and relaxation (FIRe)

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 H₂O₂ 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 temperature gradient and within the water column
2. To determine the distribution of N4-like roseoviridae along the transect
3. To collect samples for DNA analyses of microbial community structure.

Buchan Lab – UTK

Roseobacter diversity and the influence of virus lysates on natural population metabolism

Steffen & G. Johnson Group - UW/NOAA

ARGO floats

K. Johnson Group - MBARI

APEX floats

Outcomes

Johnson Lab

The Johnson group analyzed samples for numerous physical, chemical and biological variables along the meridional transects across gradients in sea surface temperature. Many of the physical and chemical variables are plotted below as part of the cruise hydrography although there are numerous additional samples that will be analyzed in the laboratory. Specifically, using a flow cytometer at sea, samples were taken throughout the water column (upper 200 m) and were analyzed for the abundance of *Prochlorococcus*, *Synechococcus* and other picophytoplankton across temperature gradients in the North Pacific. Depth profiles showed that the depth of maximum *Prochlorococcus* abundance generally decreased with increasing latitude, and ranged from 25 to 100 m. Profiles were most similar along the 19°C isotherm. Additionally, red fluorescence of *Prochlorococcus* increased with depth. Light (via depth and mixing) also appears to be important. Additional frozen and preserved samples from the water column were collected for on shore flow cytometry (~536 samples in total) for total bacterioplankton. Samples from 4 temperature shift experiments (~300 samples in total, see below) were also collected. Size fractionated chlorophyll (0.22 and 0.8 µm) was collected to determine the total and “*Prochlorococcus*”-specific biomass of phytoplankton along temperature and other environmental gradients. Chlorophyll profiles showed a deep chlorophyll maximum that became shallower as latitude increased. Active fluorescence (FIRE) was used to measure parameters of photosynthesis for both water column profiles and temperature shift experiments. pH was measured using a fixed-temperature colorimetric analytical method and the data show a strong decrease in seawater pH—increase in acidity—with both increasing latitude and depth. Ammonium (NH₄) was measured in triplicate at all of the sampling stations using a fluorometric technique. NH₄ is generally relatively low below 36°N with higher values above this latitude. Ammonium was also measured for temperature shift experiments. Frozen samples for nitrate, nitrite, phosphate, urea and silicate were taken for all profiles. An ISUS was used to estimate nitrate concentration *in situ*. *Prochlorococcus* DNA and RNA samples were collected from environmental CTD casts and from onboard temperature shift experiments using 0.2 µm polycarbonate filters. In total, we collected ~1,700 samples, which will be analyzed using qPCR and next generation sequencing. Size fractionated (0.22 and 0.8 µm) C-14 based primary production (on-deck incubations at 8 depths) and photosynthesis-irradiance curves (2 depths) were made for most early morning (i.e. ~4 am local) stations to estimate the contribution of *Prochlorococcus* and total phytoplankton to carbon cycling. Stations sampled for primary production covered sea surface temperatures from 10 to 21°C. The highest primary production for all size classes was found at the highest latitudes (cold temperatures). Higher total primary production values seemed to be associated with physical forcing, as evidenced by shallow deep chlorophyll maximum (DCM) and nitracline depth values.

In addition to measuring natural variability, four temperature shift experiments were performed whereby water from ~18°C was placed in control or -2 and +2°C incubators and various physical, chemical and biological variables tracked. The goal of these experiments was to determine how temperature affects the abundance, diversity and activity of *Prochlorococcus*. Each experiment lasted ~4 days and was sampled daily. Flow cytometry was used to determine changes in populations incubated at three different temperatures. In general, population size was augmented most greatly in the temperature increase environment, as compared to both the

control and the temperature decrease. Overall, the several 4-day temperature shift experiments generally showed decreases in chlorophyll in the beginning, with an increase by the end of the 4 days. The rates of change varied between each incubator (one control, one warm, one cool) and variability between sample bottles was also observed.

Zinser Lab

The Zinser group worked on three different projects for KM1312 (POWOW 3). The first project was to collect for molecular characterization of the *Prochlorococcus* populations along the transect. For this purpose we collected cells via filtration, which we will process back in the lab at the University of Tennessee and assay by quantitative PCR, which will allow us to quantify the different genetic lineages of *Prochlorococcus*. In a similar way we sampled the temperature shift incubation experiments. In addition, as for the KM1301 (POWOW 2) cruise, we brought on board our thermocycler which allows us to perform quantitative PCR. This instrument was used successfully to quantify two of the numerically-dominant lineages of *Prochlorococcus* during the cruise, giving us real-time information on the population structure over the transects. While only a preliminary study, it nonetheless challenged our hypothesis that the dominant lineages switch dominance at the surface at approximately 19 °C: while the “eMED4” lineage dominated colder waters, and the “eMIT9312” lineage dominated the warmer ones, the 1:1 temperature was warmer, at 22C, compared to the winter temperature, at 19C. Studies in the lab with greater replicates will be used to further develop this seasonal study.

The second project on which the Zinser lab worked consisted of collecting samples of the whole microbial community, as well as the *Prochlorococcus* community, for analysis of their metabolomes under various situations of nitrogen surplus, limitation, and starvation. Analysis of the cellular metabolites of these samples using mass spectrometry tools at UT will provide us with insight into the cellular processes taking place under different environmental conditions. We will also be able to compare results from these experiments with those of the same experiments which were performed on the POWOW2 cruise in January 2013 to consider seasonal differences in the cellular biochemistry. The Zinser lab also collected microbial community samples which had been supplemented with leucine or urea. The eventual fate of the incorporated leucine will be tracked using mass spectrometry, which will provide a better understanding of where leucine incorporation, measured in secondary production studies, is actually taking place within the cells. The same will be analyzed for the labeled urea to examine how the marine microbes are utilizing this nitrogen source.

The third project was to assess the ability of the microbial community to protect *Prochlorococcus* from hydrogen peroxide-mediated oxidative stress. *Prochlorococcus* is highly sensitive to peroxide, and our laboratory studies suggest a protective function for the other members of the marine microbial community. We used a dilution technique to vary the ability of the community to deal with a peroxide challenge, and assayed for changes in peroxide levels. We also collected samples of *Prochlorococcus* and the microbial community for further analysis back at UT.

Wilhelm Lab

We were able to complete most of our research objectives on the cruise. Approx. 680 bacterial production samples were processed at sea – sample processing stopped with about 10

days left in the cruise due to a catastrophic failure of the refrigerated microcentrifuge (see comments in cruise report on power issues in the radiation van). The group was also able to collect and process size fractionated chlorophyll from each cast (data available in swap filter), ~ 400 samples for the enumeration of *Prochlorococcus* phage by qPCR, ~ 800 samples for total virus enumeration. We completed about 25 experiments to measure the production rate of total viruses as well as cyanomyoviruses and *Prochlorococcus*-specific viruses. Nucleic acids were collected throughout the cruise (210 total samples) for metagenomic community analyses.

From our data we see anticipated trends in the north/south distribution of phytoplankton biomass. One note is that the second trip north encountered much higher chlorophyll concentrations and much of this was in a larger (> 20 μm) size class consistent with the presence of a small diatom bloom. This might be tied to spikes in surface N noted by the Johnson lab in the preceding days and is potentially tied to storm events leading to upwelling of nutrient rich deeper waters.

ARGO / APEX Deployments

Two APEX floats from MBARI and four ARGO floats from UW, were deployed on the cruise. All are successfully transmitting and details of the deployment are listed below.

Data

CTD Locations & Times

Station #	Cast	Event ID	UTC Day	UTC Time	Lat	Lon	Bottom Depth (m)
1	1	CTD01	183	04:15	21.3911	-158.2908	1323
2	1	CTD02	183	21:54	22.7508	-158.0002	4743
2	2	CTD03	184	13:53	22.7500	-158.0004	4742
3	1	CTD04	184	21:57	24.0883	-157.9989	4496
4	1	CTD05	185	13:58	27.0658	-157.9972	5392
5	1	CTD06	185	21:56	28.2896	-157.9988	5531
6	1	CTD07	186	11:53	30.7293	-157.9991	5936
7	1	CTD08	186	21:54	32.3886	-157.9982	6000
8	1	CTD09	187	11:58	34.8567	-157.9987	5956
9	1	CTD10	187	21:55	36.5312	-157.9996	5729
9	-	APEX1	187	23:02	36.5300	-158.0000	5729
10	1	CTD11	188	11:55	38.5622	-158.0003	5794
10	2	CTD12	188	14:00	38.5625	-158.0004	5792
11	1	CTD13	188	21:52	39.9691	-157.9953	4676
12	1	CTD14	189	11:51	42.4880	-158.0017	5774
13	1	CTD15	189	21:50	44.2346	-158.0016	5450
14	1	CTD16	190	12:01	46.6888	-157.9983	5261
15	1	CTD17	190	21:58	45.7133	-156.9056	5351
16	1	CTD18	191	21:54	42.4440	-153.3645	5302
17	1	CTD19	192	10:57	40.6128	-151.4675	5016
18	1	CTD20	192	21:10	39.2704	-150.1040	5634
18	2	CTD21	193	10:55	39.2698	-150.1050	5636
18	3	CTD22	193	12:57	39.2706	-150.1057	5638
19	1	CTD23	193	21:00	38.1501	-148.9866	5540
20	1	CTD24	194	10:56	36.1986	-147.0872	5316
21	1	CTD25	194	20:53	34.8719	-145.8207	5490
22	1	CTD26	195	10:56	33.9998	-145.0002	5497
22	2	CTD27	195	12:54	33.9991	-144.9995	5496
22	3	CTD28	195	20:59	33.9999	-145.0000	5497
22	-	APEX2	196	02:50	34.0000	-145.0000	5497
23	1	CTD29	196	10:51	34.2264	-143.1959	5532
24	1	CTD30	196	20:55	34.4795	-140.8300	5373
25	1	CTD31	197	10:54	36.8847	-140.6035	5263
25	2	CTD32	197	12:56	36.8851	-140.6045	5248
26	1	CTD33	197	20:59	38.2074	-140.2855	4712
27	1	CTD34	198	10:54	40.5756	-139.7030	4583
28	1	CTD35	198	20:54	42.2378	-139.2789	4291
29	1	CTD36	199	10:55	44.7148	-138.6276	4192
30	1	CTD37	199	18:53	46.0273	-137.7319	4297
31	1	CTD38	200	09:56	43.9999	-135.9999	3591
31	-	ARGO1	200	10:55	43.9988	-135.9976	3591
32	1	CTD39	200	20:04	42.4038	-135.0143	3975
33	-	ARGO2	201	00:21	41.8333	-134.6667	-
34	1	CTD40	201	10:01	40.1517	-133.6961	3658

35	1	ARGO3	201	14:00	39.6218	-133.3991	4703
36	1	CTD41	201	19:56	38.5964	-132.8343	4544
37	1	ARGO4	202	05:38	37.0000	-132.0000	4743
38	1	CTD42	202	09:56	36.2049	-131.9981	4875
39	1	CTD43	202	19:53	34.4794	-131.9997	5084
39	2	CTD44	202	21:34	34.4796	-132.0002	5077
40	1	CTD45	203	09:56	33.1783	-130.8152	4494
40	2	CTD46	203	11:58	33.1781	-130.8147	4495
41	1	CTD47	203	19:51	32.2434	-129.7091	4621
42	1	CTD48	204	09:51	31.4365	-128.7450	4326
43	1	CTD49	204	19:53	30.7744	-127.8760	4385
44	1	CTD50	205	09:56	29.7999	-126.6083	4283
45	1	CTD51	205	19:01	29.6510	-125.6354	4345
45	1	CTD52	205	20:09	29.6511	-125.6351	4345
46	1	CTD53	206	08:53	29.8331	-124.0005	4373
47	1	CTD54	206	18:56	29.5726	-123.2563	4234
47	2	CTD55	206	20:13	29.5725	-123.2564	4237
48	1	CTD56	207	08:54	29.2500	-122.3336	4380
49	1	CTD57	207	17:54	30.4719	-121.3331	3987
50	1	CTD58	208	13:21	33.0838	-118.6674	418
51	1	CTD59	208	16:25	33.5171	-118.6673	349
52	1	CTD60	208	19:10	33.2489	-118.3160	1059

Note: some stations not shown here correspond to ARGO or APEX deployment-only stations.

CTD Notes – niskin mis-trips

CTD19 niskin 12 (50 m) potential mis-trip based on pH

CTD26 niskin 3 (150 m) potential mis-trip based on pH

CTD31, niskin 17 (25 m) likely a mis-trip based on FCM, Chl and nutrient data

CTD42, niskin 1 (200 m) potential mis-trip based on pH

APEX & ARGO deployments

Float Type	Float #	WMO ID#	Station #	Latitude	Longitude	Deployment UTC Day	Deployment UTC Time
APEX	7642		9	36.5300	-158.0000	187	23:02
APEX	7698		22	34.0000	-145.0000	196	02:50
ARGO	F0231	4901558	31	43.9988	-135.9976	200	10:55
ARGO	F0232	4901559	33	41.8333	-134.6667	201	00:21
ARGO	F0233	4901560	35	39.6218	-133.3991	201	14:00
ARGO	F0230	4901557	37	37.0000	-132.0000	202	05:38

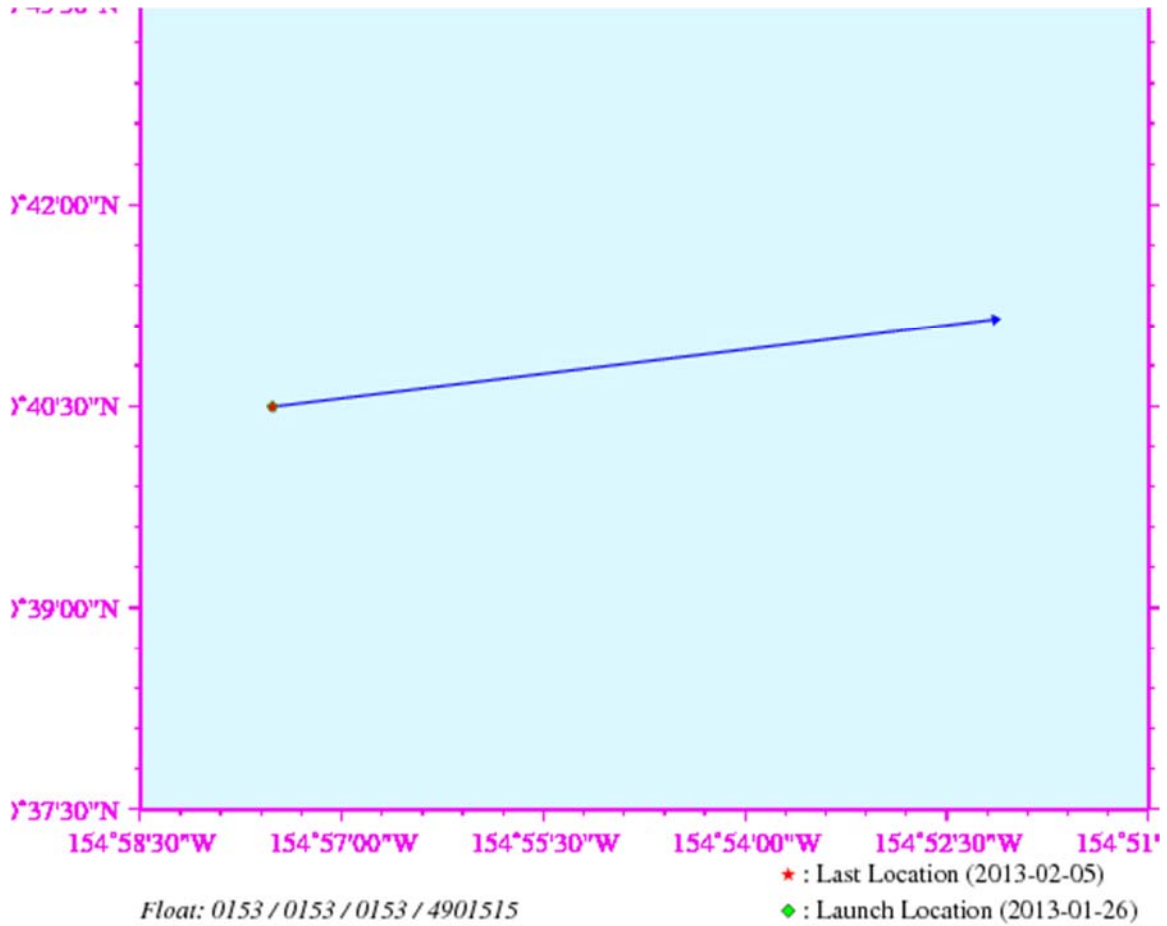


Figure 2: Sample Float Trajectory: WMO ID#4901515

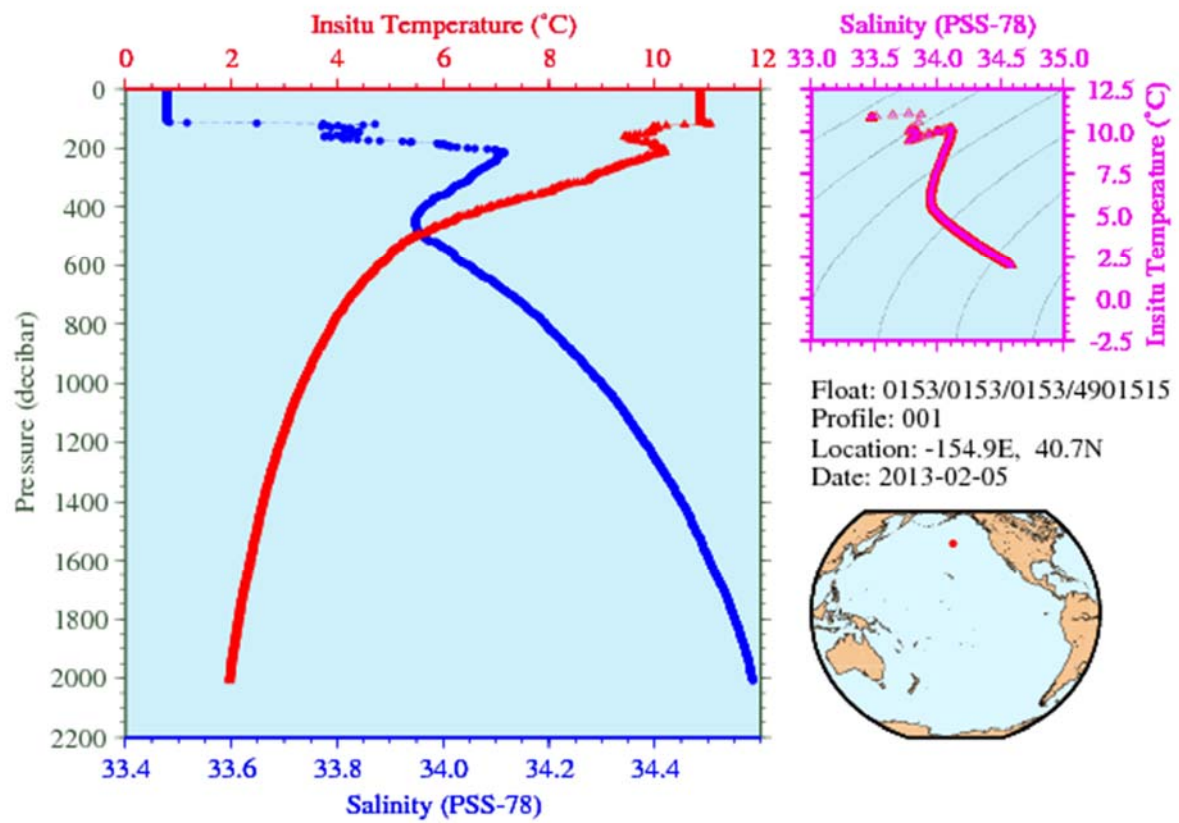


Figure 3: Sample profile for ARGO float (WMO ID#4901515)

Maps

Satellite Images

iA20131822013212.L3m_MO_SST_9_July2013.hdf

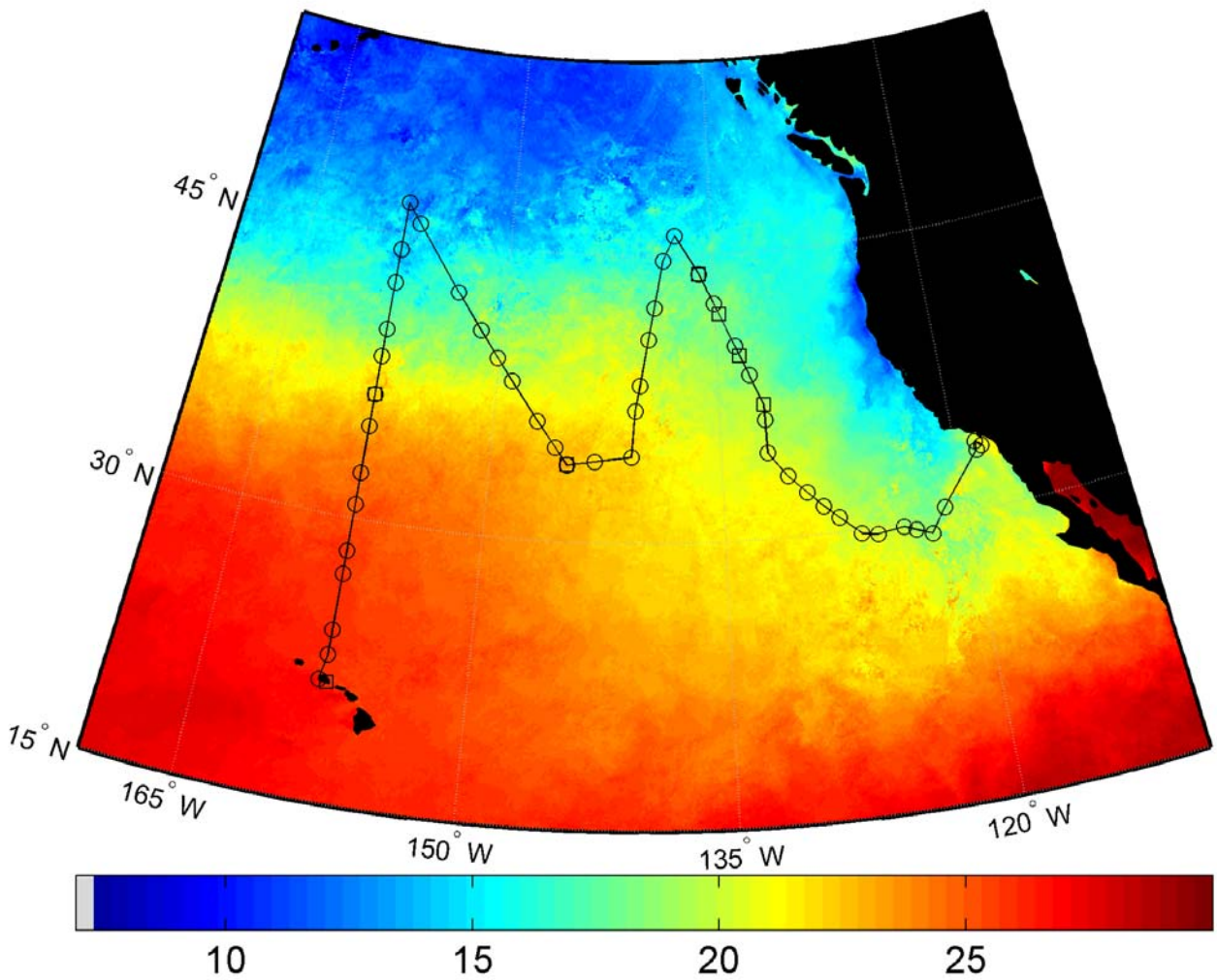


Figure 4: NASA Aqua MODIS estimated day time (11 μ) sea surface temperature (July 2013 composite) with overlaid cruise track and stations. SST interpolated for pixels where data not available (e.g. clouds).

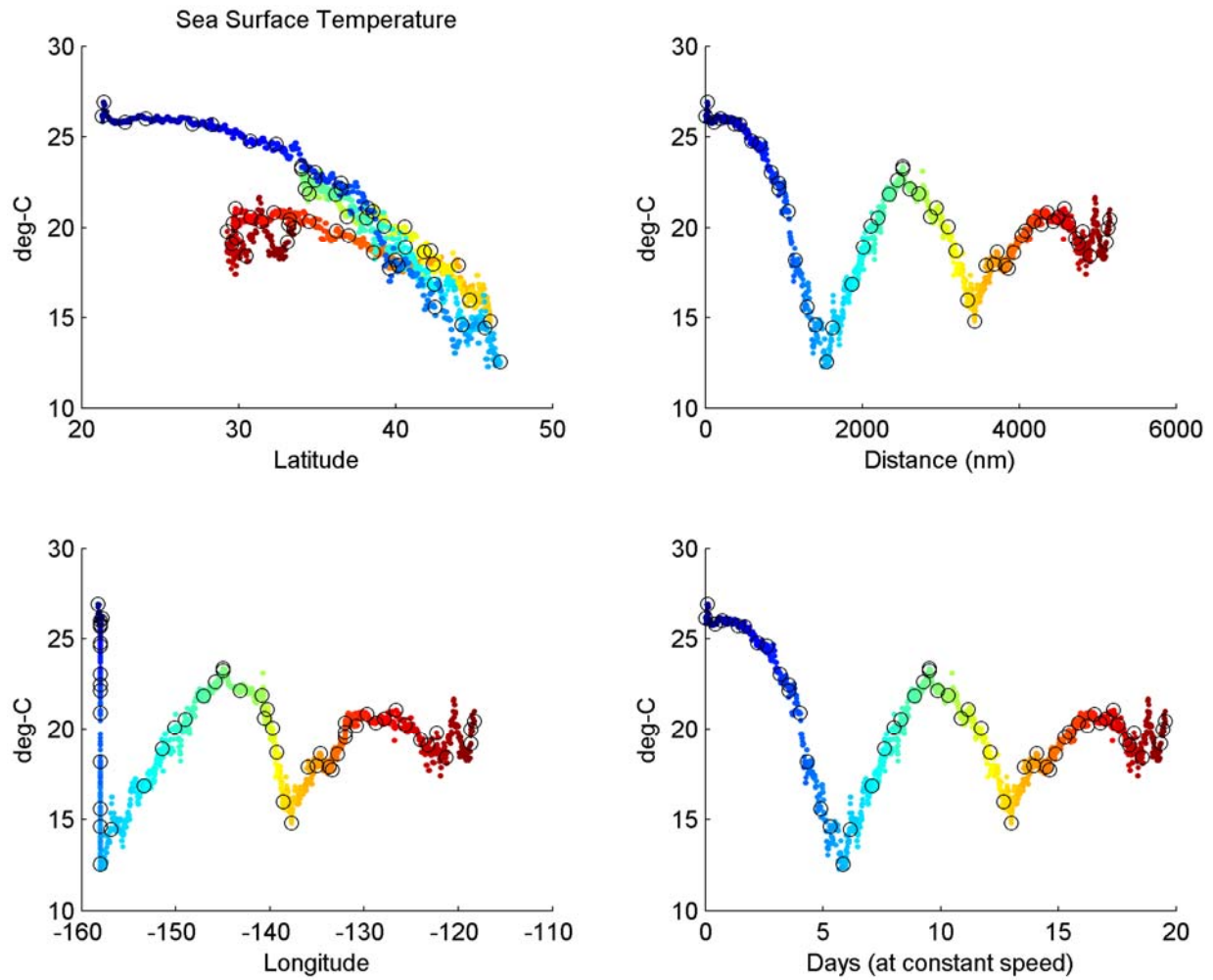


Figure 5: NASA Aqua MODIS estimated day time (11 μ) sea surface temperature (July 2013 composite) along the cruise track as a function of latitude, distance, longitude and days at sea (assuming constant 11 knots). Circles denote station locations.

A20131822013212.L3m_MO_CHL_chlor_a_9km_July2013.hdf

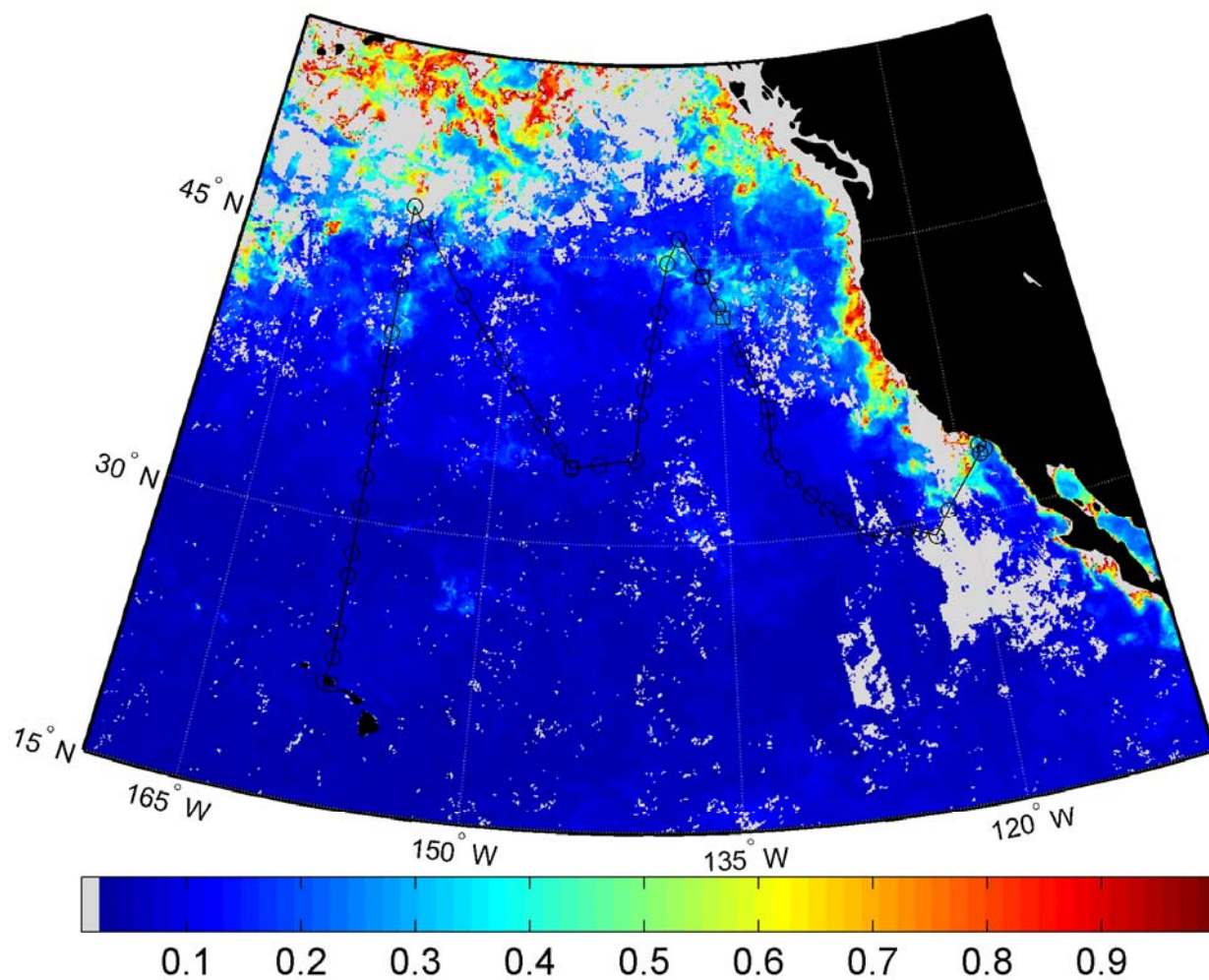


Figure 6: NASA Aqua MODIS estimated chlorophyll concentrations (July 2013 composite) with overlaid cruise track and stations. Note the large number of clouds (gray pixels) in the composite image.

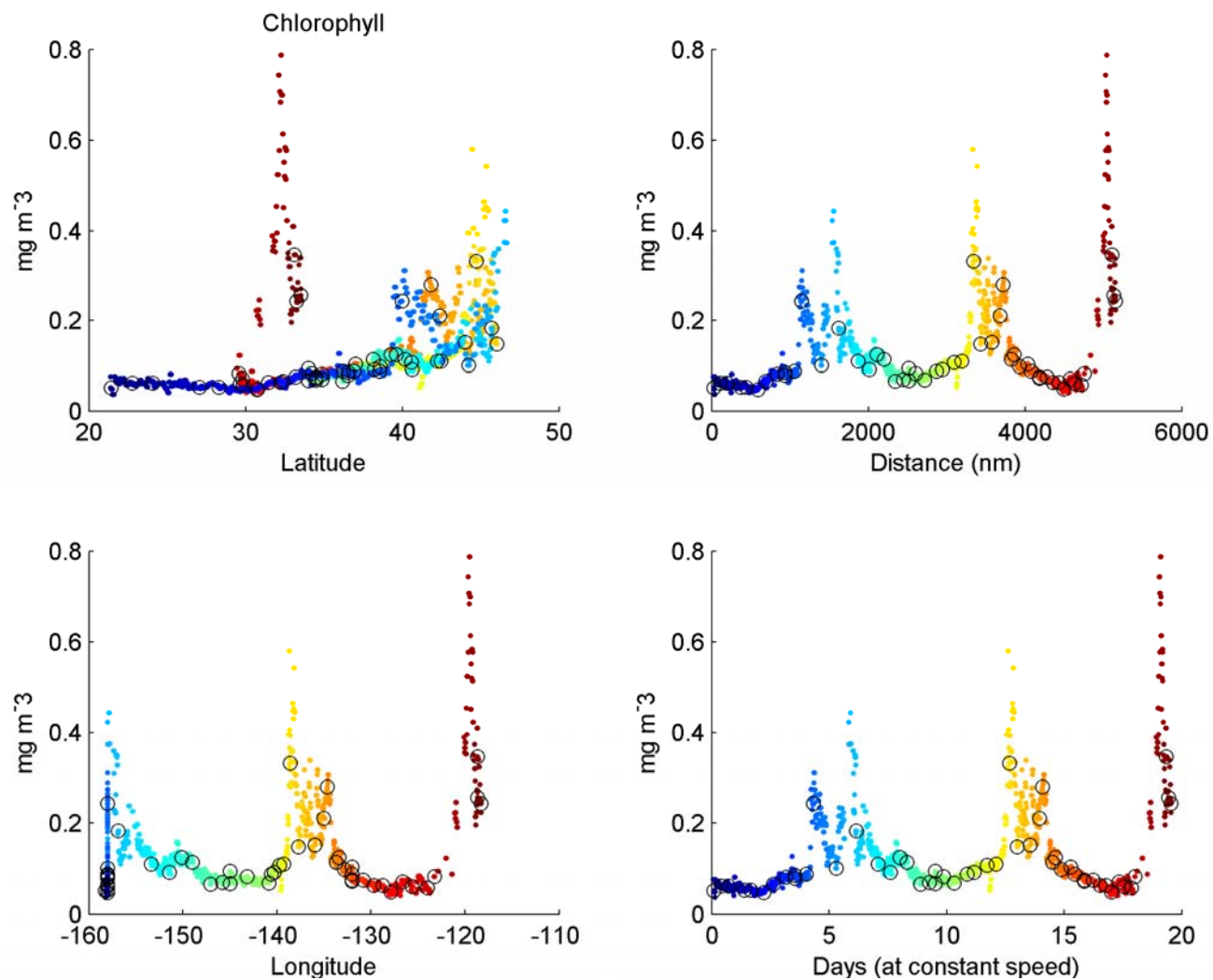
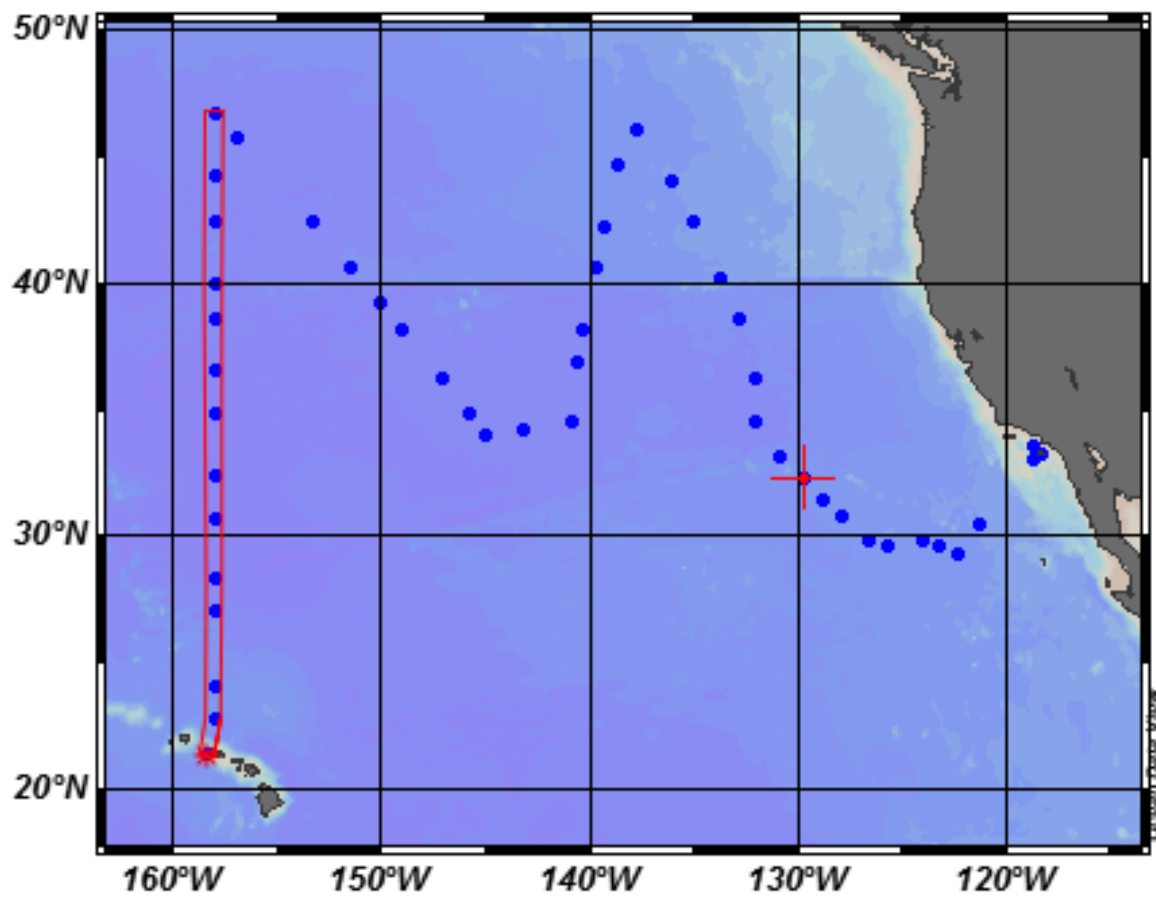
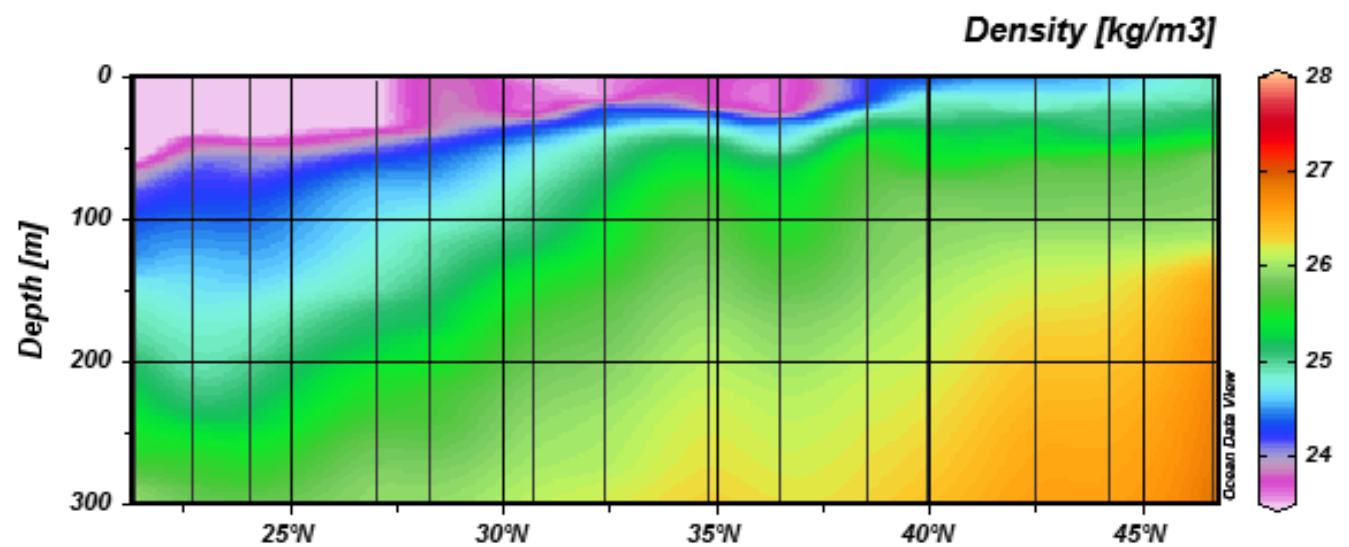
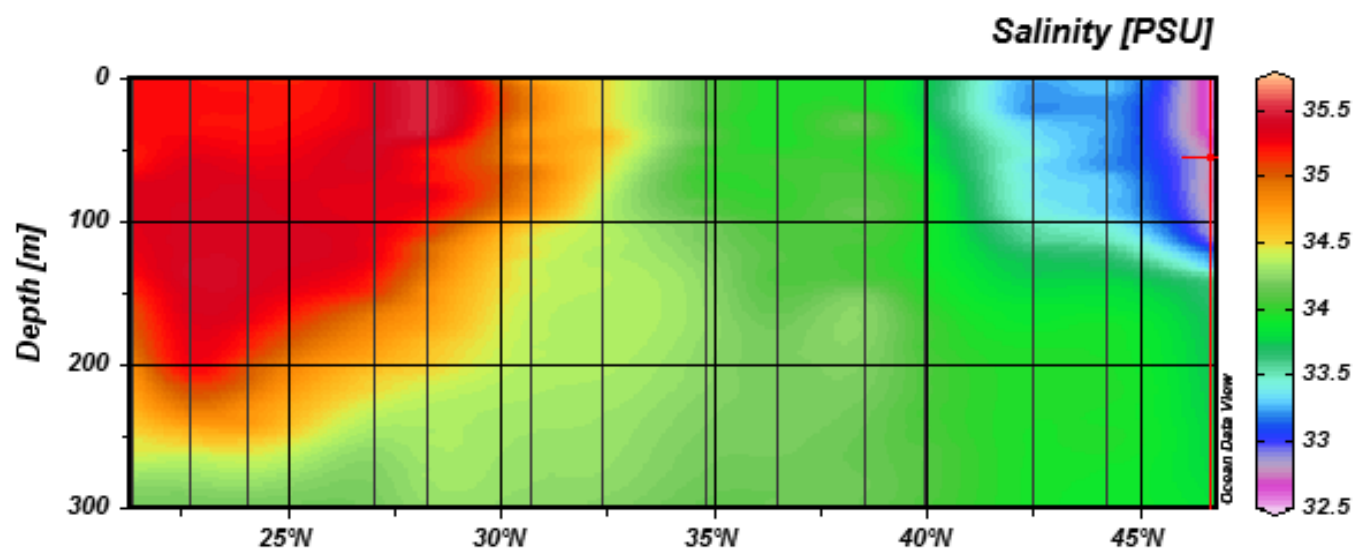
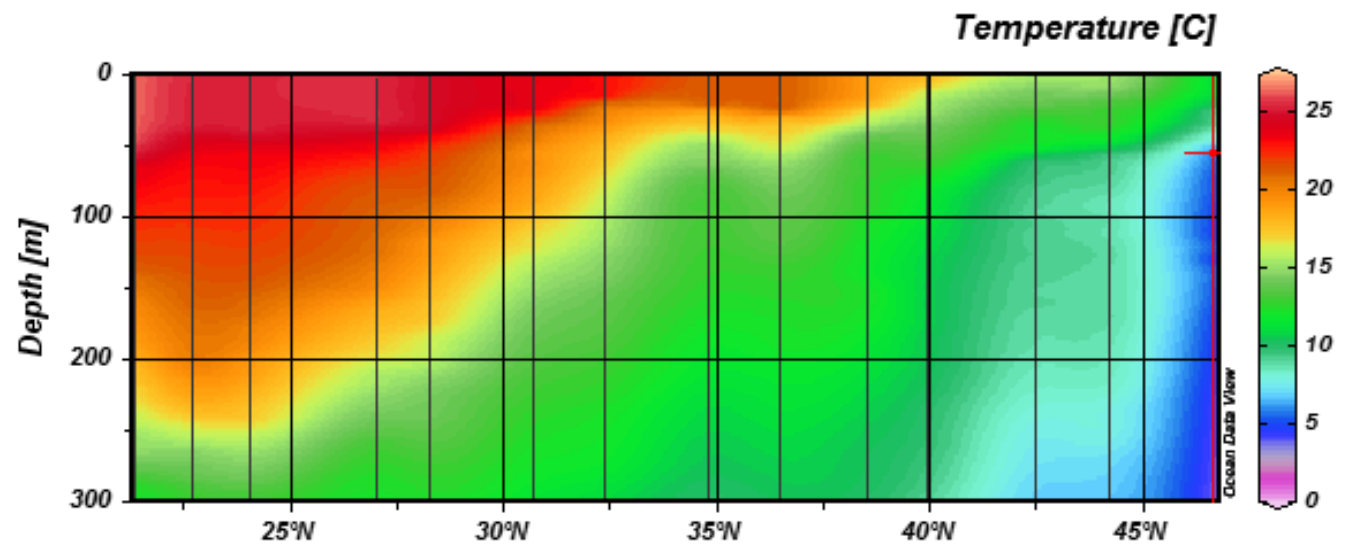


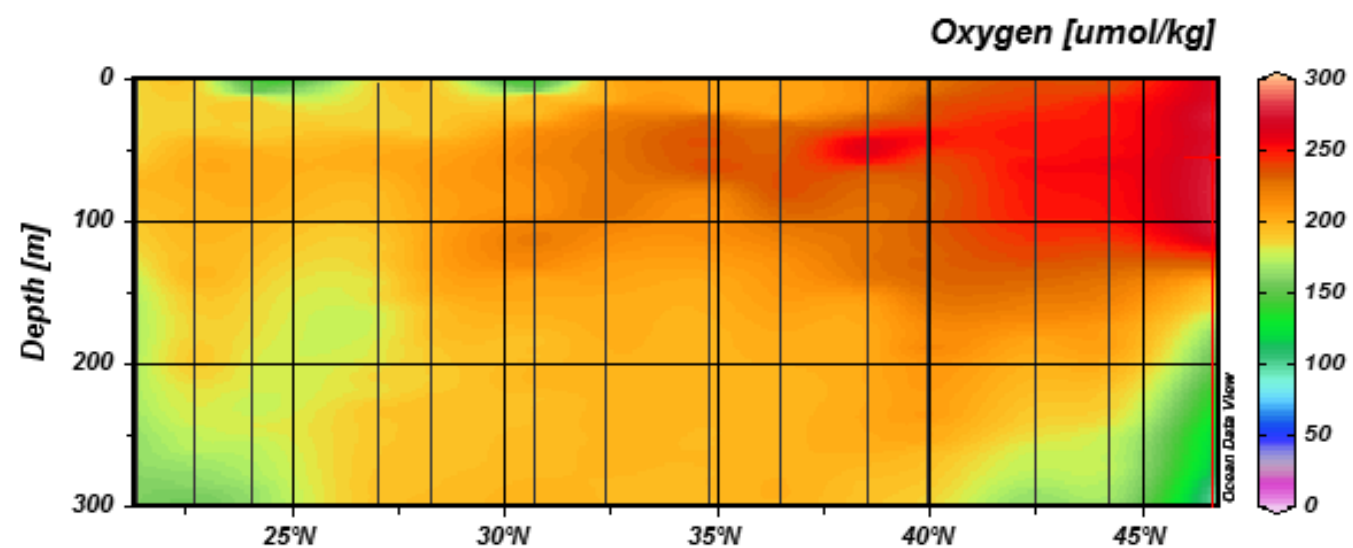
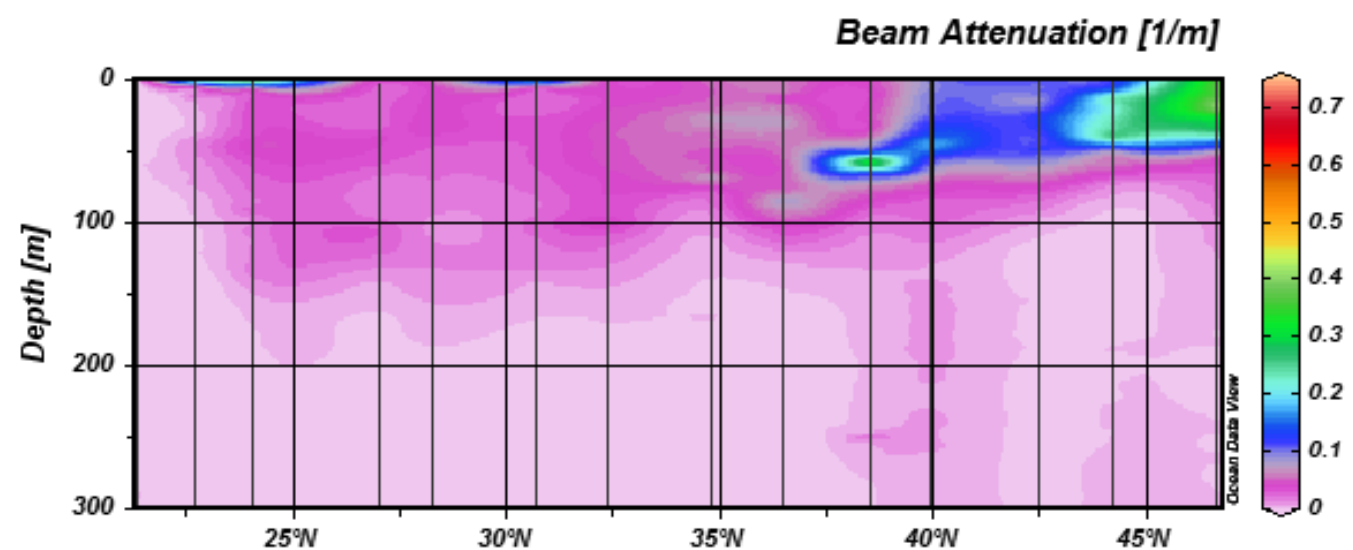
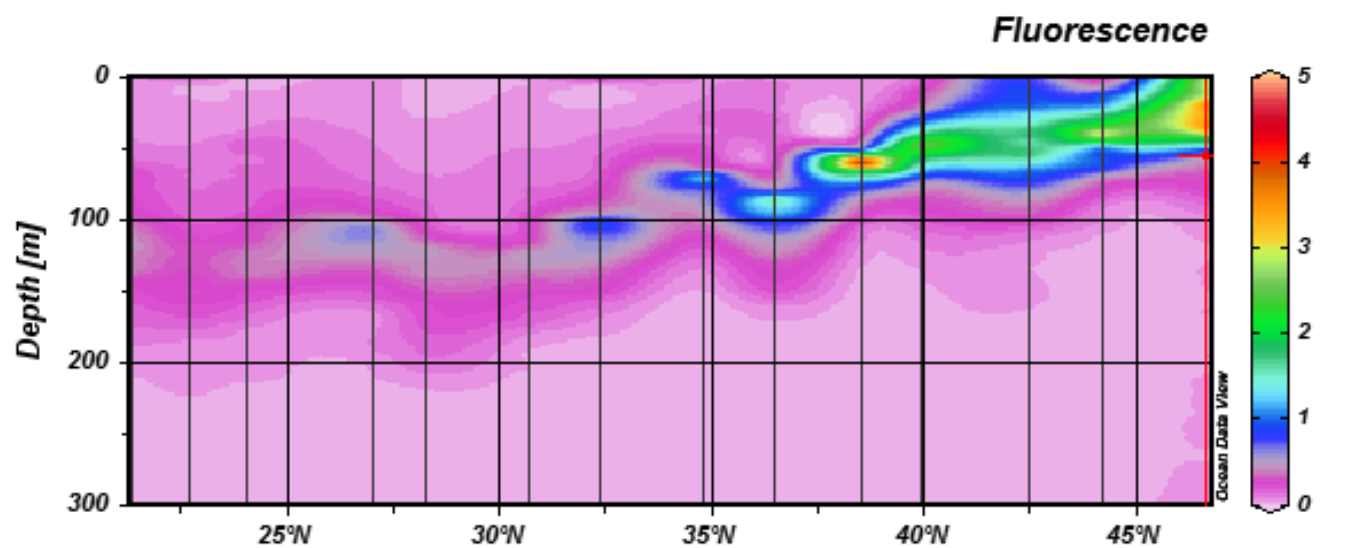
Figure 7: NASA Aqua MODIS estimated chlorophyll concentrations (January 2013 composite) along the cruise track as a function of latitude, distance, longitude and days at sea (assuming constant 11.5 knots). Circles denote station locations.

Hydrography: Sectional Data

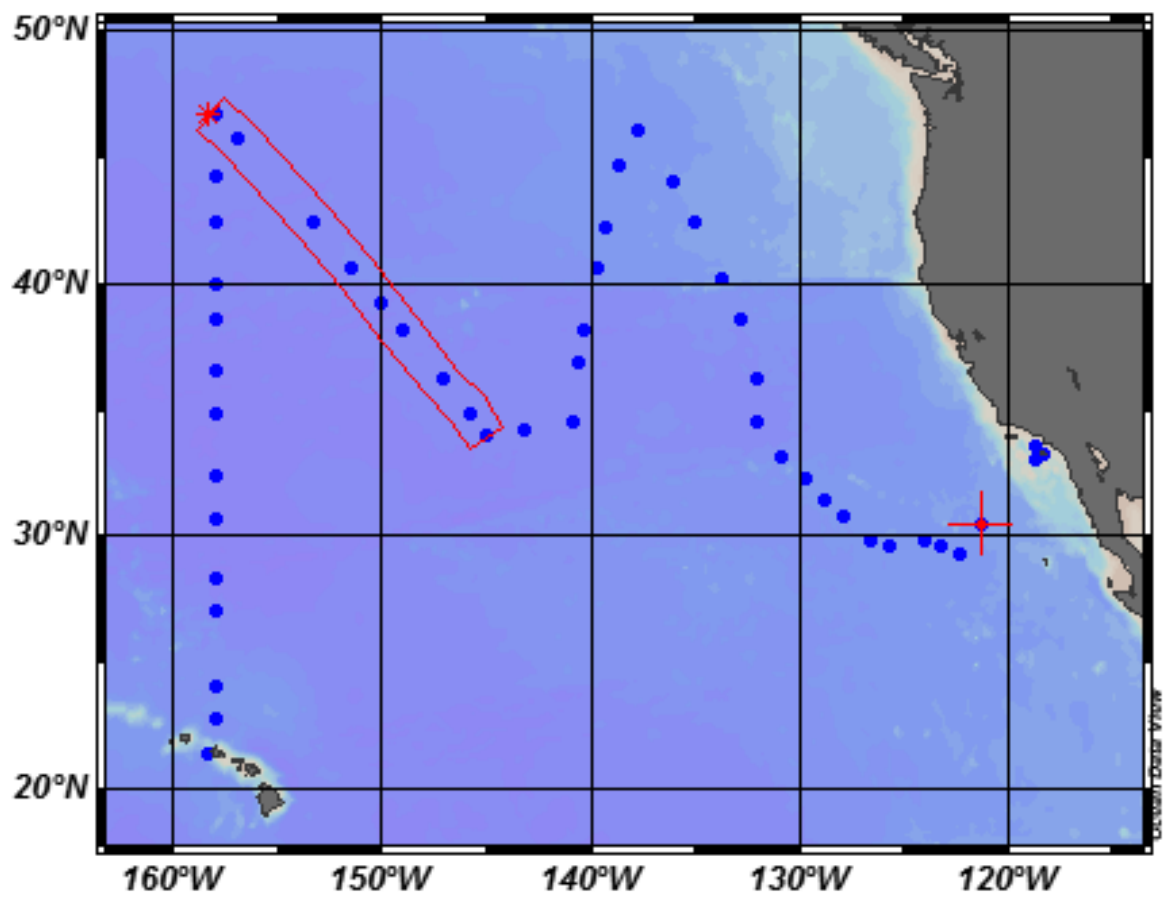
Section #1

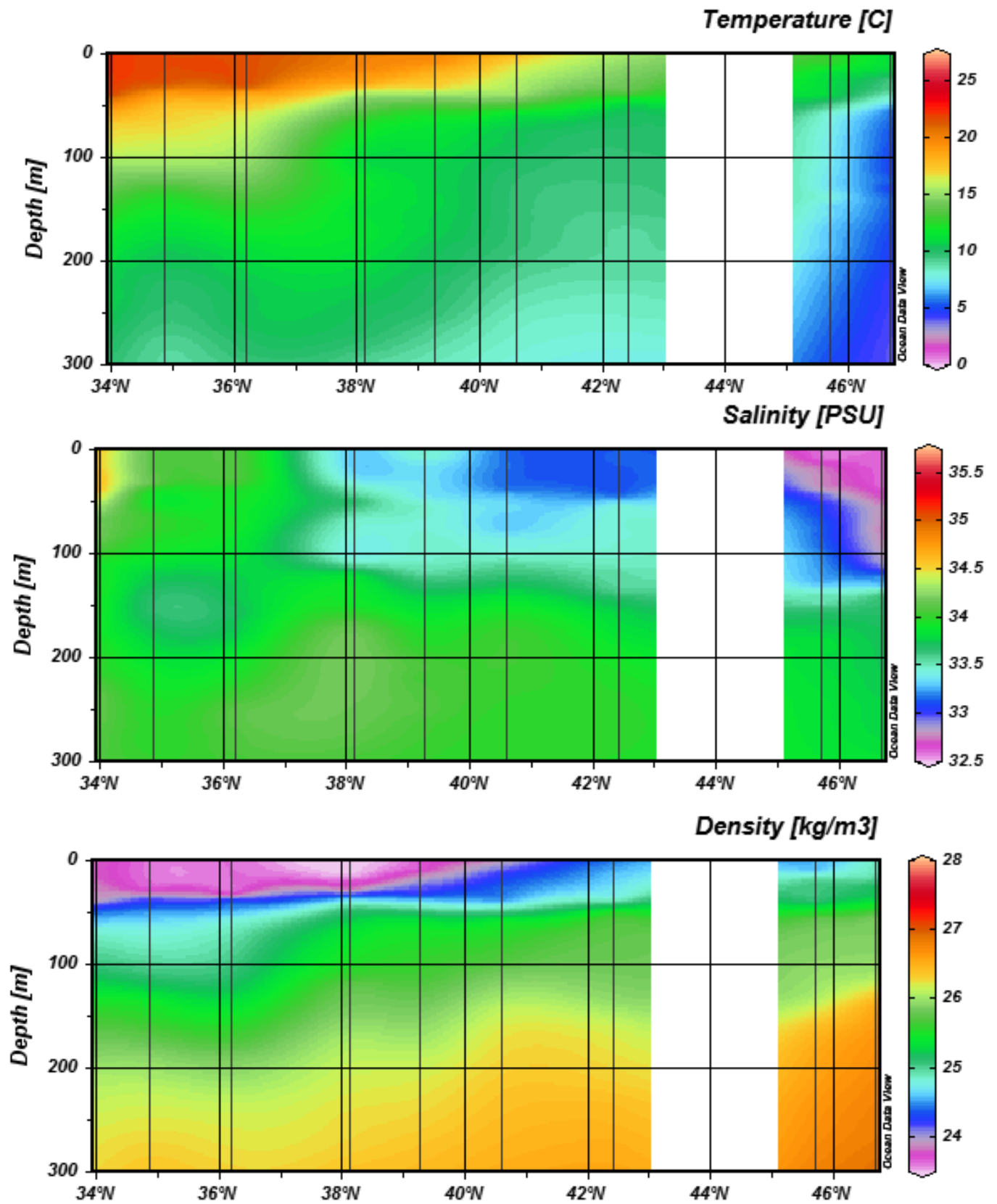


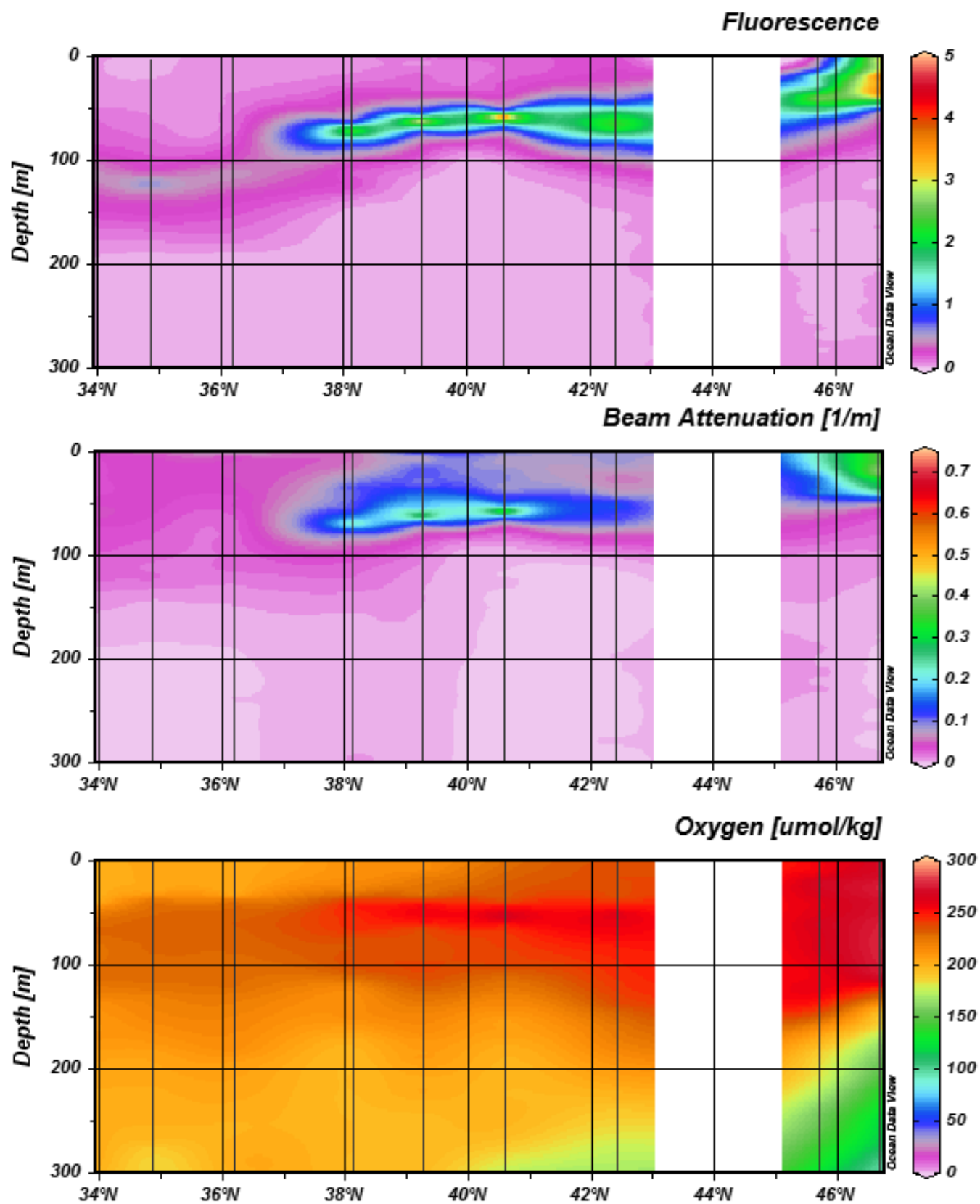




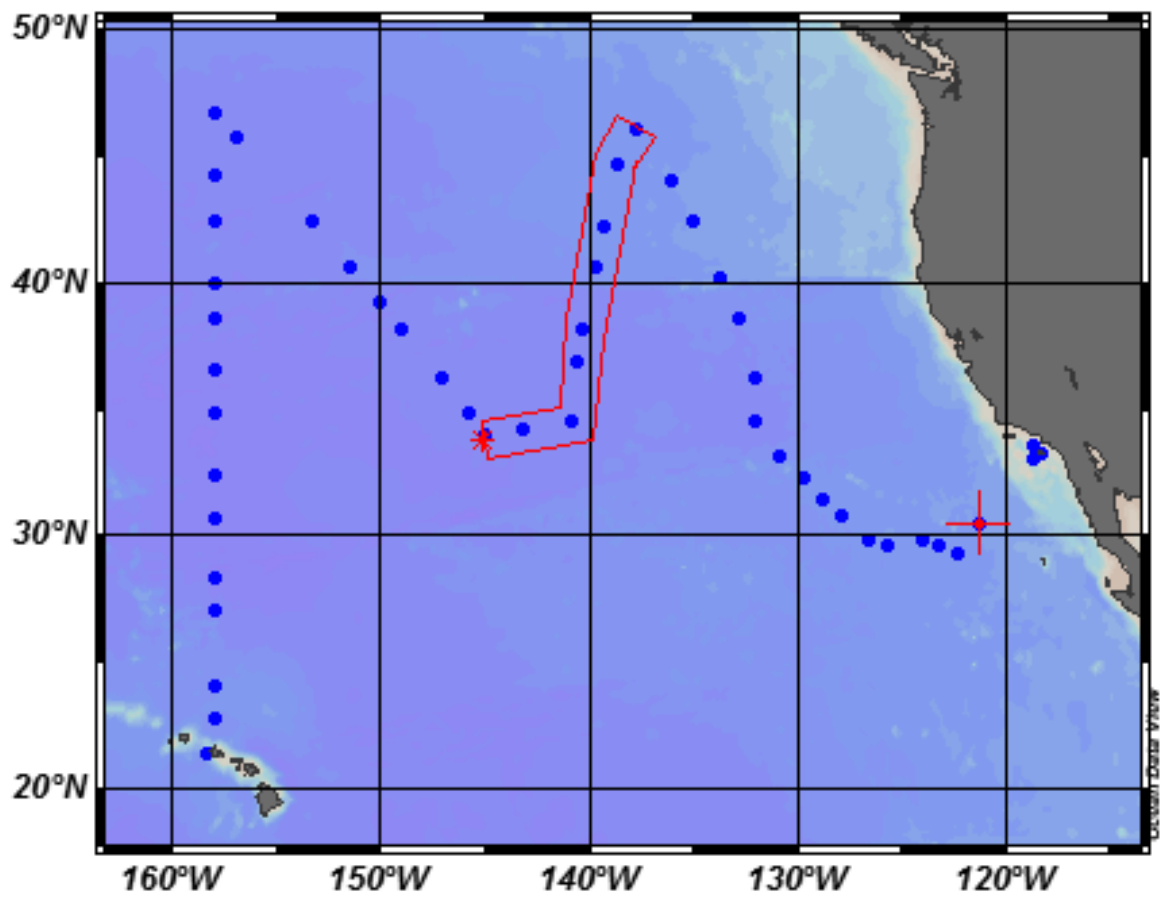
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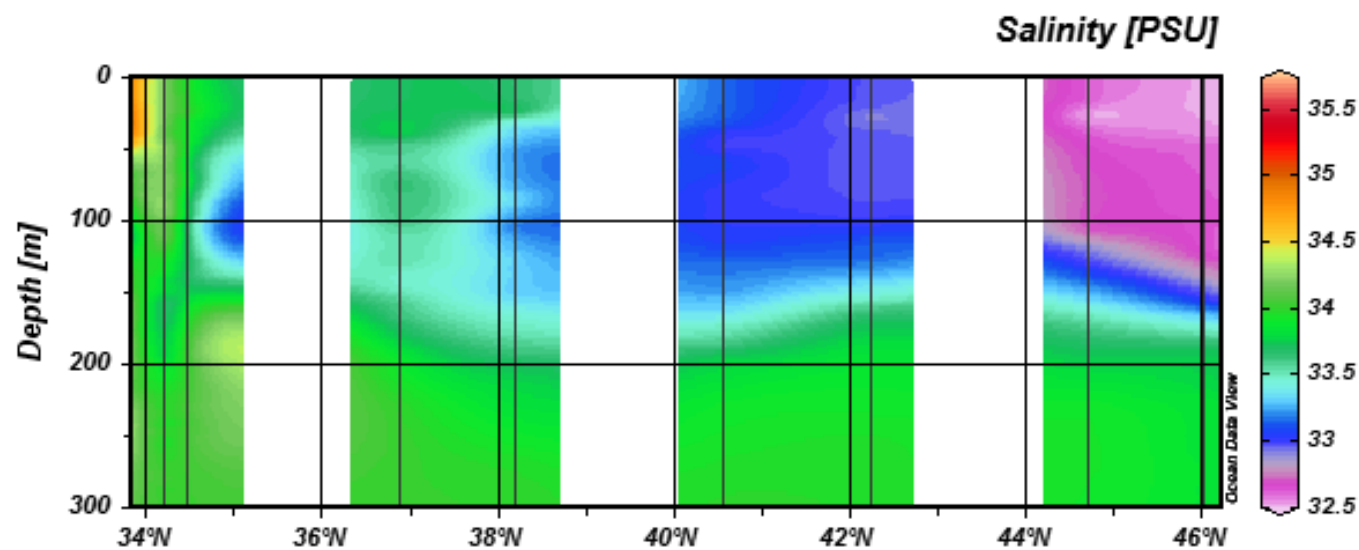
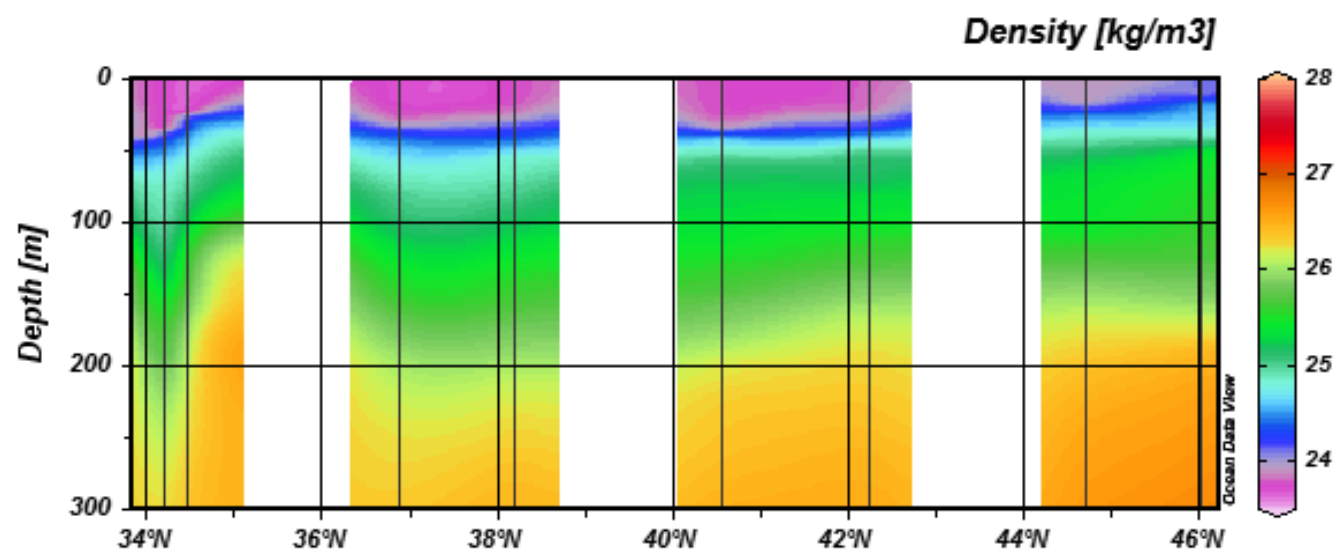
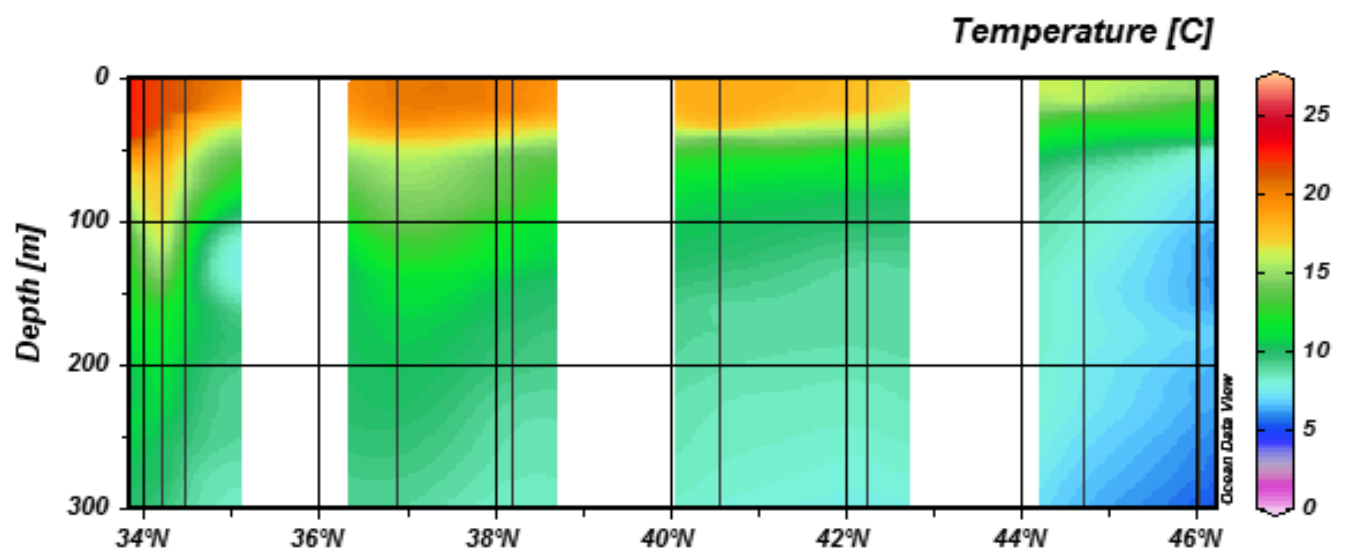


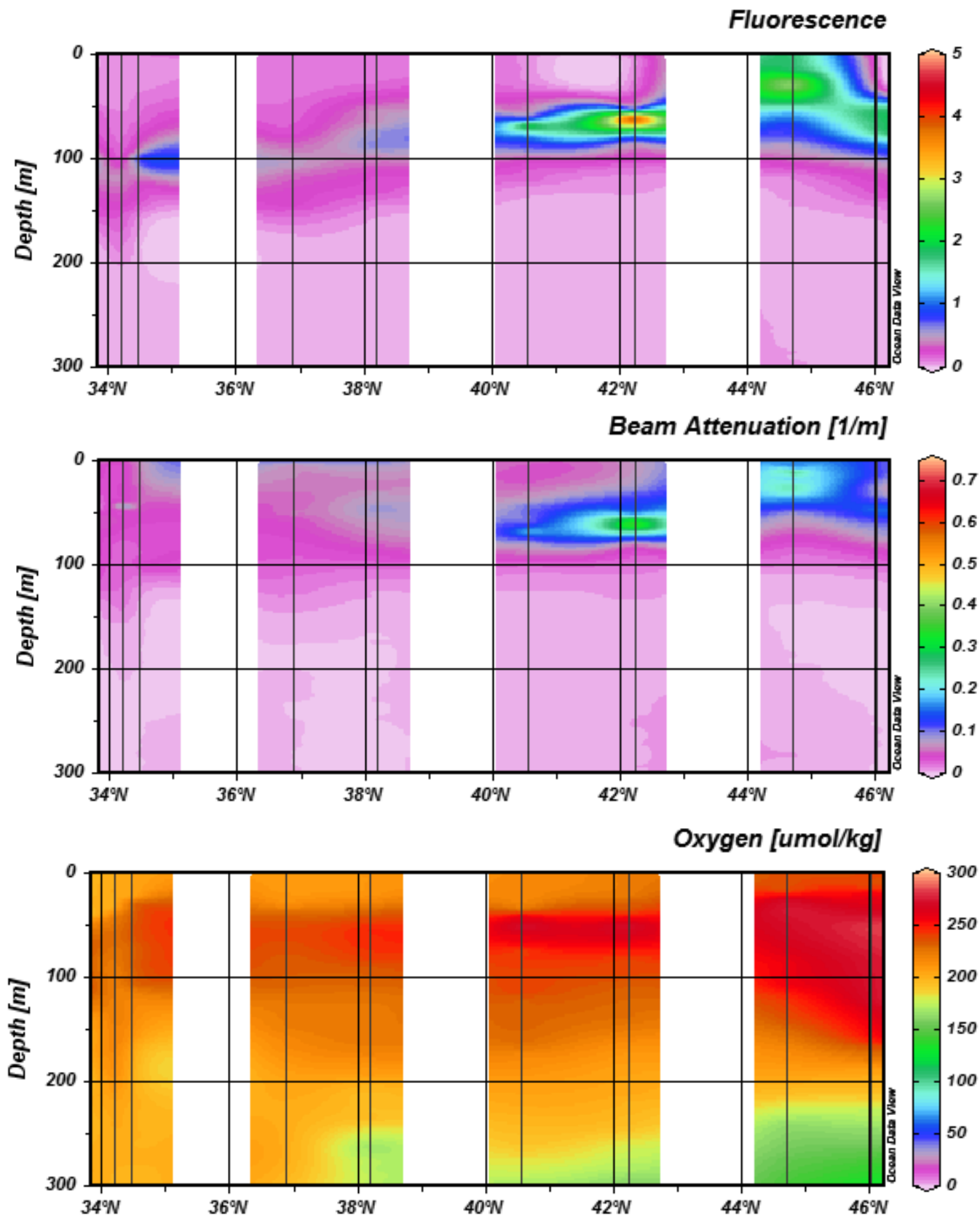




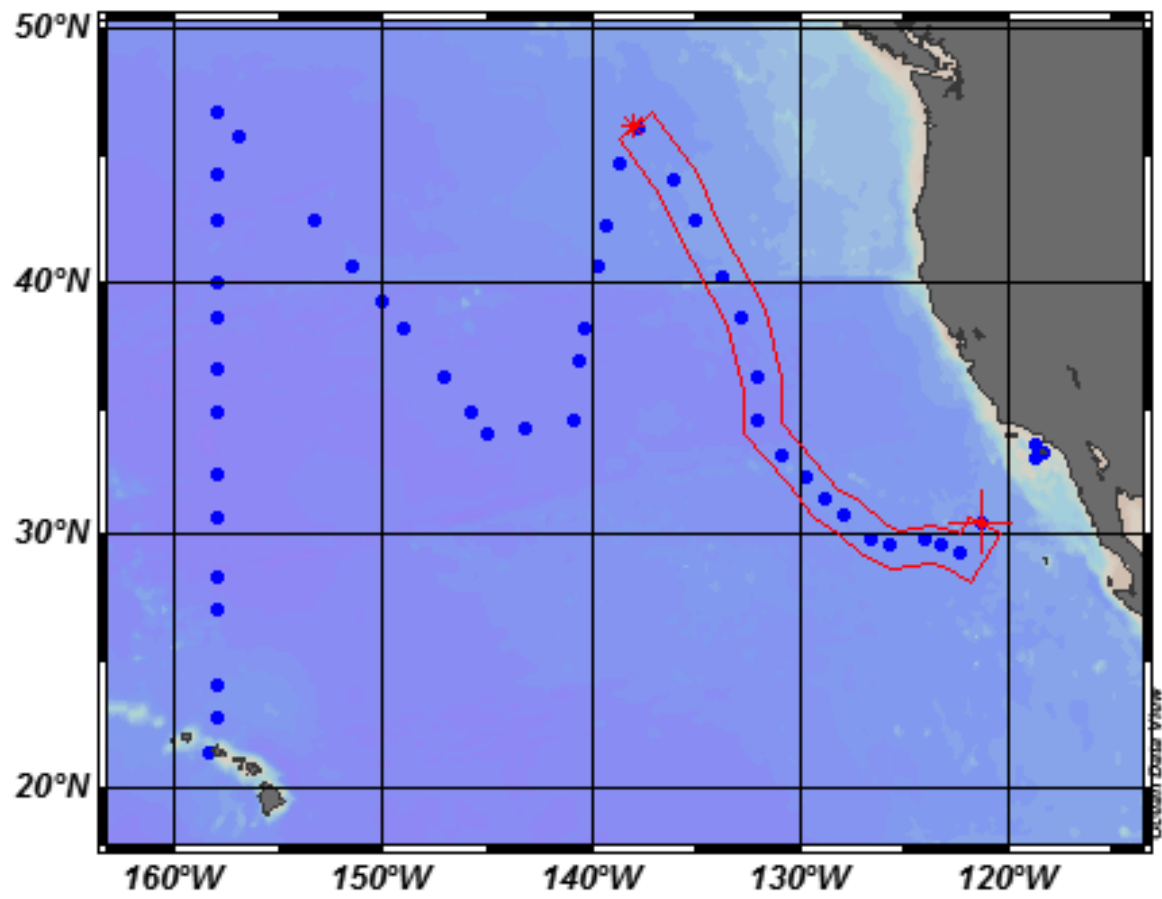
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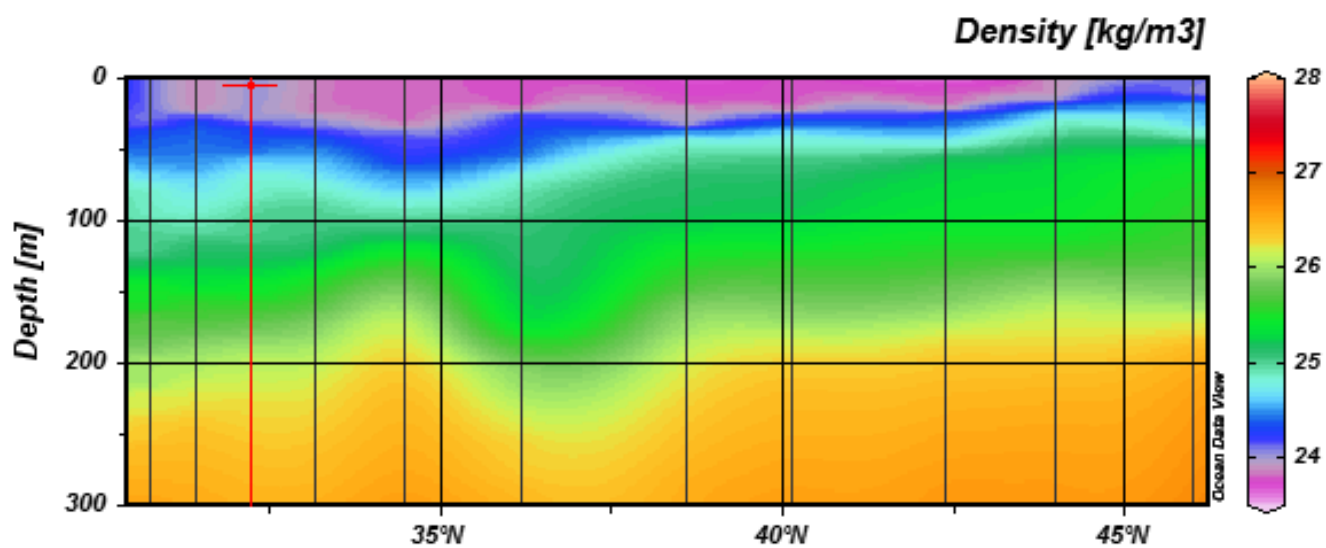
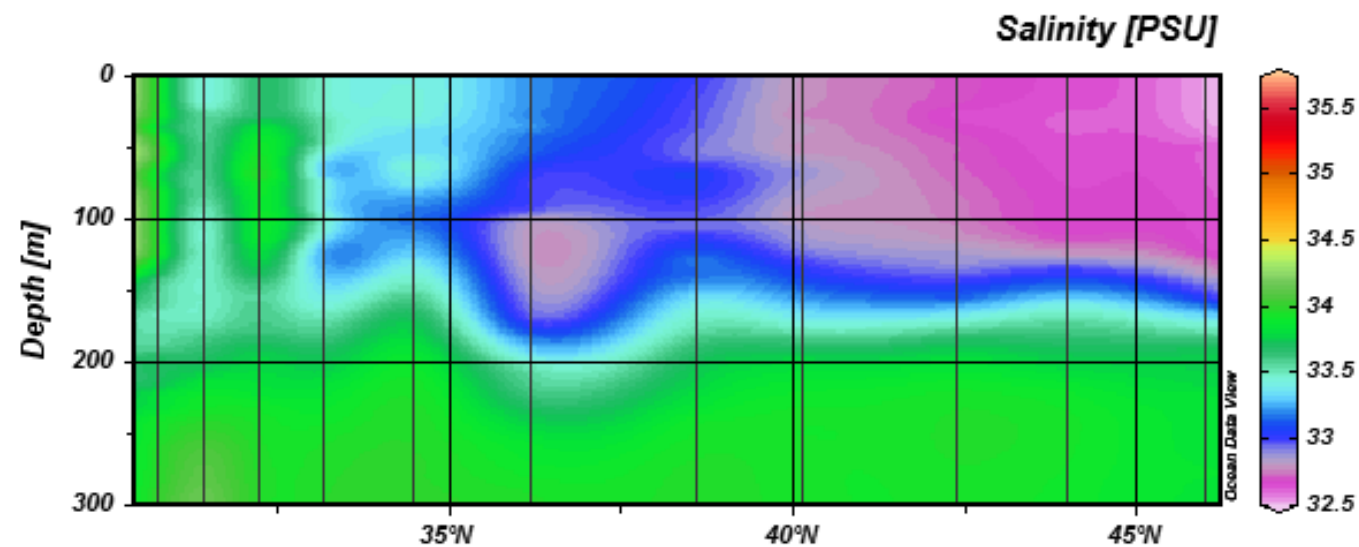
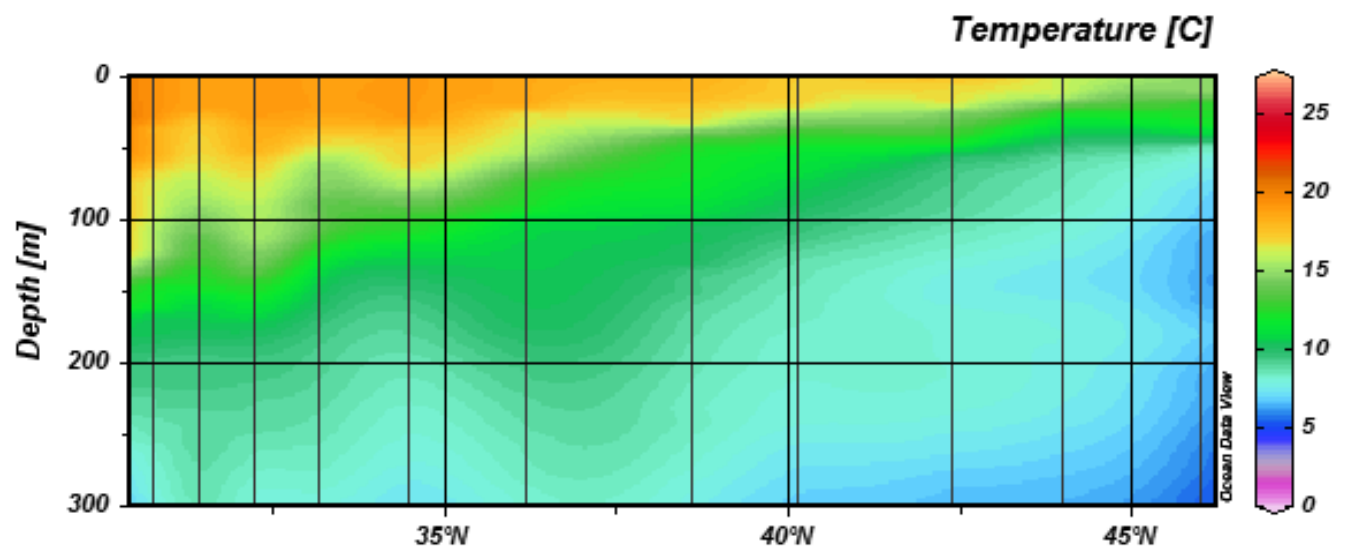


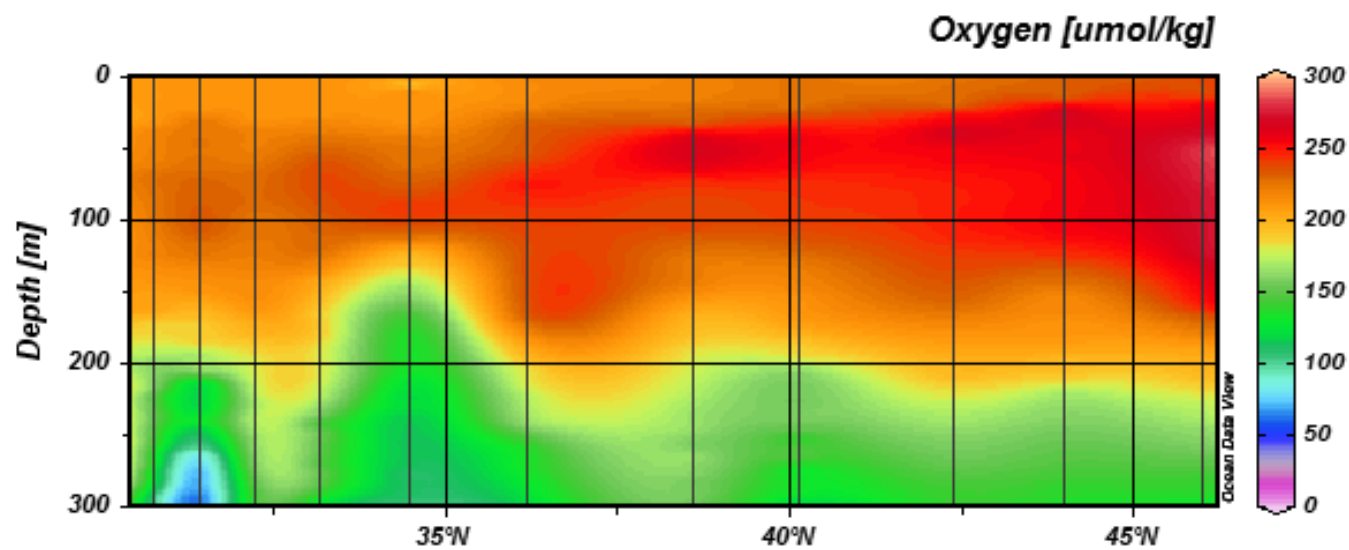
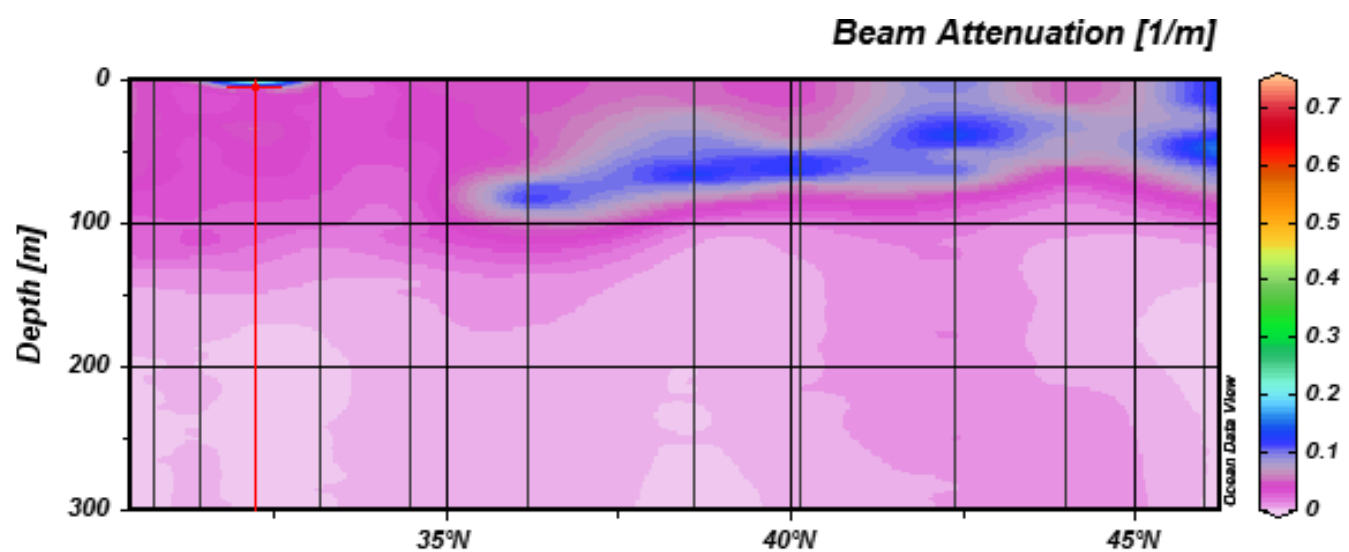
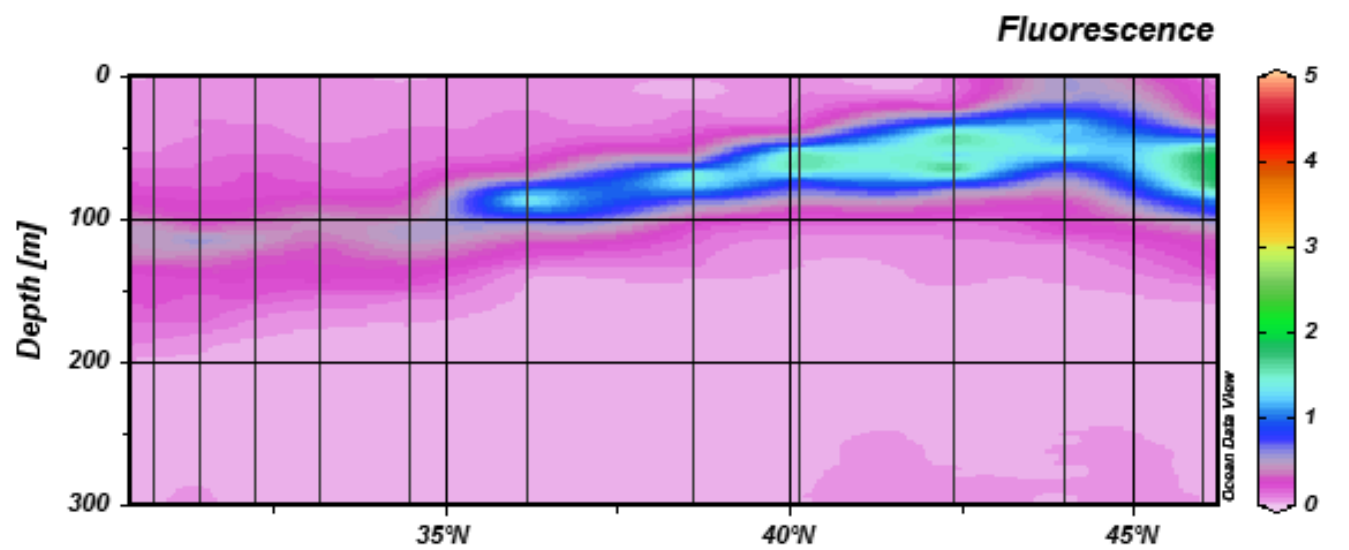




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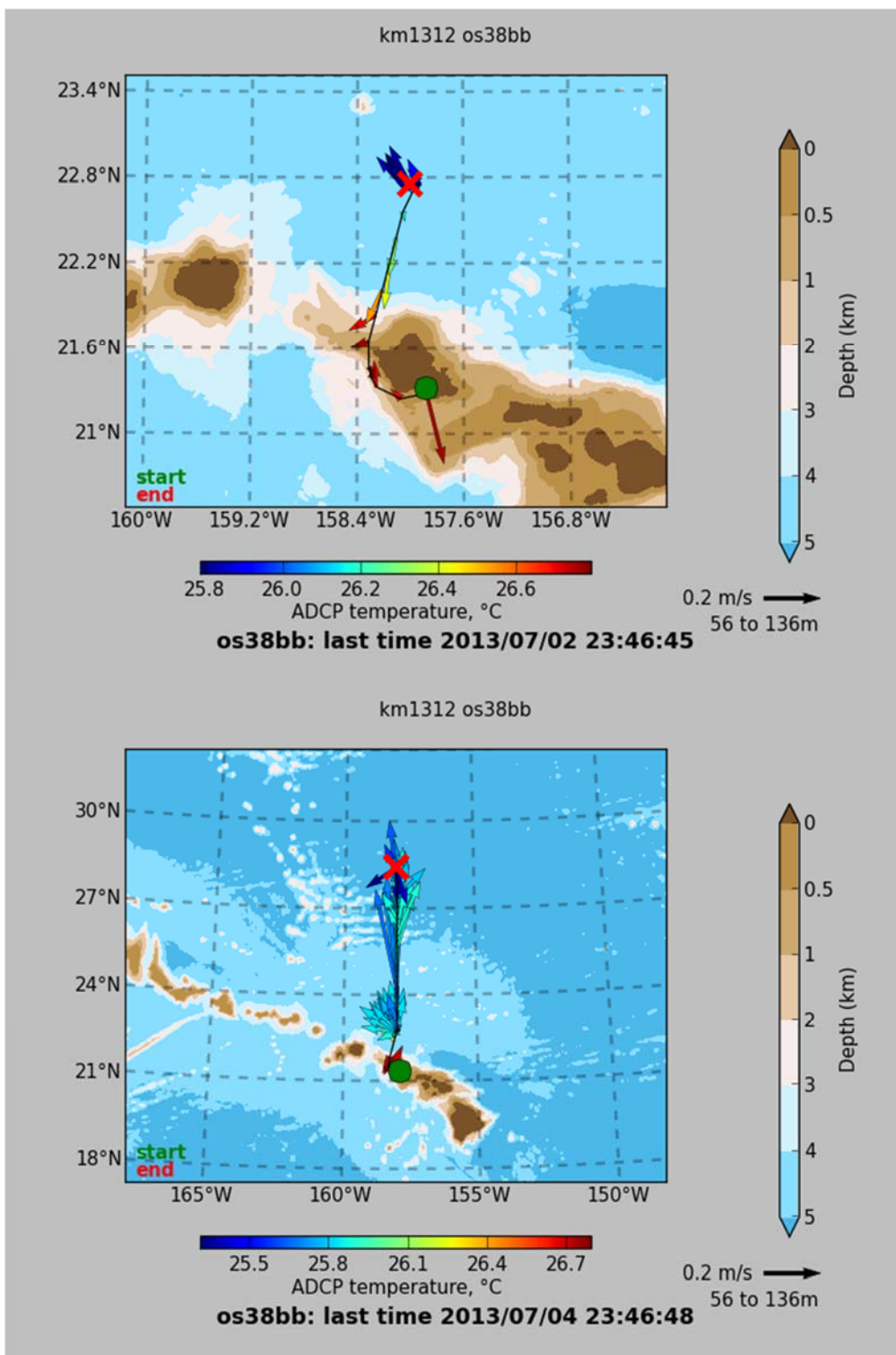




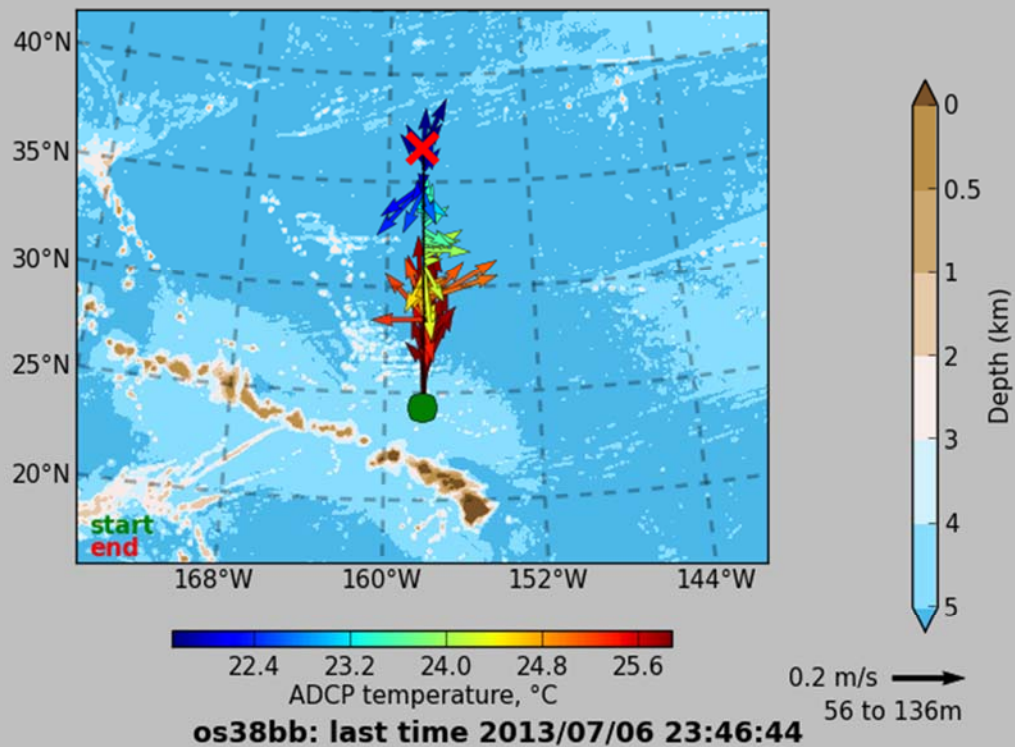


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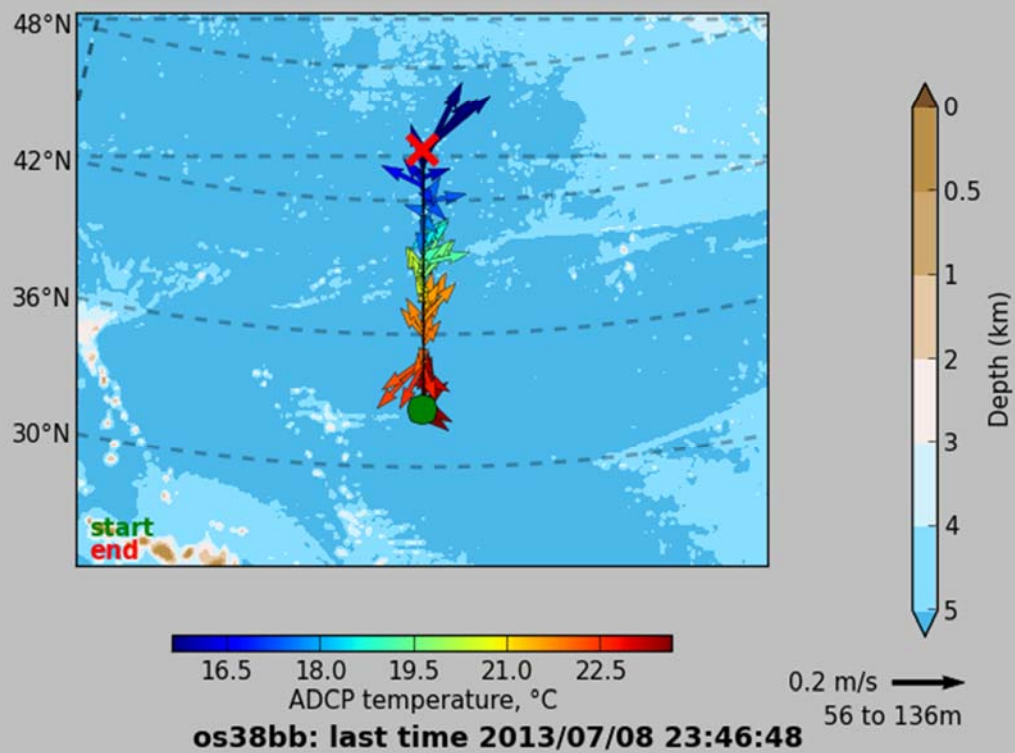
Surface Vectors



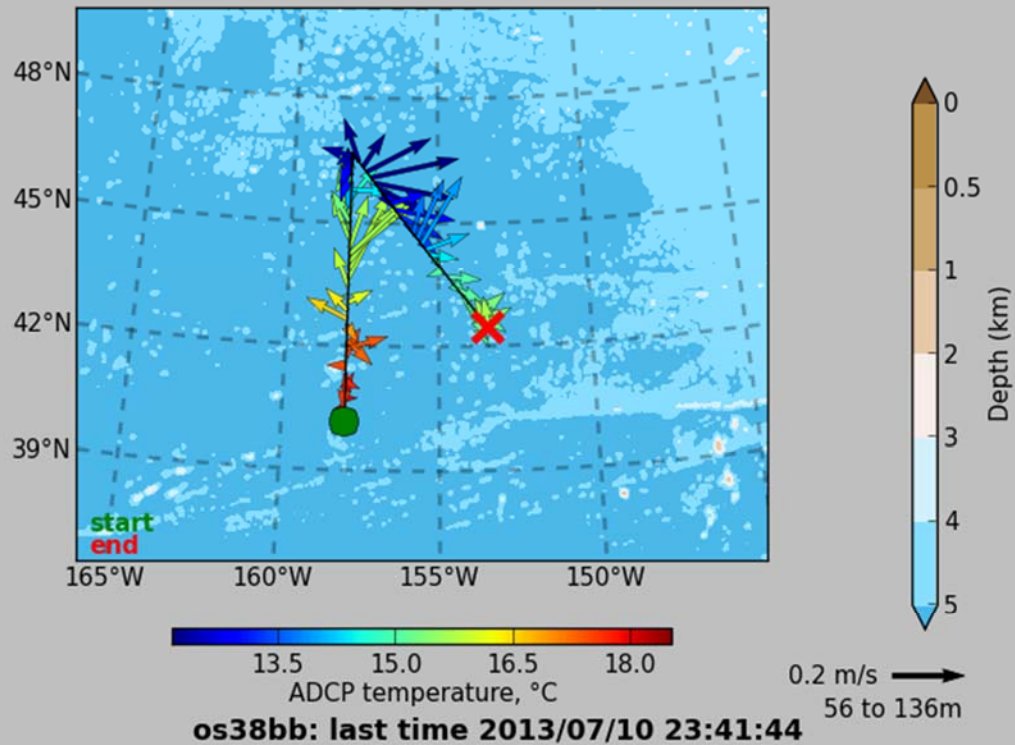
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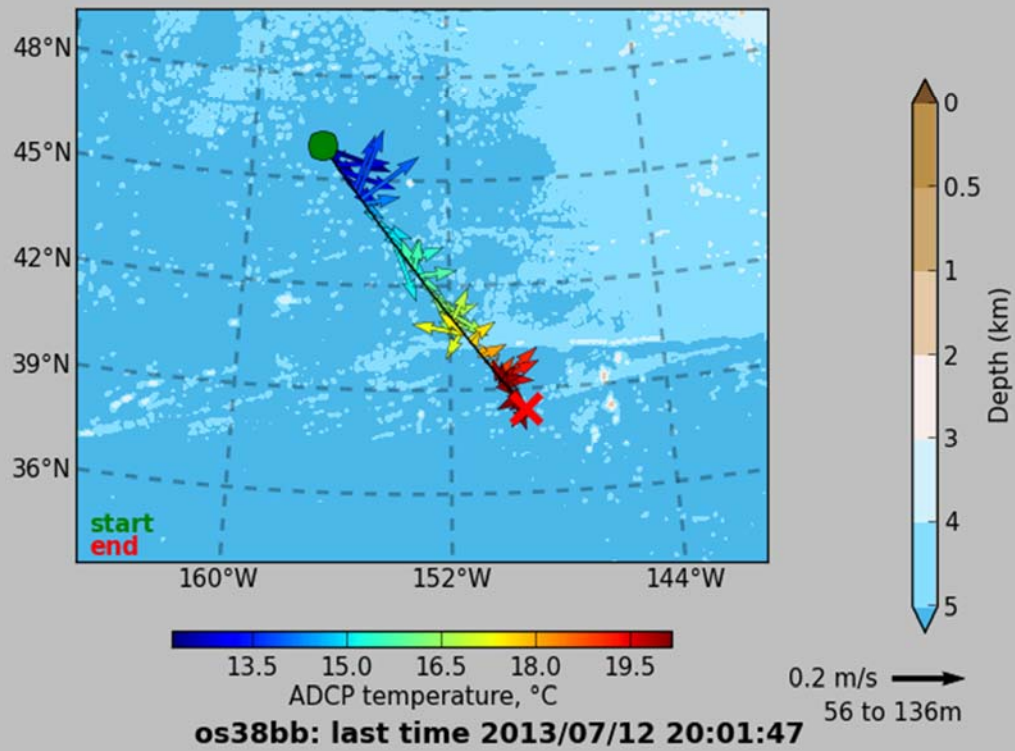
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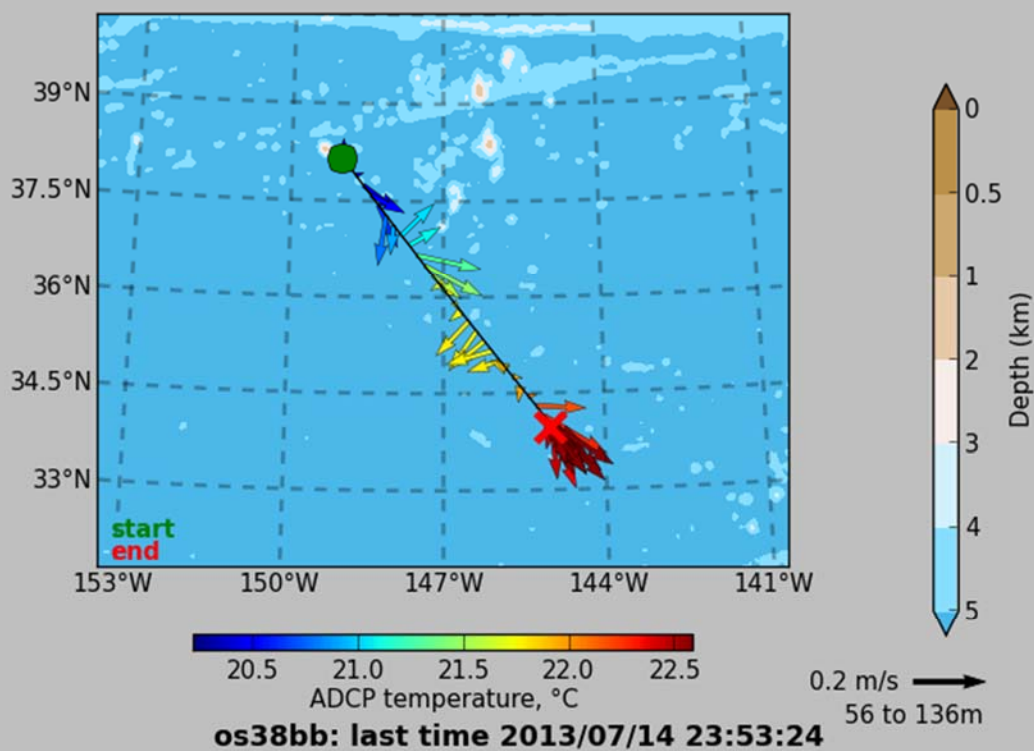
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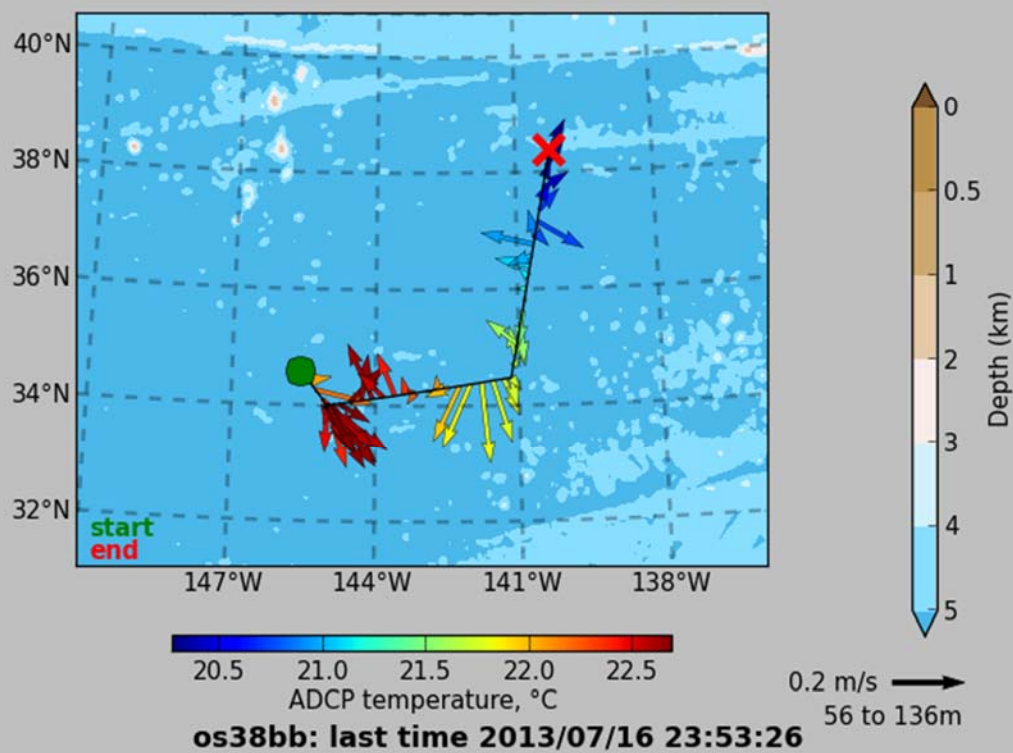
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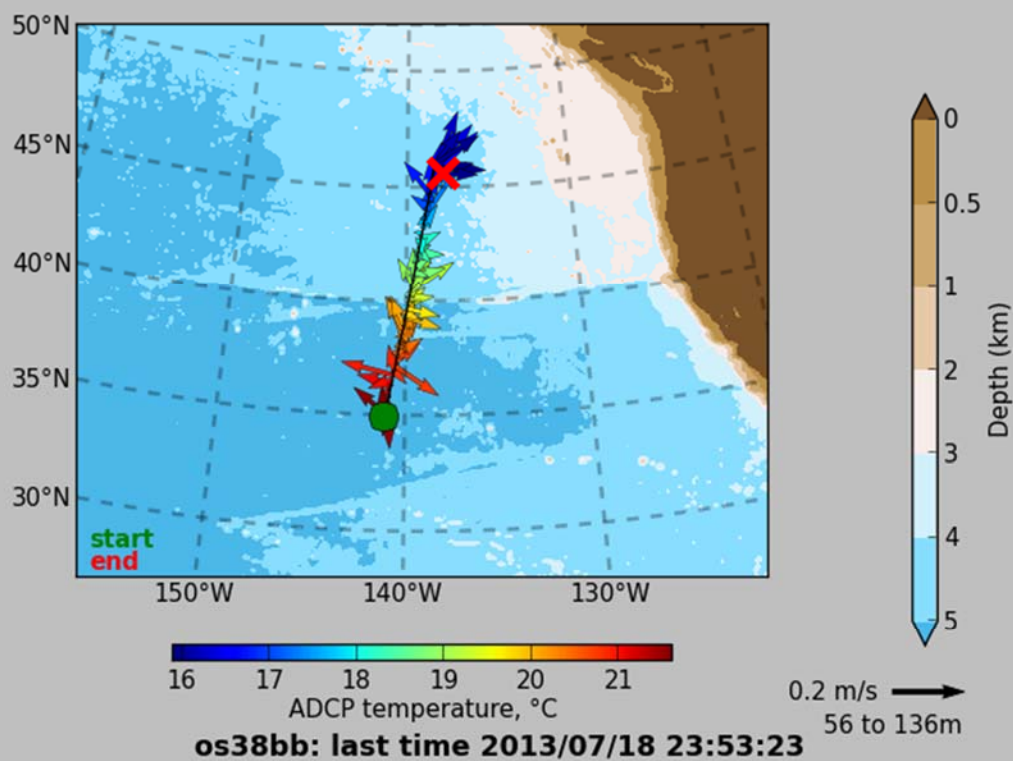
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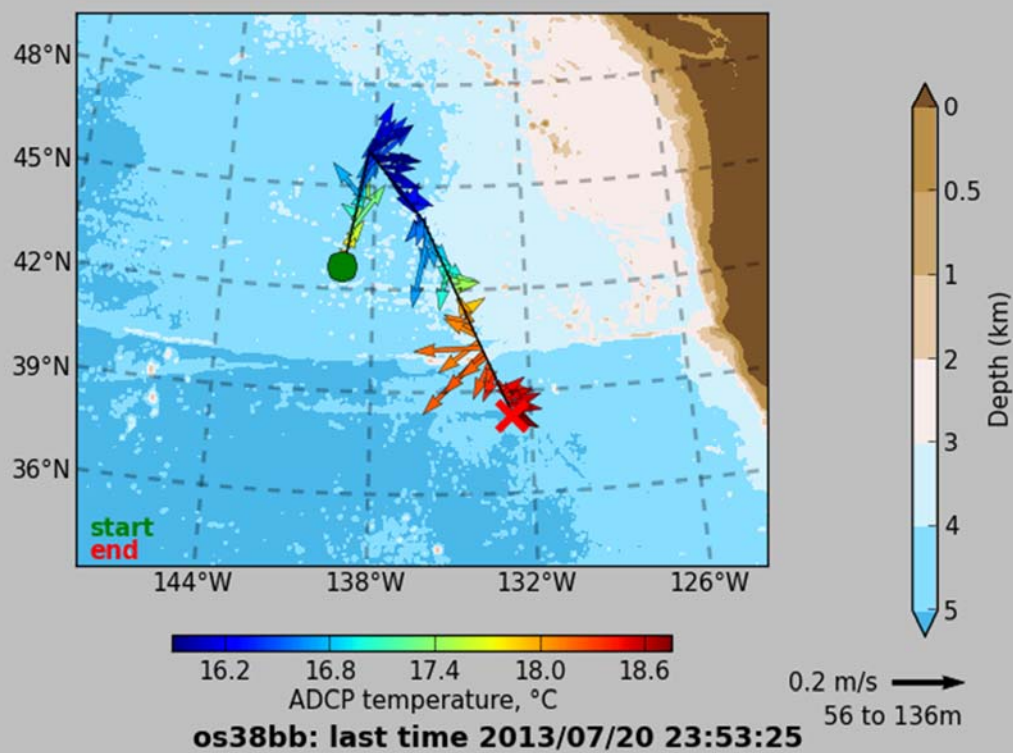
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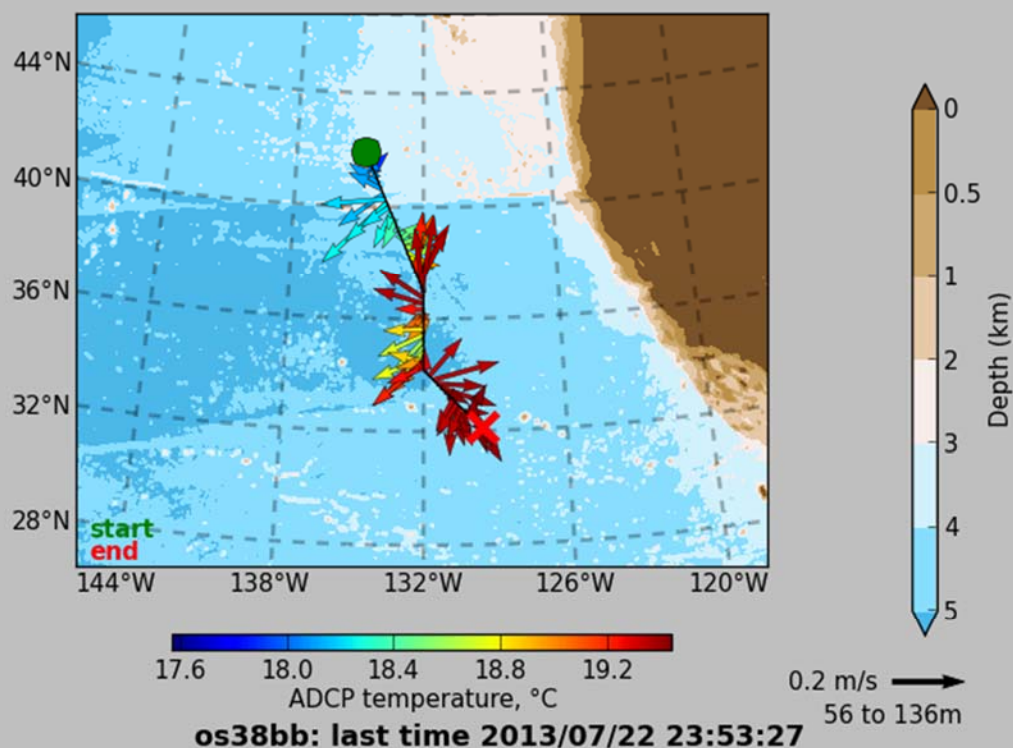
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km1312a os38bb

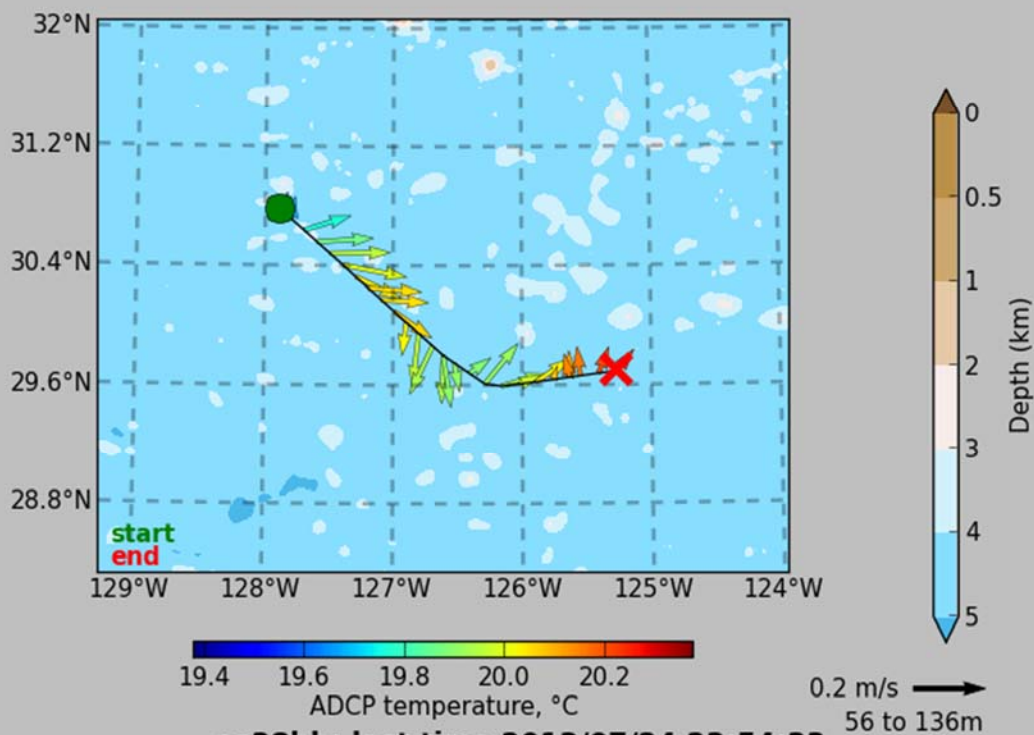


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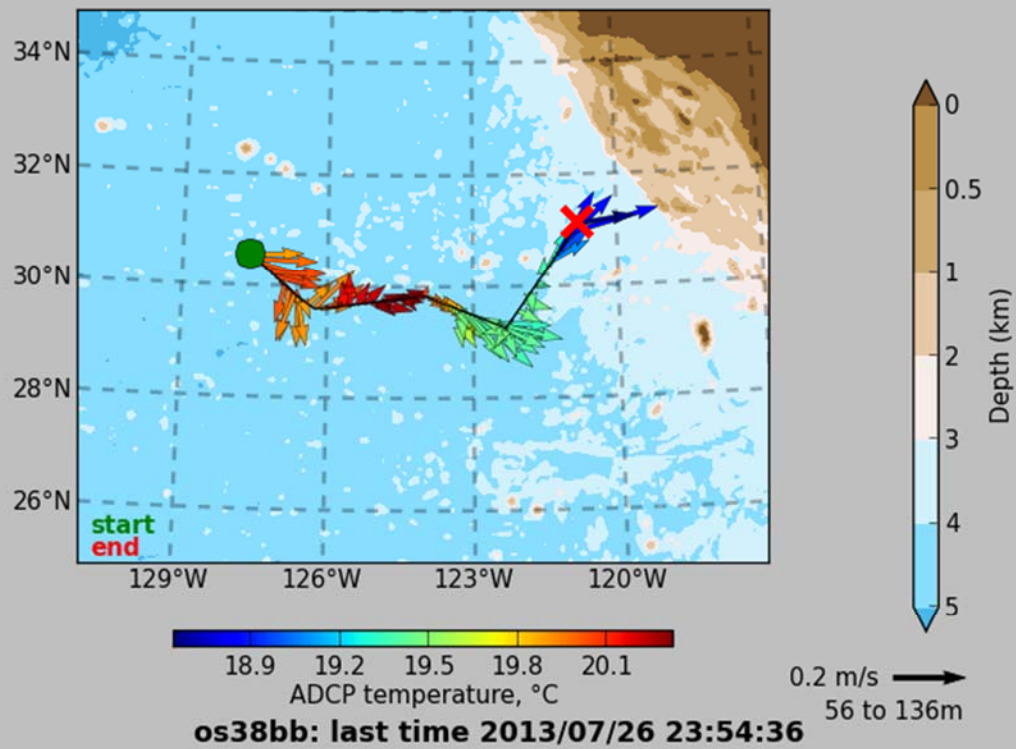
os38bb: last time 2013/07/22 23:53:27

km1312b os38bb

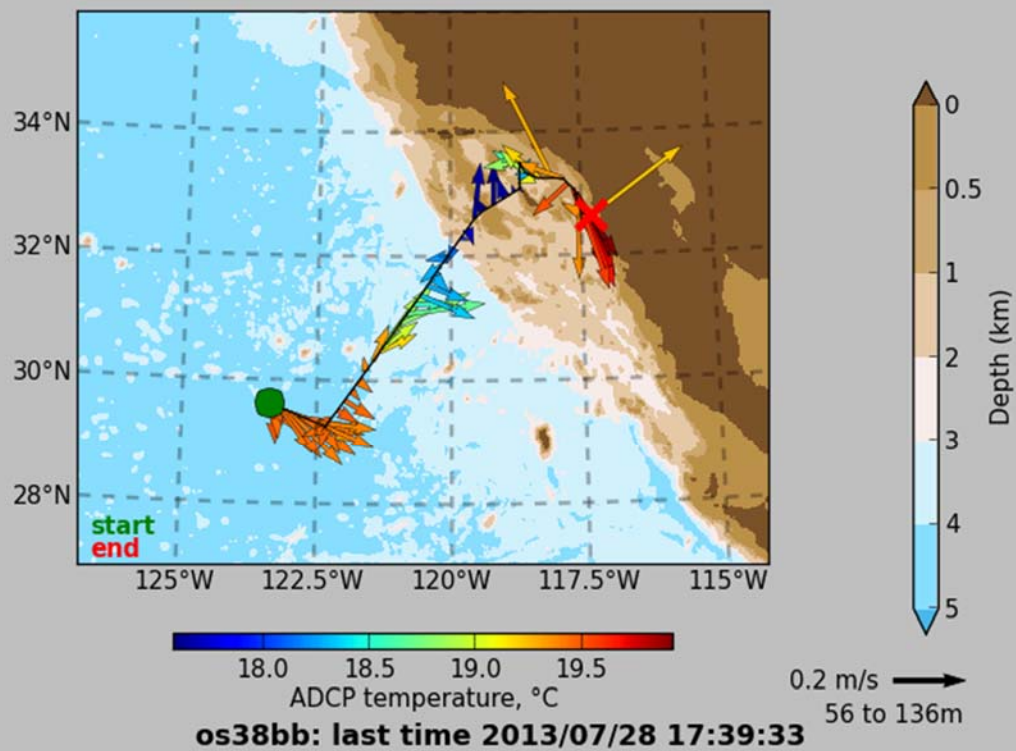


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km1312b os38bb

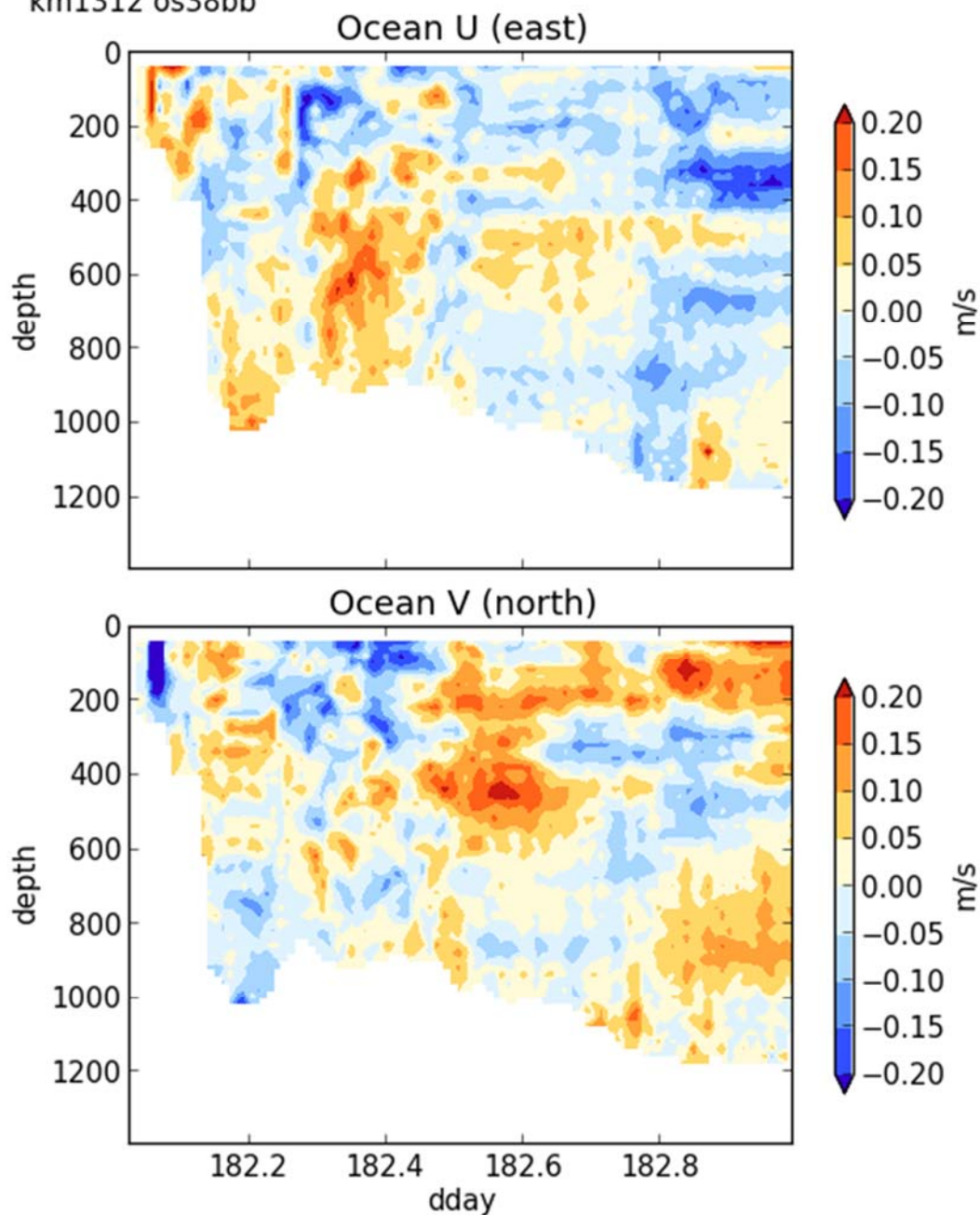


km1312b os38bb

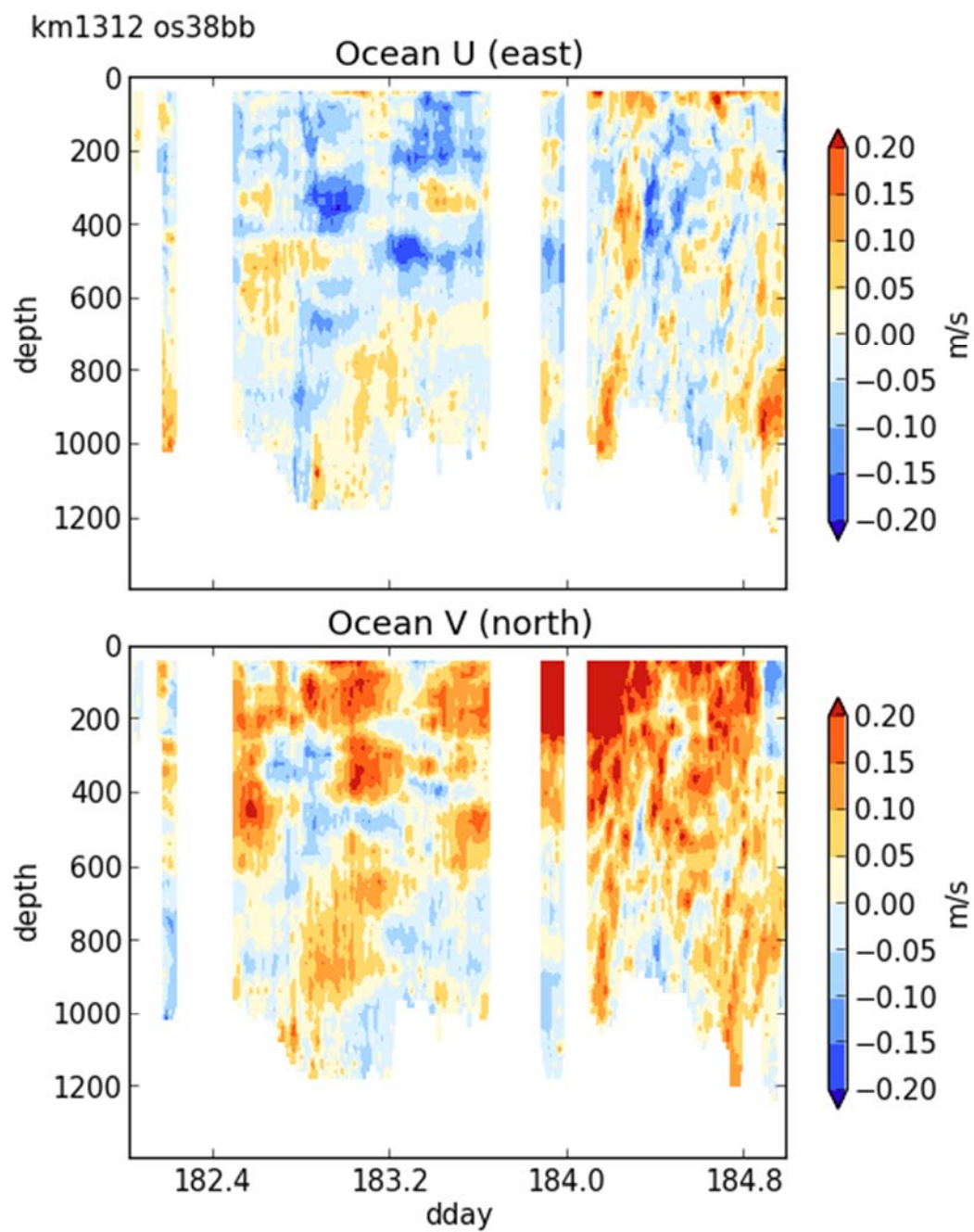


Depth Vectors (decimal day)

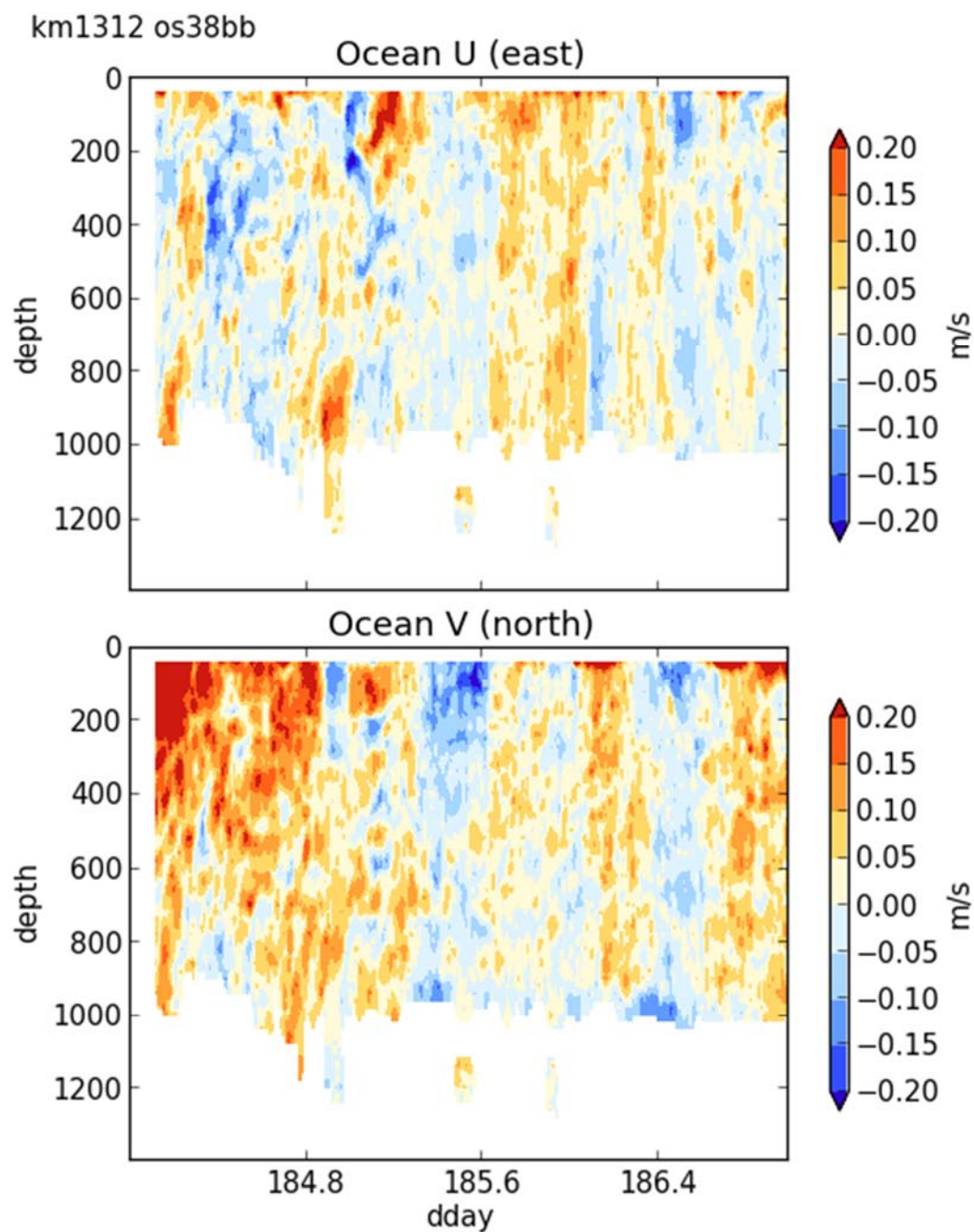
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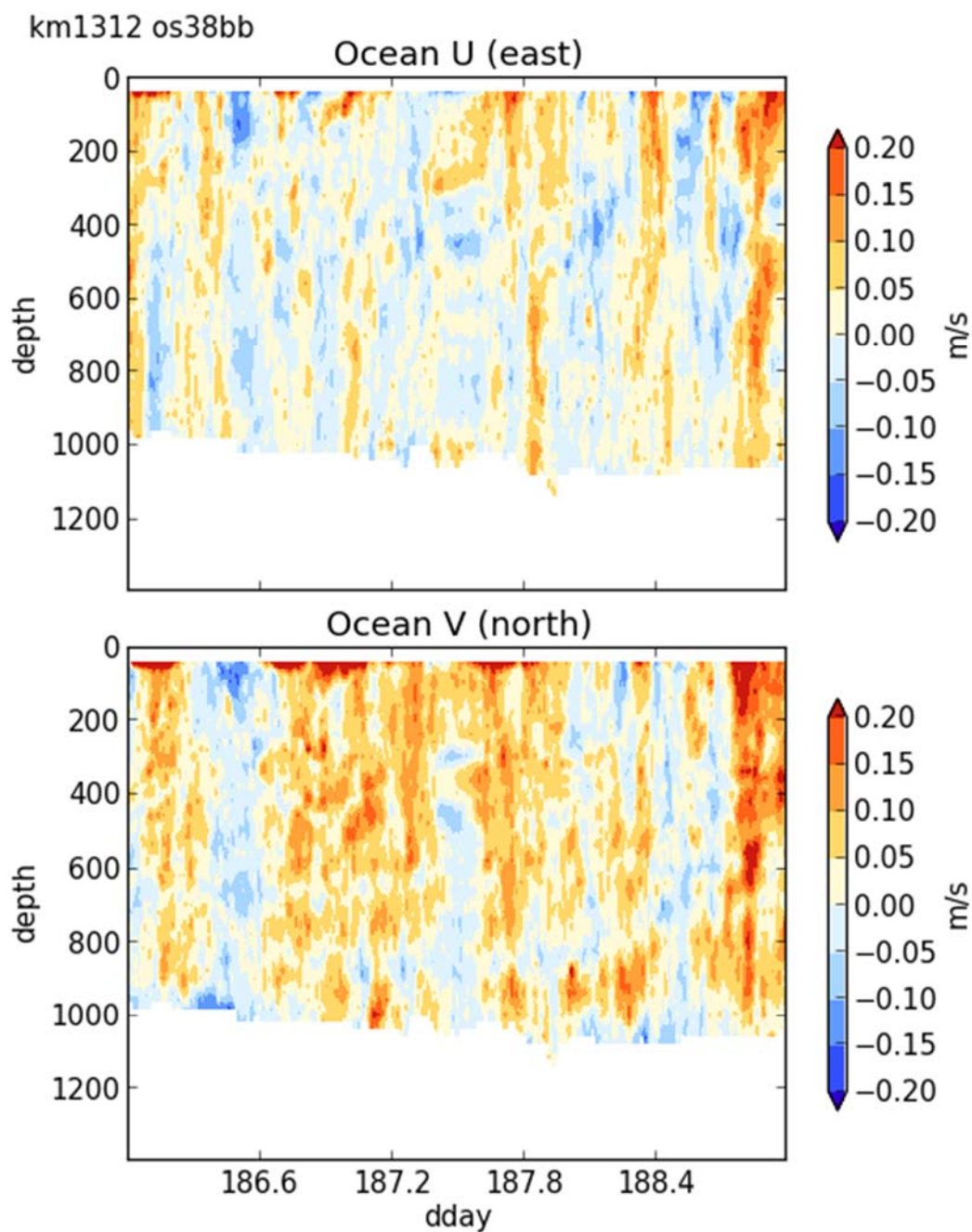
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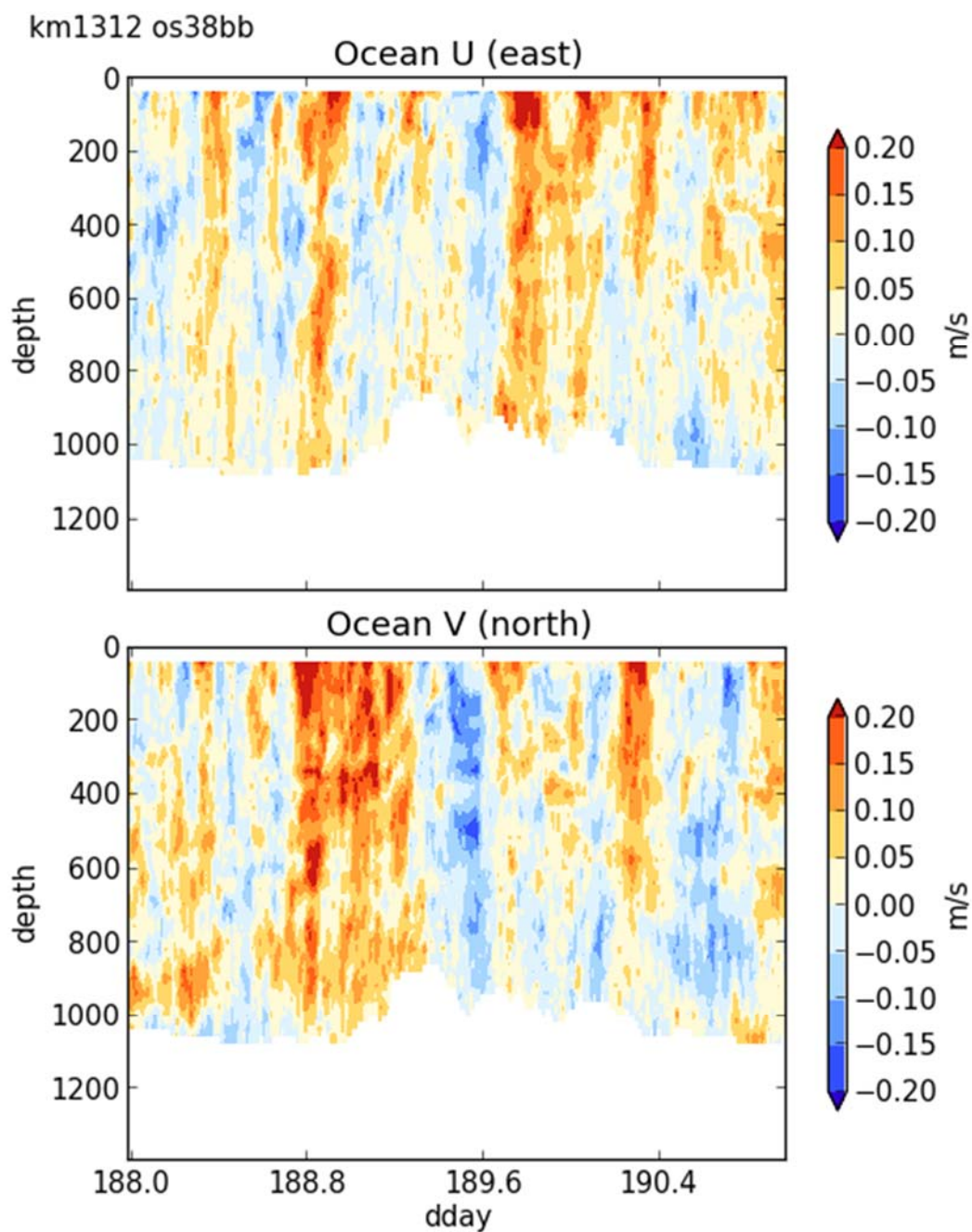
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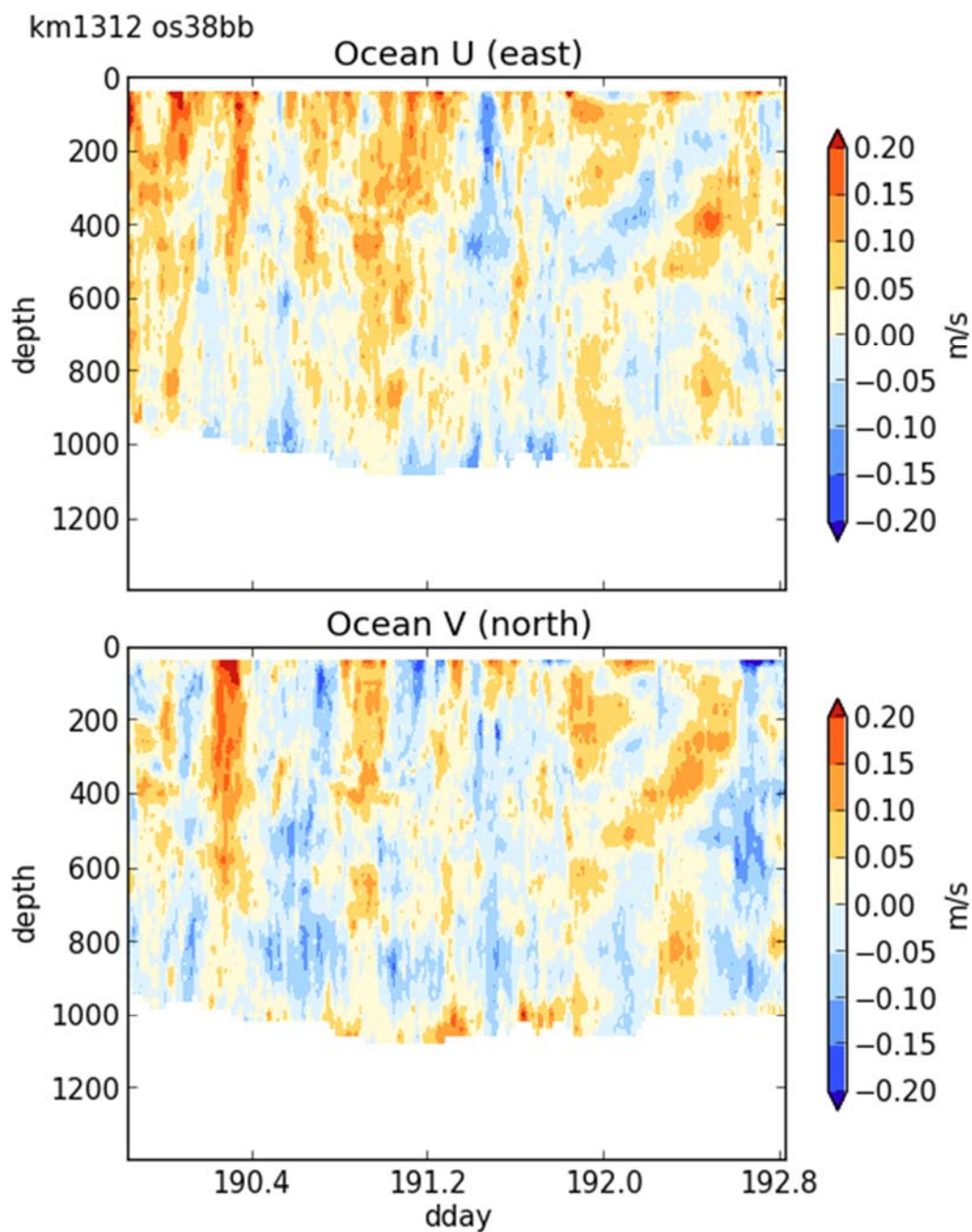
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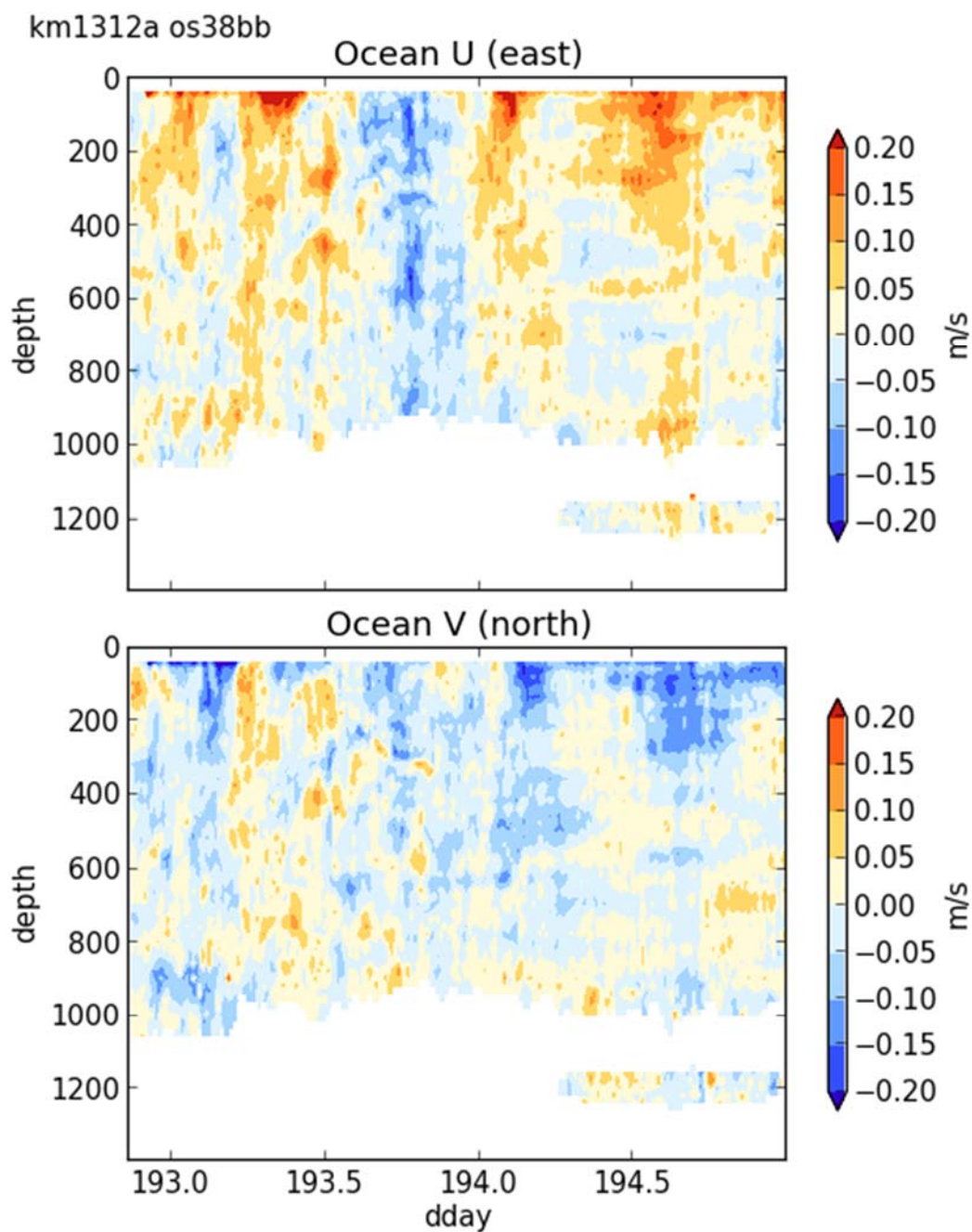
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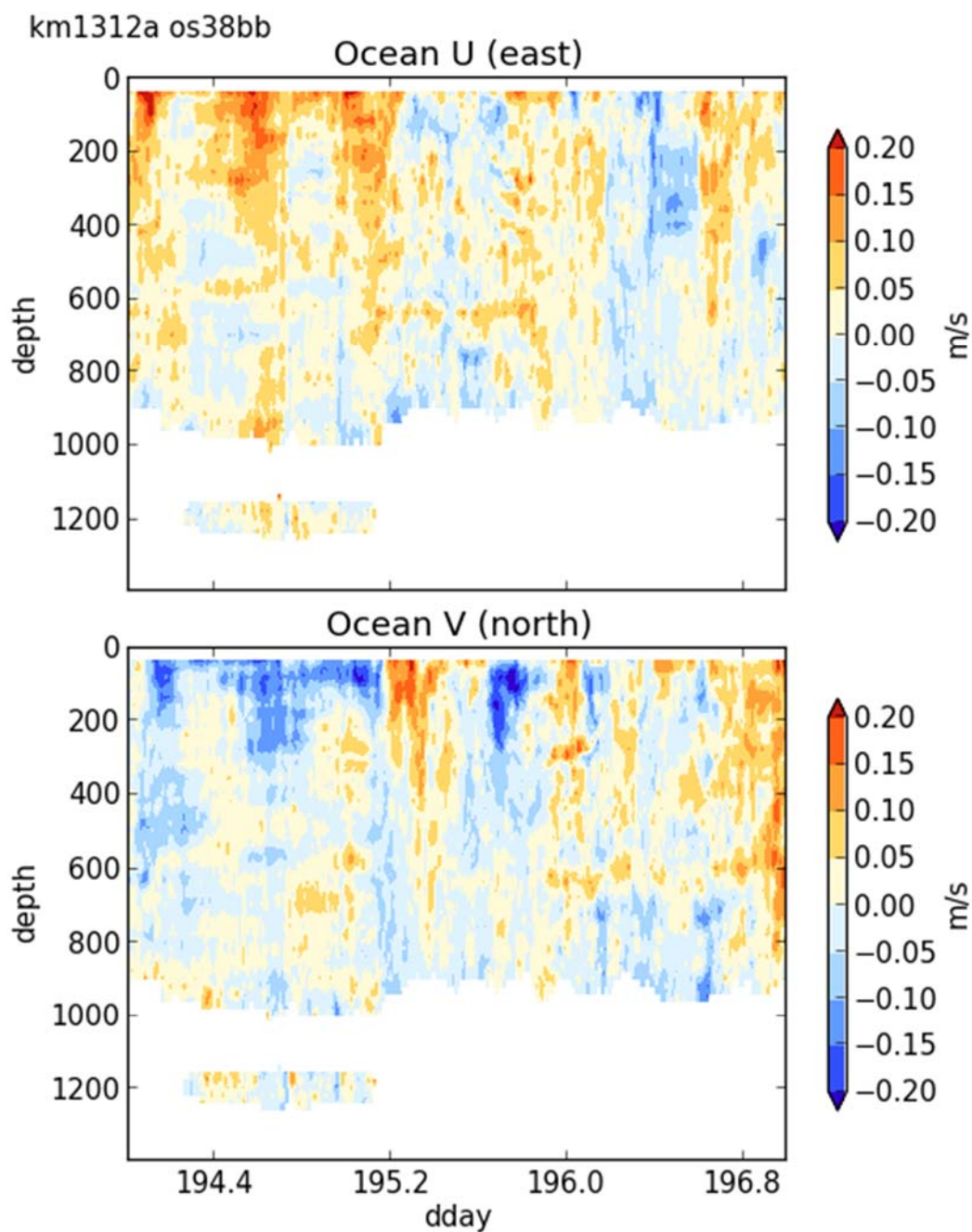
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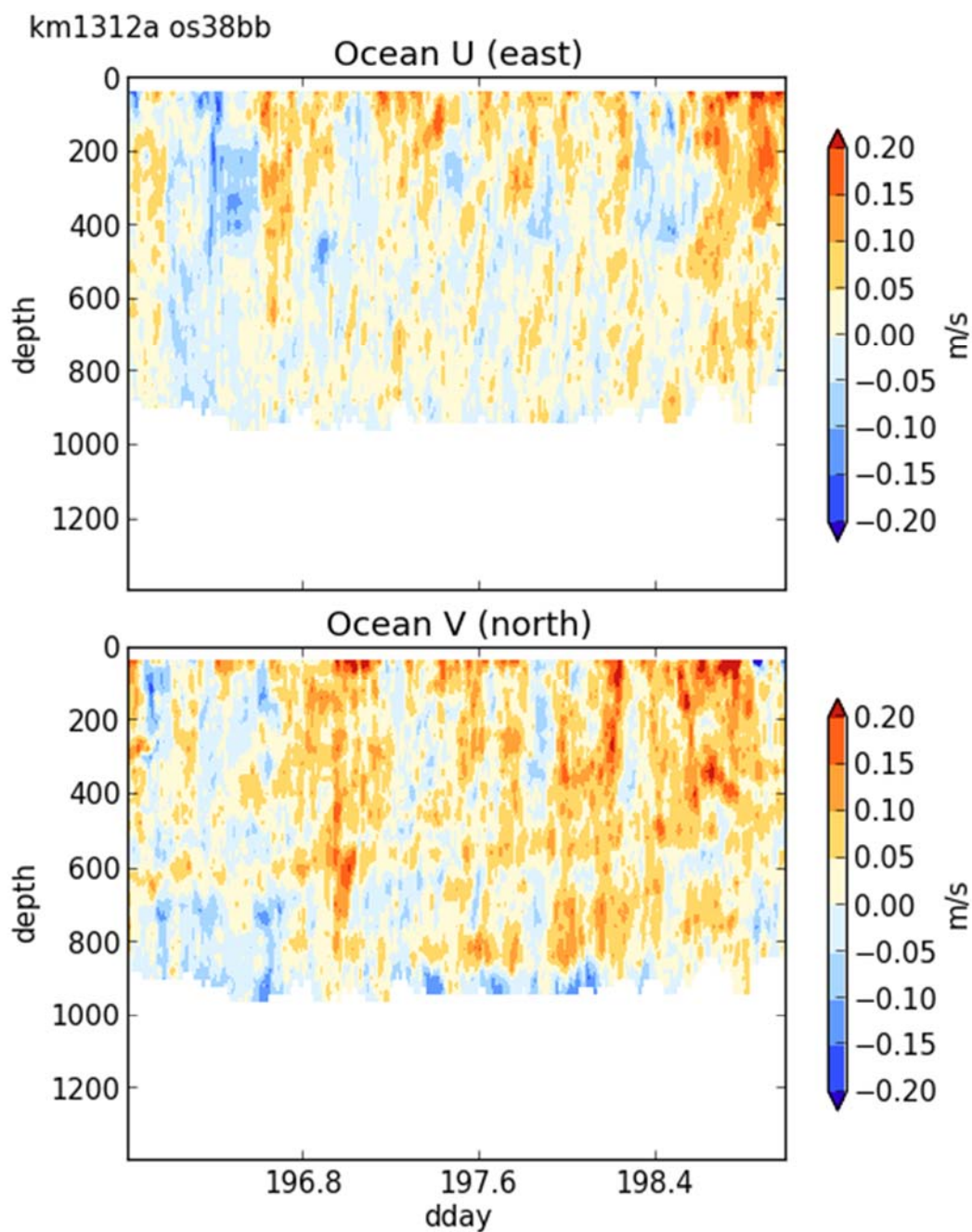
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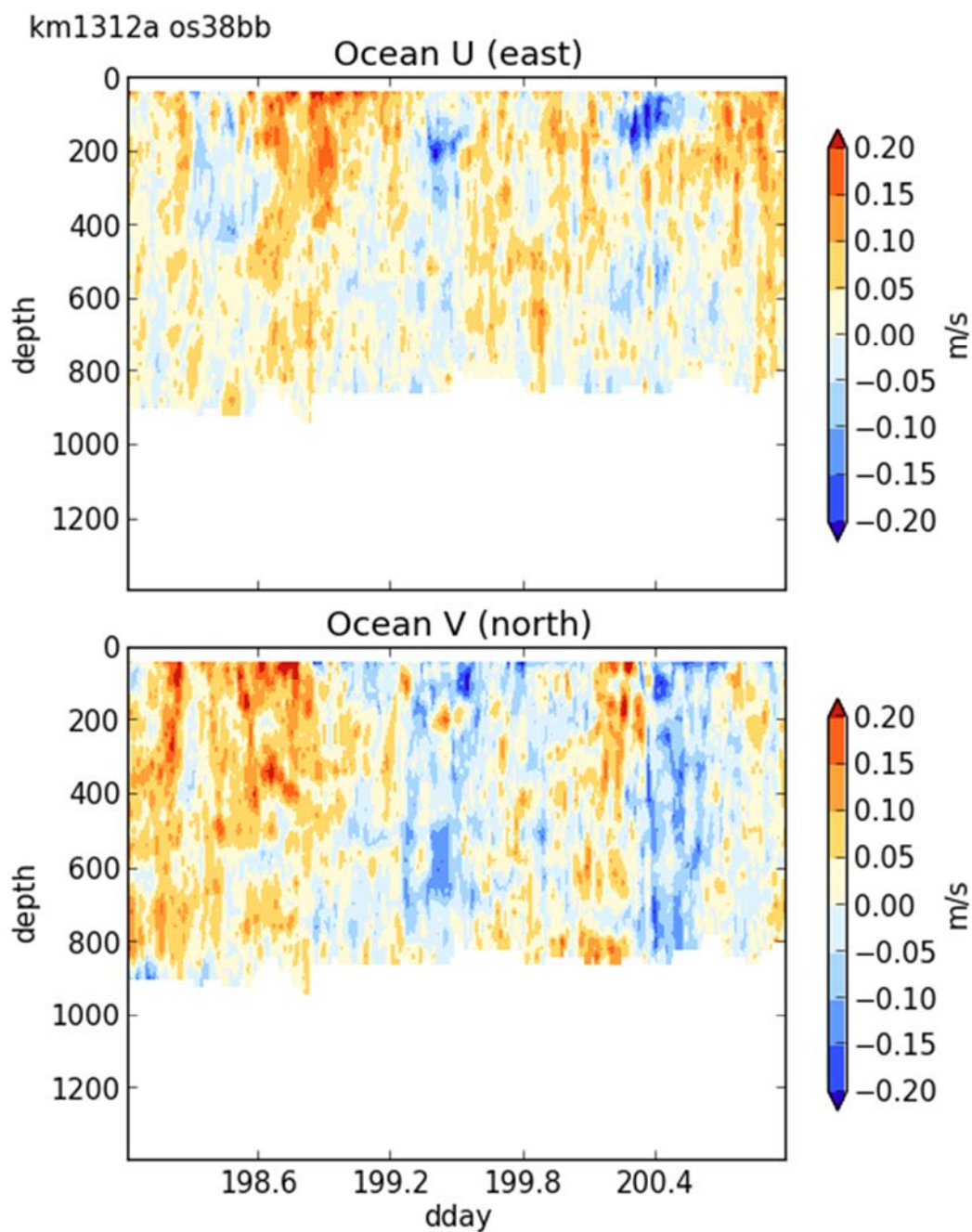
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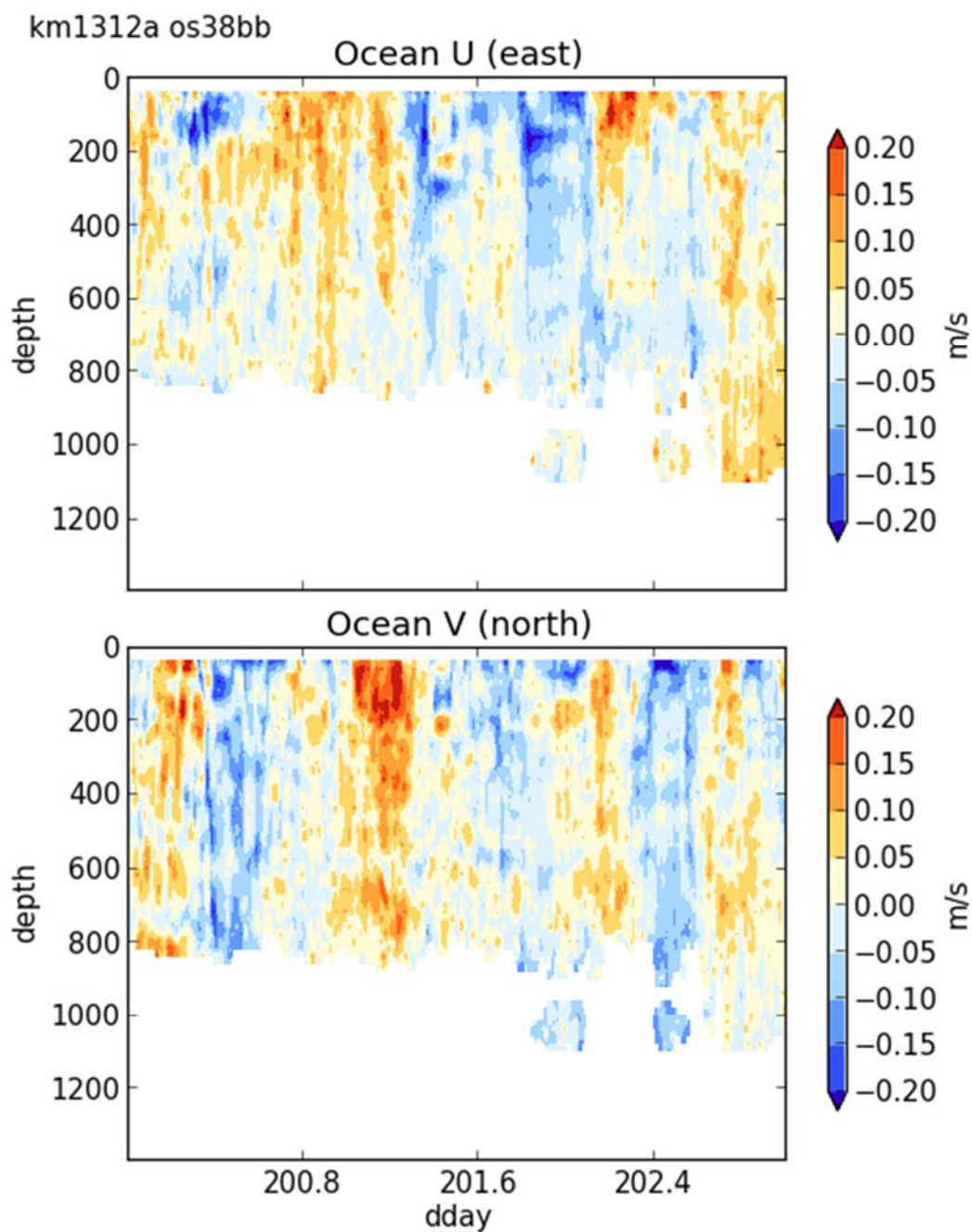
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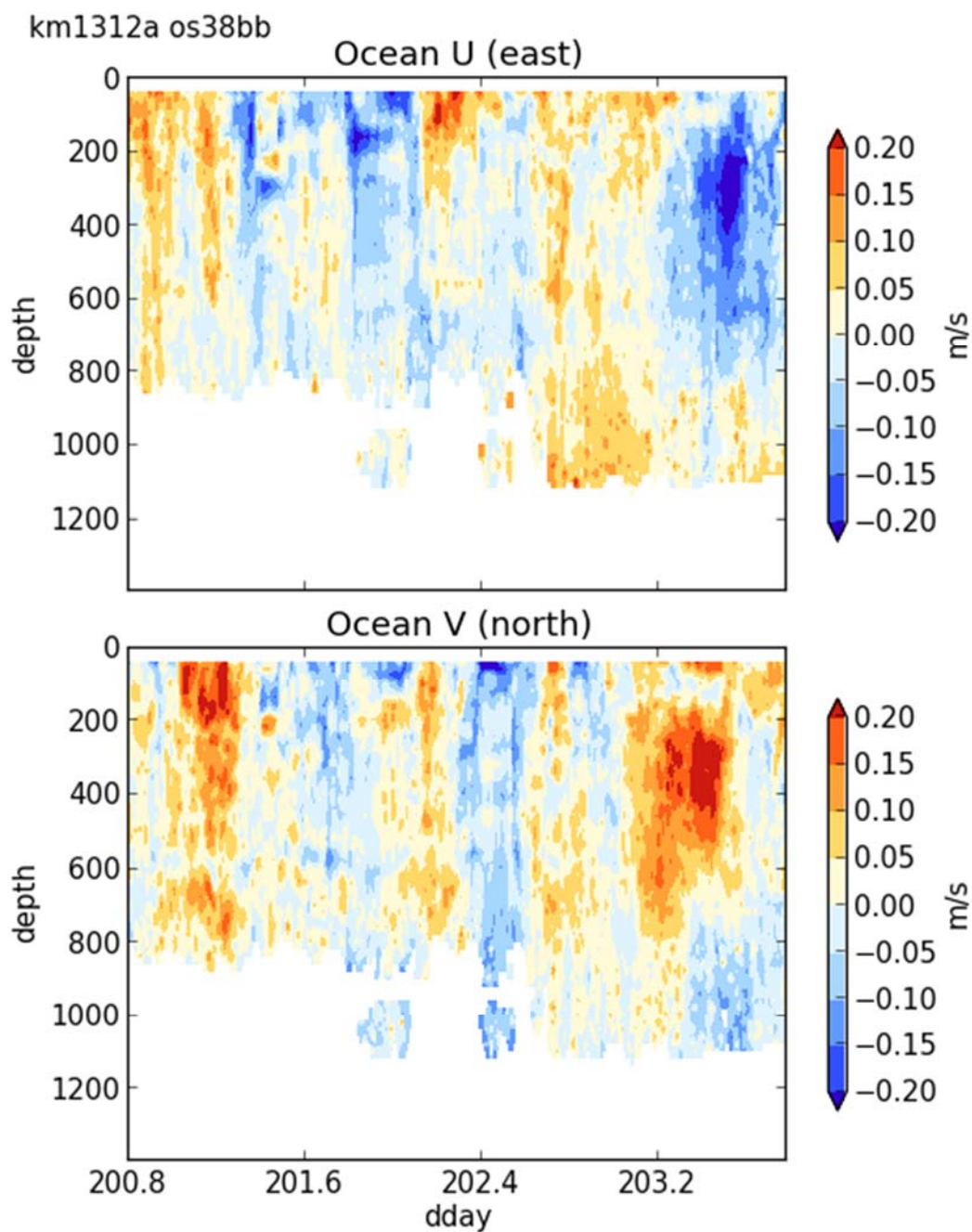
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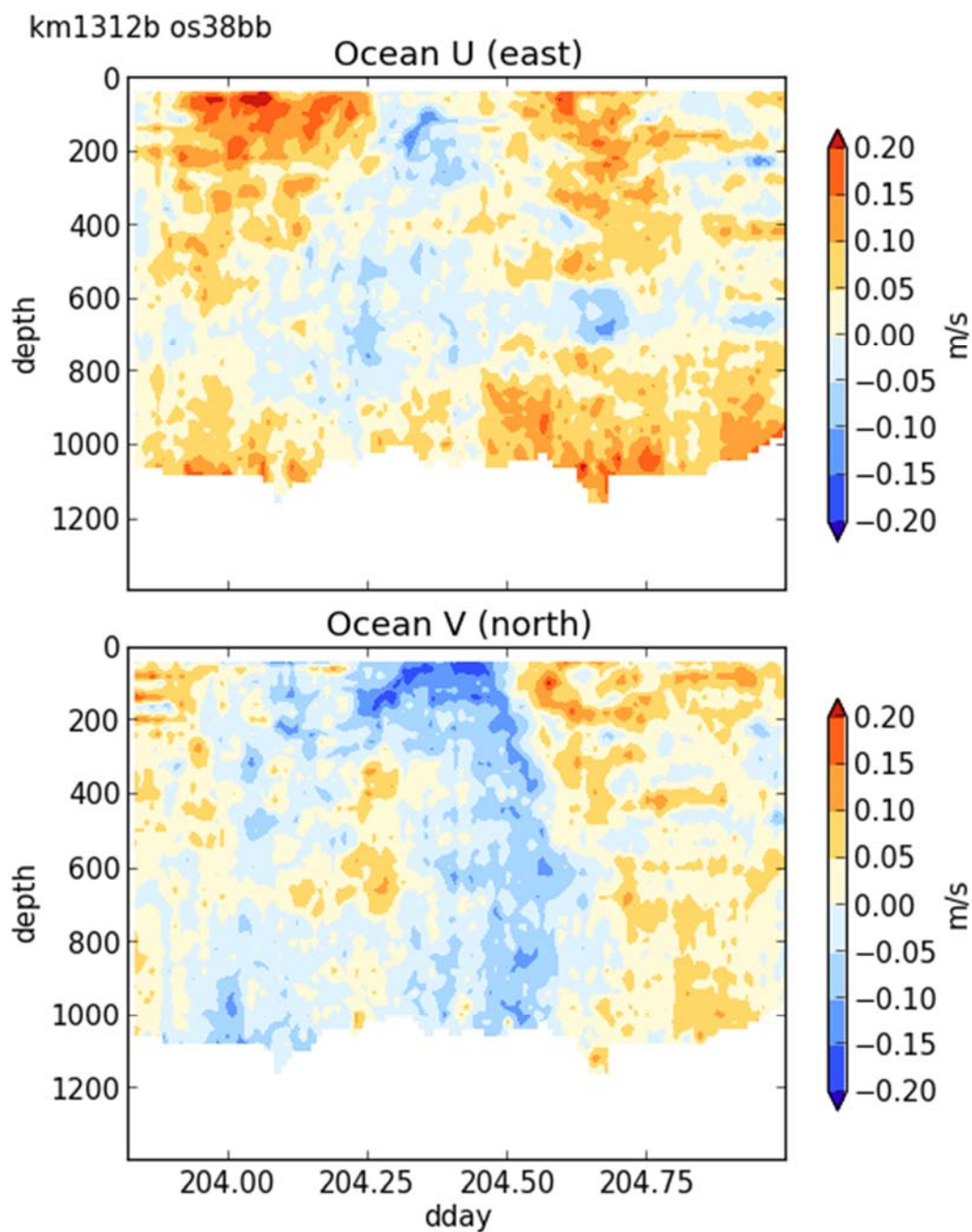
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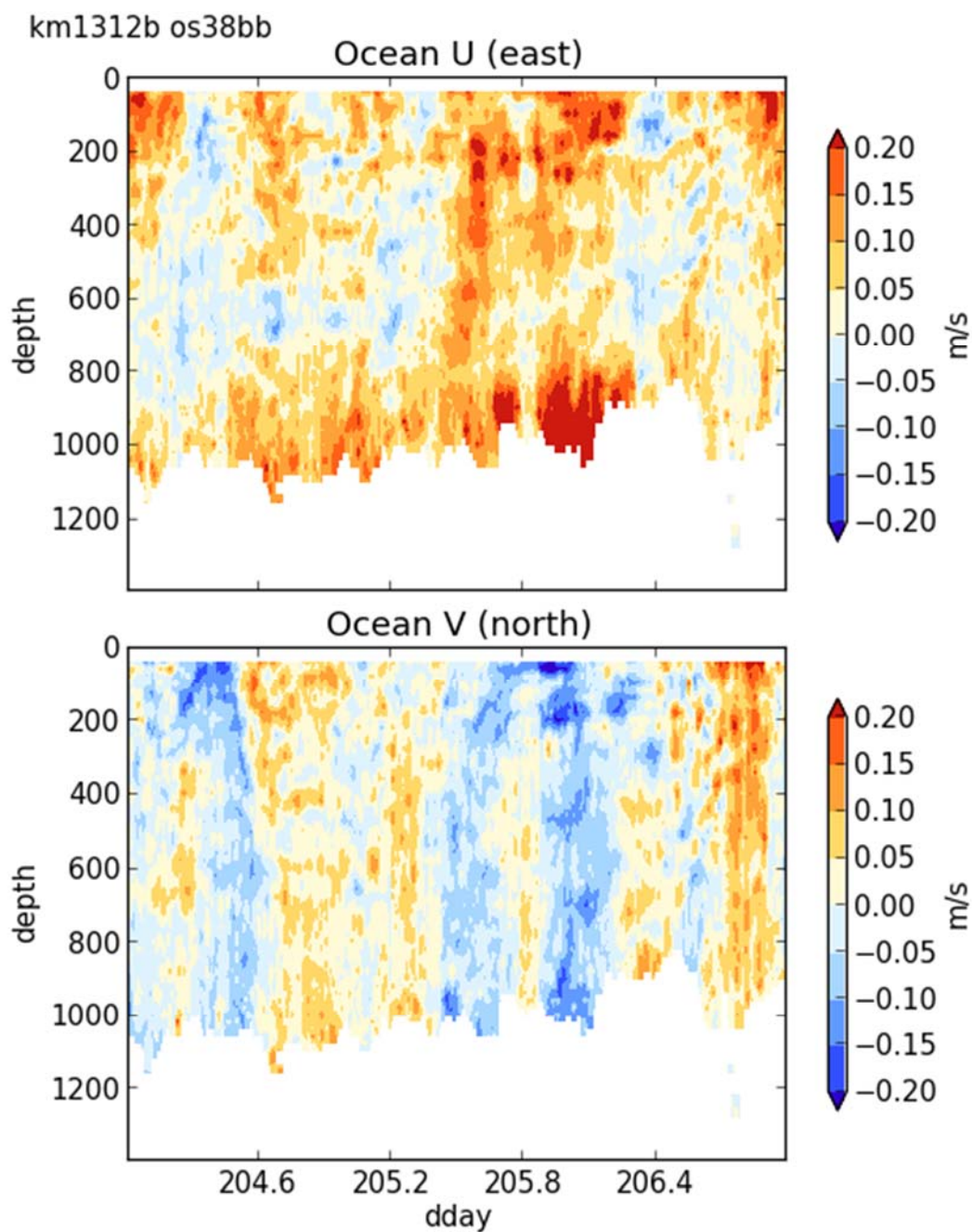
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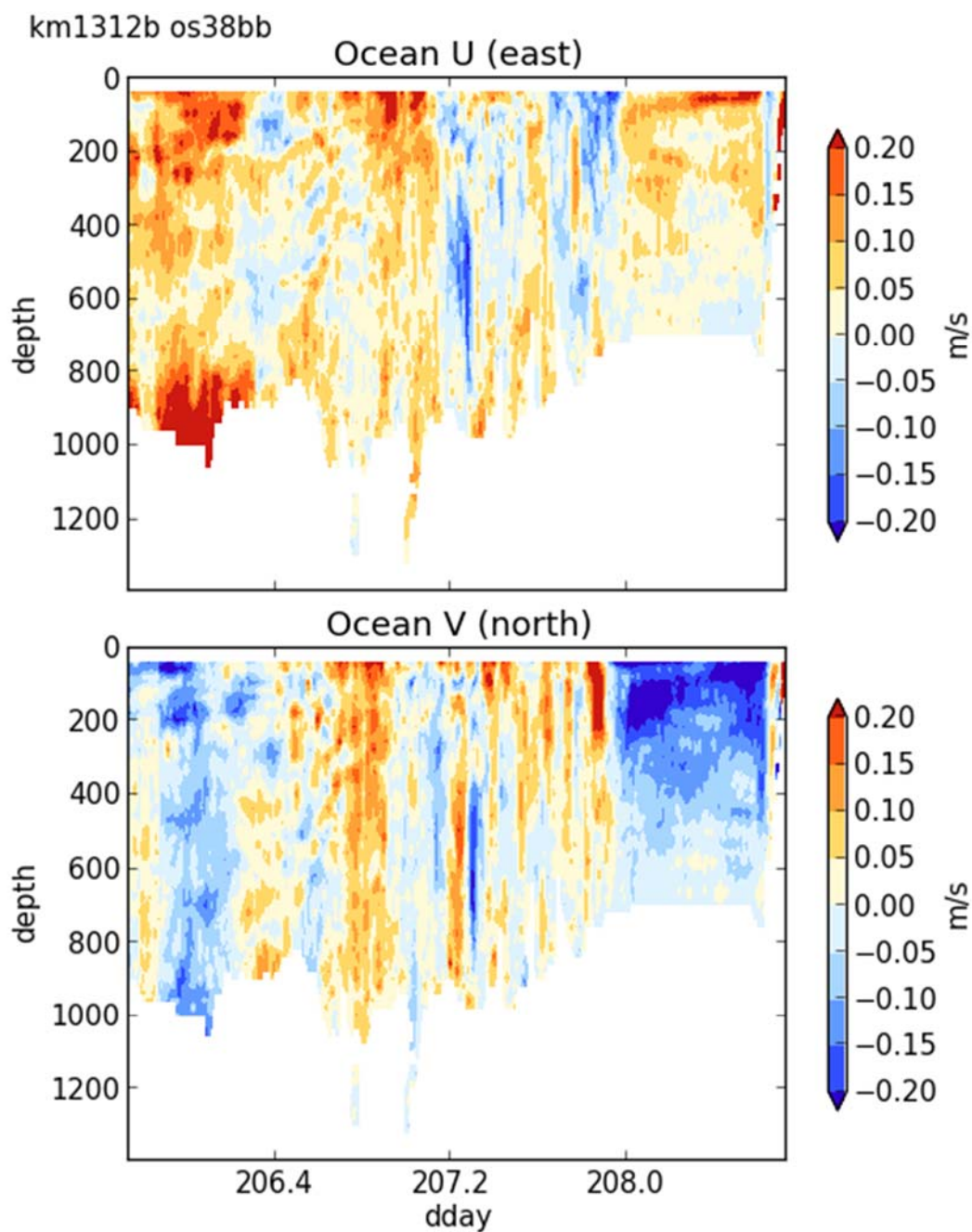
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os38bb: last time 2013/07/24 23:58:15



os38bb: last time 2013/07/26 23:54:38



os38bb: last time 2013/07/28 17:24:36

References

Project Website:

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

Data Archive

<http://bcodmo.org> (Oceanographic)

<http://www.ncbi.nlm.nih.gov/> (Molecular)

Major Funding Agency:

National Science Foundation

4201 Wilson Boulevard

Arlington, VA 22230

<http://www.nsf.gov>

University of Hawaii Marine Operations (R/V Kilo Moana)

<http://www.soest.hawaii.edu/UMC/cms/kilo-moana/>

Satellite Imagery Data (NASA MODIS)

<http://modis.gsfc.nasa.gov/>

Ocean Data View: Data Visualization Software

<http://odv.awi-bremerhaven.de/>

ARGO / APEX floats

<http://www.argo.ucsd.edu/>

ARGO project description

<http://www.usgodae.org/argo/argo.html>

ARGO data storage site

http://www.usgodae.org/cgi-bin/argo_select.pl

ARGO data retrieval

<http://runt.ocean.washington.edu/argo/heterographs/rollcall.html>

UW ARGO float information

<http://floats.pmel.noaa.gov/>

PMEL ARGO float information

<http://www.mbari.org/chemsensor/floatviz.htm>

MBARI Apex/ISUS Data Visualization