APPENDIX D - SHIP SURVEY DATA SUMMARIZED

Calibration of Chlorophyll Fluorescence CTD Measurements

CTD fluorescence measurements (voltages) were compared to chlorophyll concentrations measured by chemical methods in discrete bottle samples. Subsequently, new calibration coefficients were determined for algal biomass assessment; these coefficients were compared to factory calibration and analyzed the applicability of these CTD chlorophyll calibration coefficients (i.e., examine the dependence of chlorophyll calibration on sampling areas, dates of sampling and the parameters potentially affecting calibration: water turbidity (beam-C), CDOM and underwater irradiance). In addition, an extensive dataset collected by 5 organizations was analyzed (see Bight'08 Offshore Water Quality Field Operations Manual (2009)) to identify differences between instruments as a potential source of calibration inconsistencies.

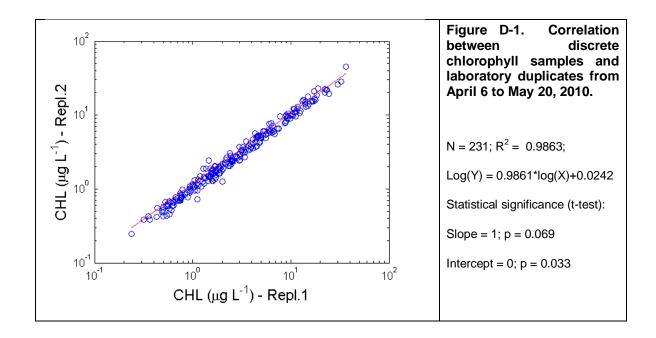
Chlorophyll Discrete Samples

Discrete water samples were collected at 149 stations, typically from two depths, surface and maximum chlorophyll fluorescence layer, at three CTD stations along any given transect. Water samples were typically obtained on the upcast using Rosette/CTD samplers. A few organizations sampled using a bucket (or something appropriate for surface only), and Niskin bottle during/after the CTD cast for deep samples. One liter of seawater was collected from each depth. Field processing used new GF/F glass fiber filter, clean equipment (i.e., forceps, filtering tower apparatus), and gentle sample mixing and vacuum filtration techniques. Standard sample volume was 100 ml, but smaller volumes could be used on water containing high particle concentrations. Filters containing chlorophyll samples were folded onto themselves, placed in separate glass test tubes, sealed, labeled, covered in foil, then frozen immediately and kept cold (dark) until laboratory analysis. Duplicate samples were taken from the same container. In the laboratory, chlorophyll-a was extracted, 24 hour minimum, using 90% acetone and measured using a Turner 10-AU fluorometer following Parsons *et al.* (1984).

All samples fit within the minimum detection limit. Only the samples with Fo/Fm<2 (99%) were taken for analysis. About one-half of chlorophyll samples were duplicates taken from the same water bottle. Most samples (22%) where the difference between duplicates exceeded 20% were eliminated from the analysis (this work was done by Meredith Howard). The remaining pairs of observations demonstrated high correlation (Figure D-1; $R^2 = 0.9863$; with bias close to zero [0.0242] and slope close to one [0.9861]), indicating good laboratory accuracy. Relative percent differences (RPD) averaged 10% (+/- 1, confidence limit) with a range of 0–50%. High RPD were generally limited to chlorophyll a values less the 2 µg/L with 6 values exceeding 25%. Duplicate samples were averaged to the initial samples to create one chlorophyll value for each depth at a particular station (Table D-1).

Region	Organization	Apr. 6-7	Apr.	May 3-7	May	May	Total
			27-28		10-14	18-21	
Ventura	ABC Labs	-	-	15	16	-	31
Santa Monica Bay	Hyperion	-	-	17	16	15	48
Palos Verdes	LACSD	20	16	-	-	-	36
Orange County	OCSD	24	12	-	18	13	67
San Diego	City of San Diego	-	26	10	13	-	49
То	tal	44	54	42	63	28	231

Table D-1.	Number of discrete	CHL samples	(excluding	duplicates)	collected	during	Bight'08
Offshore W	ater Quality Program.					-	-



Factory Calibration

All CTD instruments used in relation to discrete samples were factory calibrated [*SeaBird Electronics Inc* and Wet Labs Inc.] within a year of the survey starting date. These chlorophyll-a sensors (excitation: 460 nm, emission: 695 nm) measure fluorescence then convert the associated voltage (0-5 VDC, analog) to concentration (μ g L⁻¹) using a linear scaling factor. Factory calibration ensures that the scaling factor correctly responds to internal standards and a reference lab culture of *Thalassiosira weissflogii* phytoplankton used by the manufacture, in addition to setting the clean water offset. Sensor derived chlorophyll concentrations were calculated as follows for all CTD instruments.

 $CHL (\mu g/L) = (V - Vcwo) * ScaleFactor$

Where:

V is chlorophyll sensor voltage of sample;

Vcwo is the clean water offset voltage (pure filtered de-ionized water, provided by factory calibration sheet);

ScaleFactor is a multiplicative value (provided by factory calibration sheet).

Sensor calibrations show similar slopes and slightly different intercepts and resolutions (Table D-2).

Table D-2.Chlorophyll calibration values from the manufacture for sensors used during theBight'08 Water Quality survey, April 6 to May 20, 2010.

Organization	ScaleFactor (µg/L/V)	Vcwo (V)	Max Output (V)	Resolution (mV)	Cal Temp (°C)
ABC Labs	15.1	0.054	5.50	0.43	22 ± 1
LACSD/Hyperion*	15.0	0.068	5.44	0.68	22 ± 1
OCSD	14.8	0.048	5.47	0.41	22 ± 1
San Diego	15.0	0.064	5.46	0.61	22 ± 1

* organizations used the same sensor; μg = microgram; L =

liter; V = voltage.

For most organizations chlorophyll sensor voltage and resulting chlorophyll measurements were highly correlated (Figure D-2, Table D-3). The coefficients of regression were close to factory calibration coefficients. In the San Diego region, some chlorophyll voltages and concentrations demonstrated significant disagreement.

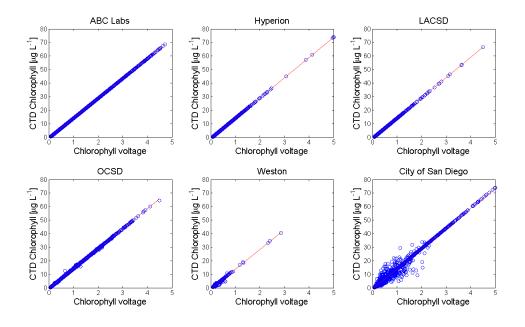


Figure D-2. Correlation between CHL voltage and concentration.

Table 3. Linear regression between chlorophyll sensor voltage and CTD chlorophyllmeasurements for the Bight'08 dataset.

Organization	Number of measurements (at 1-m depth intervals)	Goodness-of-fit (R ²)	ScaleFactor (µg/L/V)	Vcwo (V)
ABC Labs	6639	1.0000	14.6997	0.0510
Hyperion	6930	0.9999	14.9951	0.0658
LACSD	8698	0.9999	14.9758	0.0652
OCSD	14100	0.9998	14.7833	0.0456
Weston	732	0.9921	14.577	0.0504
San Diego	10909	0.9918	14.9254	0.0636
Total	48008	0.9971	14.8333	0.0567

In San Diego area, a disagreement between chlorophyll sensor voltage and concentration was observed on April 7-8 (all 28 stations) and April 28 (stations 401, 403, 702, 704, 802, 803, F23). This disagreement was associated with evident vertical displacement between CHL and voltage profiles (Figure D-3).

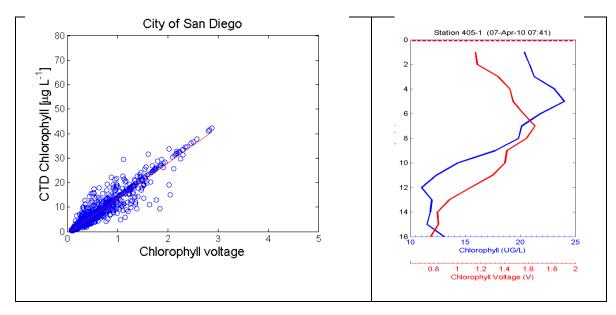


Figure D-3. Disagreement between chlorophyll sensor voltage and chlorophyll measurements during Bight'08 observations by City of San Diego on April 7-8 and April 28 (left) and an example of the displacement of vertical profiles of chlorophyll sensor voltage and chlorophyll at station 405, April 7, 2010 (right).

All CTD stations where discrete chlorophyll samples were collected did not demonstrate vertical displacement and, as such, the correlation between the Bight'08 discrete chlorophyll samples and CTD chlorophyll voltage could be used for calibration purposes.

Depth of CTD versus Discrete samples

Aligning CTD measured chlorophyll concentration during downcasts with discrete samples that were collected near real time, typically on the upcast, can be difficult. Disagreement between the CHL concentrations in discrete samples and the CTD CHL fluorescence at the same depth may result from heterogeneity of phytoplankton biomass and composition (i.e., chlorophyll concentration in phytoplankton cells). This disagreement should be proportional to the time lag between downcasts and upcasts (i.e., the period between CTD measurements and collection of discrete samples) and heterogeneity of chlorophyll in water column. As such, it was hypothesized that maximum correspondence (correlation) between CTD CHL fluorescence and discrete CHL measurements can be achieved by optimization of the depth interval (centered at the depth of discrete sample), within which CTD CHL voltage is averaged for comparison with discrete sample CHL concentration. For all samples collected during Bight'08 program, the optimal depth interval was ±3 m (Figure D-4).

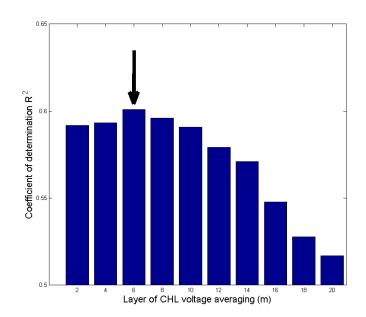


Figure D-4. Correlation (R^2) between discrete CHL samples and CTD CHL voltage averaged over the layer centered at the depth of discrete sample. Arrow indicates optimal layer of 6 m (±3 m). R^2 = 0.601.

CHL ($\mu g L^{-1}$) = (V + 0.0316) * 6.1265,

The slope coefficient (6.13) indicates that the factory equation (slope=14.78) overestimates CHL concentrations in the Southern California Bight by 200%. The new coefficients were used instead of the factory calibration for phytoplankton biomass assessment in the SCB during the Bight'08 Program.

CTD Fluorescence and CHL Concentration: Linear vs. Power

Power relationship between CTD CHL voltage and discrete CHL measurements appears to be more realistic than linear, because statistical distributions of both parameters are far from normal (Figure D-5). Lognormal distributions, although still significantly different from the measured values, fit CHL data much better (compare χ^2 and KS statistics for normal and lognormal distributions in Table D-4).

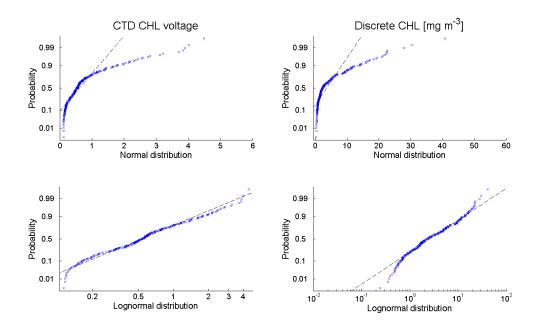


Figure D-5. Normal and lognormal probability plots for CTD CHL voltages (averaged over 6-m layers centered at the depths of discrete samples) and discrete CHL measurements.

Table D-4. Statistics (χ 2 and Kolmogorov-Smirnov, KS) of correspondence of CTD CHL voltages (averaged over 6-m layers centered at the depths of discrete samples) and discrete CHL measurements to normal and lognormal distributions.

Parameter	χ2	d.f.	р	KS
CTD CHL voltages	48.51	3	1.66*10 ⁻¹⁰	0.5449
Log-transformed CTD CHL voltages	14.74	6	0.0224	0.3078
discrete CHL	48.69	2	2.68*10 ⁻¹¹	0.6598
Log-transformed discrete CHL	24.17	7	0.0011	0.3430

Numerous studies have demonstrated that statistical distribution of CHL concentrations in the ocean is close to lognormal (Banse and English 1994, Campbell 1995). Assuming lognormal distributions of CHL in marine phytoplankton, this study used an alternative method of calculating CHL concentration from CTD voltage based on the power equation:

$CHL (\mu g L^{-1}) = A * V^{B}$,

which coefficients can be derived from the linear regression between log-transformed CHL and log-transformed *V*:

Log (CHL) = Log (V) * Slope + Intercept.

A = exp (Intercept) and B = Slope.

Both regressions (linear vs. power) are very close (Figure D-6). Although, the power equation demonstrates some advantages over linear. Fit was better for the power equation than for the linear equation with $R^2 = 0.665$ vs. 0.601, respectively. For the power equation, residuals random errors appeared to be independent of CHL concentration. In contrast, the linear equation substantially overestimated the weight of high CHL concentrations, which may result in an incorrect regression slope. Both the linear and power regressions were significantly different from the factory calibration coefficients (dashed red line in Figure D-6).

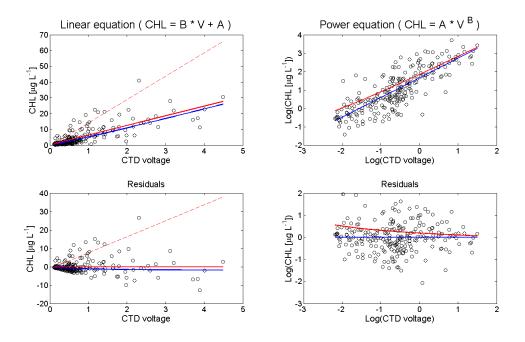


Figure D-6. Linear (red) and power (blue) relationships between discrete CHL (µg L⁻¹) and CTD CHL voltages averaged over 6-m layers centered on the depths of discrete samples. Red dashed line indicates CHL estimated from voltage using CTD factory calibration coefficients.

Both the power equation CHL ($\mu g L^{-1}$) = 5.2127 * V^{1.0689} and the linear equation CHL ($\mu g L^{-1}$) = (V + 0.0316) * 6.1265 can be used for CHL biomass assessment in the entire Southern California Bight.

CTD CHL for Regions and Dates

The equations transforming CTD chlorophyll sensor voltage to chlorophyll concentrations brought the original, factory derived, CHL concentration spread close to the expected values from the discrete samples (Figure D-7). These transformations are equally applicable to all SCB regions during the entire period of the Bight'08 Offshore Water Quality Program data (April–May 2010). We make this conclusion because the slopes of the regression equations between the CTD voltage and the discrete CHL were independent of regions, Ventura to San Diego, and demonstrated no evident trend during April–May 2010 (see next two paragraphs). The regression intercepts were slightly different, and these differences varied with the extended time periods. The variability of the CTD voltage/CHL relationship indicates potential interaction from the phytoplankton community composition. Differing technical characteristics of the various instruments did not appear to cause differences between the regional chlorophyll measurements.

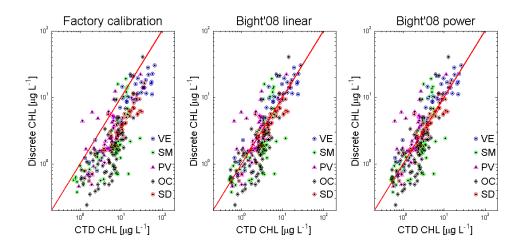


Figure D-7. The three graphs compare CTD derived chlorophyll-*a* concentration to laboratory measured discrete sample values. a) Factory values compared to discrete samples; b) Post-survey linear transformation of voltage to concentration; c) Post-survey power transformation of voltage to concentration; C) Post-survey power transformation of voltage to concentration. Regions: Ventura (VE); Santa Monica Bay (SM); Palos Verdes (PV); Orange County (OC); San Diego (SD).

Regional analysis was performed on values derived from the power equation. Comparison between the slopes and intercepts of the regression equations between log-transformed CTD CHL voltages and CHL concentrations in discrete samples were performed using a one-way analysis of covariance (ANOCOVA) statistical model. The data were classified into five groups or regions (Ventura, Santa Monica Bay, Palos Verdes, Orange County, and San Diego). The results of the analysis of covariance demonstrate that chlorophyll concentrations were significantly dependent on the "group" factor,) but were independent of the interaction between "log(V)" and "group" factors (Table 5). Generally, differences between the regression slopes were insignificant; only the intercepts of the regression equations were significantly different (Table 6). A multiple comparison test revealed two groups of regions: Ventura and Palos Verdes, had regression equation intercepts significantly higher than Santa Monica, Orange County, and San Diego. Note that this difference was observed between Palos Verdes and Santa Monica, which used the same CTD instrument for their surveys. However, phytoplankton composition rather than instrument characteristics is a likely explanation for the regional differences.

Table 5. Analysis of Co-Variance (ANOCOVA) table demonstrates that log-transformed chlorophyll concentration (a dependent variable) was independent of the interaction between Agency/Region ("group") and log-transformed sensor voltage ('log(V)").

Source	d.f.	Sum of Squares	Mean Square	F	Prob.>F

group	4	13.4978	3.3745	9.1961	0.0000
log (V)	1	130.5600	130.5600	355.8026	0.0000
group*log(V)	4	1.0528	0.2632	0.7173	0.5809
Error	221	81.0949	0.3669		

Table 6. Regression coefficients (estimates for "All groups" and the offsets from these values for each group) and statistical significances of the differences between the coefficients. Mind no difference between the slopes and significant differences between the intercepts (all regions but San Diego).

Group		Intercept			Slope				
	Estimate	Std. Err.	Т	Prob.> T	Estimate	Std. Err.	Т	Prob.> T	
All groups	1.7256	0.0622	27.7558	0.0000	1.0329	0.0578	17.8565	0.0000	
Ventura	+0.2549	0.1066	2.3916	0.0176	-0.1231	0.1010	-1.2188	0.2242	
Santa Monica Bay	-0.2747	0.1183	-2.3224	0.0211	-0.0462	0.1071	-0.4315	0.6666	
Palos Verdes	+0.3990	0.1733	2.3030	0.0222	+0.0920	0.1489	0.6175	0.5375	
Orange County	-0.2299	0.1130	-2.0343	0.0431	+0.1143	0.1030	1.1095	0.2684	
San Diego	-0.1494	0.0957	-1.5616	0.1198	-0.0370	0.1117	-0.3309	0.7410	

Date or survey event differences between the regions were inconsistent (Figure D-8). In particular, negative residuals from Orange County (the only region where discrete CHL was collected during the entire two-month period) demonstrate that CTD CHL fluorescence somehow overestimated CHL concentration in the beginning (early April) and the end (late May) of the data collection period. In late April, CHL in Orange County was underestimated (residuals were mostly positive). An opposite pattern was observed off Palos Verdes. Positive residuals mean CHL was underestimated in early April and normalized around zero by late April. Such unstable differences cannot be explained by instrument sensor drift alone and point to taxonomic variations of phytoplankton. Note that these date variations were observed on time scales of weeks. No daily variation was observed within a given sampling event (e.g., 3 days to complete a cruise).

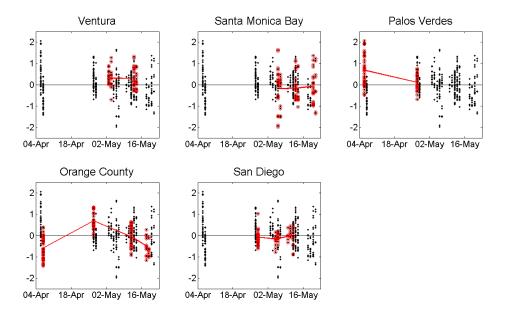


Figure D-7. Residuals (the differences between discrete CHL and CTD CHL, both log-transformed) in different regions during the Bight'08 sampling events. Each region was done by separate sampling organizations. Black dots indicate all residuals. Red circles indicate the residuals observed in each of the five regions. Red lines connect the means of residuals in each region during each sampling event.

Effect of Water Turbidity (beam-C), CDOM and Irradiance

The effect of turbidity (beam-C), underwater irradiance, and CDOM concentration on the accuracy of CTD measurements of chlorophyll concentration was either small or absent. Analysis of correlations between beam-C, CDOM, and irradiance against the residuals of CTD chlorophyll demonstrated that CTD CHL fluorescence slightly underestimated CHL concentrations under high turbidity and high irradiance. The relationship between CTD CHL fluorescence and discrete CHL was independent on CDOM concentration.

The correlation between residuals and beam-C was low but significant ($R^2 = 0.0423$; p = 0.0017; d.f. = 229). Also, residuals were uncorrelated with CDOM ($R^2 = 0.0051$; p = 0.3297; d.f. = 186) and somehow correlated with underwater irradiance ($R^2 = 0.0650$; p = 0.0373; d.f. = 65). However, low coefficients of determination (R^2) indicate that including beam-C and irradiance would hardly improve the relationship between CHL voltage and CHL concentration. Beam-C and irradiance explained only a small part of the residuals (~4% and ~6%, respectively). Irradiance was measured at a limited number of stations (see d.f. = 65 vs. 229 for beam-C and 186 for CDOM).

As such, beam-C and underwater irradiance shouldn't be used as correction factors for CTD CHL measurements.

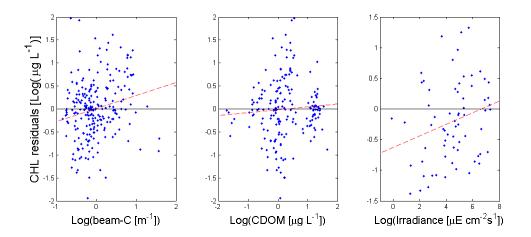


Figure D-8. Relationship between CHL residuals and beam-C, CDOM and underwater irradiance.

Conclusions

- CTD measurements of chlorophyll fluorescence in the Southern California Bight (SCB) during Bight'08 Offshore Water Quality Program (April-May 2010) based on factory calibration coefficients varied significantly (by a factor ~2.5) and consistently overestimated chlorophyll concentration. For a rough evaluation of chlorophyll concentration (as a measure of phytoplankton biomass), CTD chlorophyll measurements should be multiplied by a factor of 0.4.
- 2. Better assessment of chlorophyll biomass in SCB should be based on CTD fluorescence voltage, using either the linear equation CHL ($\mu g L^{-1}$) = (V + 0.0316) * 6.1265 or the power equation CHL ($\mu g L^{-1}$) = 5.2127 * V^{1.0689}. The resulting CHL concentrations are close; fit of the power model was slightly better (R² = 0.665 vs. 0.606).
- 3. Inconsistency (displacement of vertical profiles) between CTD sensor voltages and CTD chlorophyll measurements was found at 35 stations collected by the City of San Diego during April 7-8 and April 28.
- 4. The calibration coefficients obtained in this study are equally applicable to all locations within the SCB (from Ventura to San Diego) and all B'08 sampled periods (early April–late May). Local variations in model disagreement demonstrate no consistent spatial bias or temporal trend.
- CTD measurements slightly underestimated CHL concentrations under high turbidity (beam-C) and high irradiance. This underestimation was small and did not significantly improve the model "goodness-of-fit".

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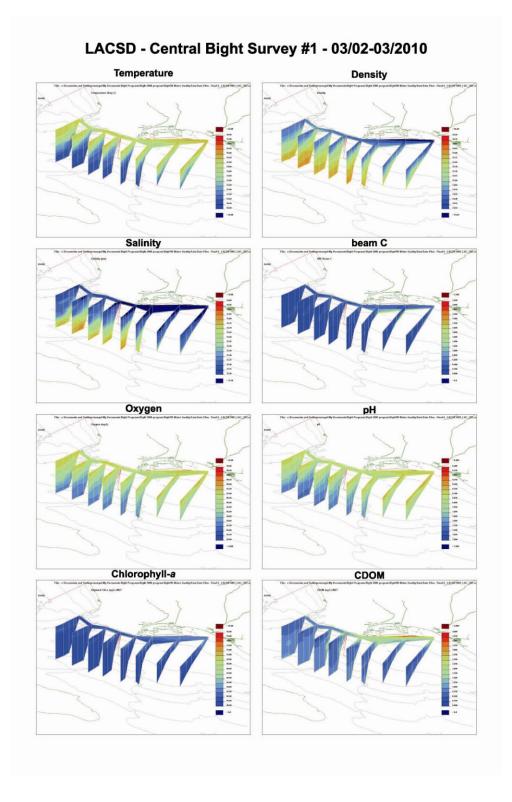
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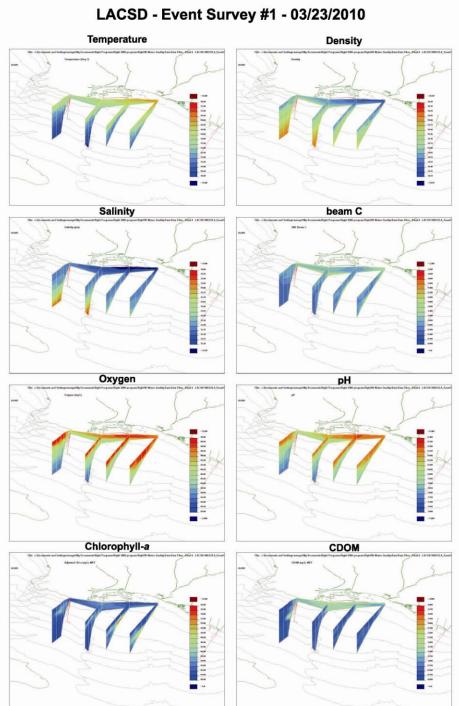
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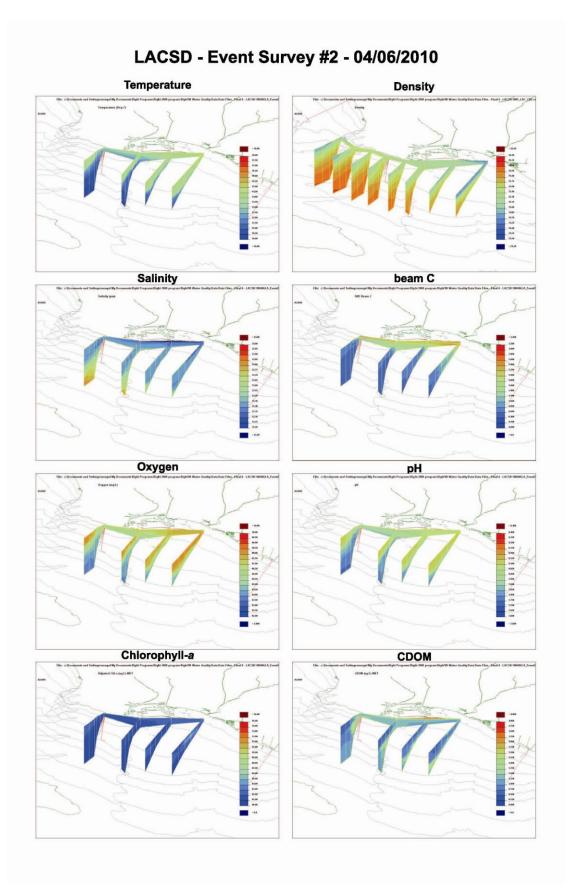
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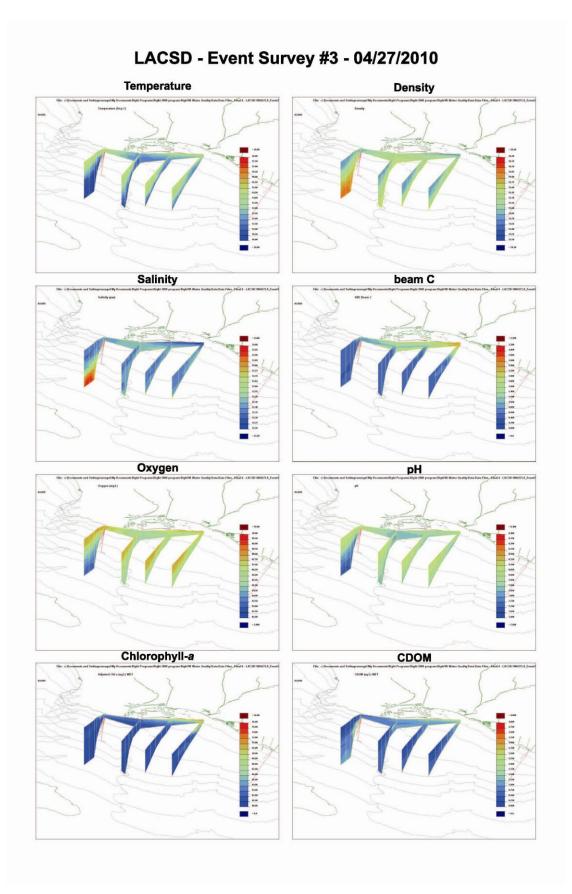
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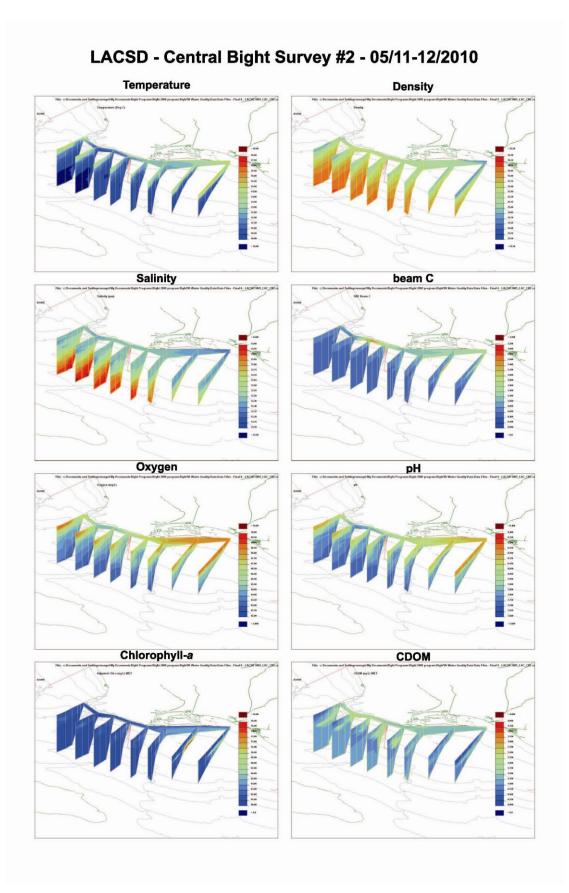
The Los Angeles County Sanitation District ship survey CTD data summarized for each survey.





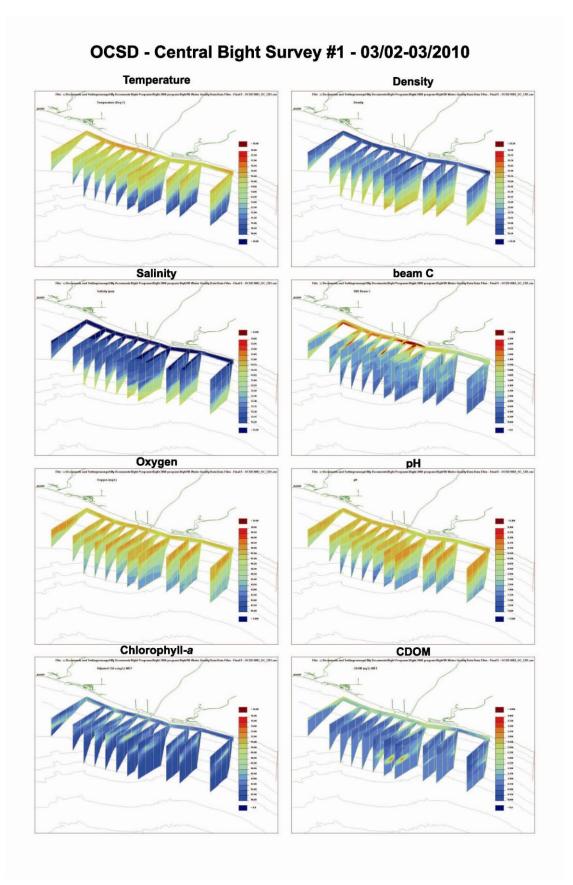


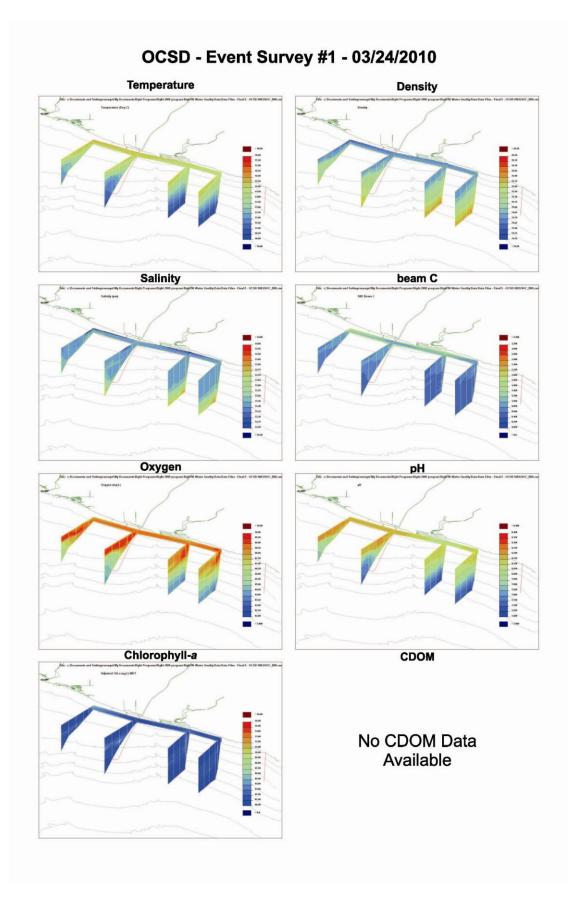


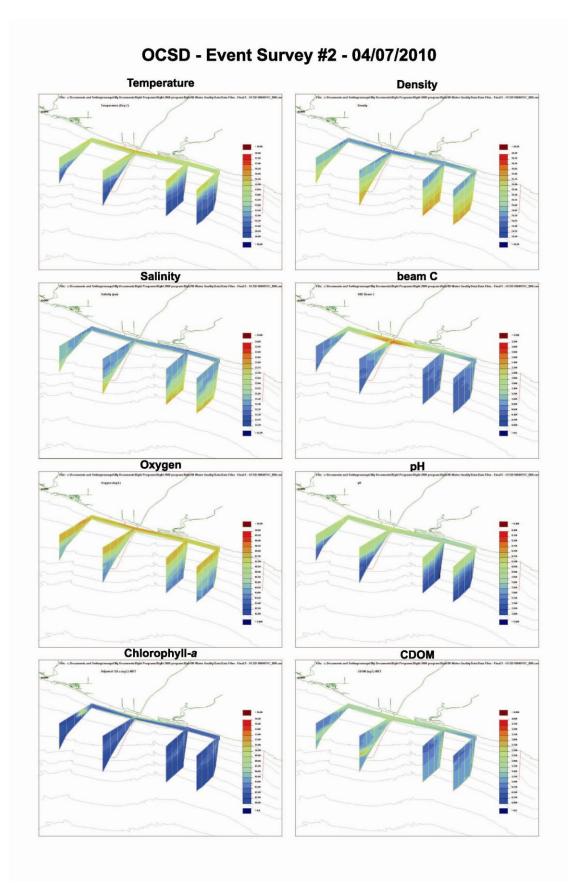


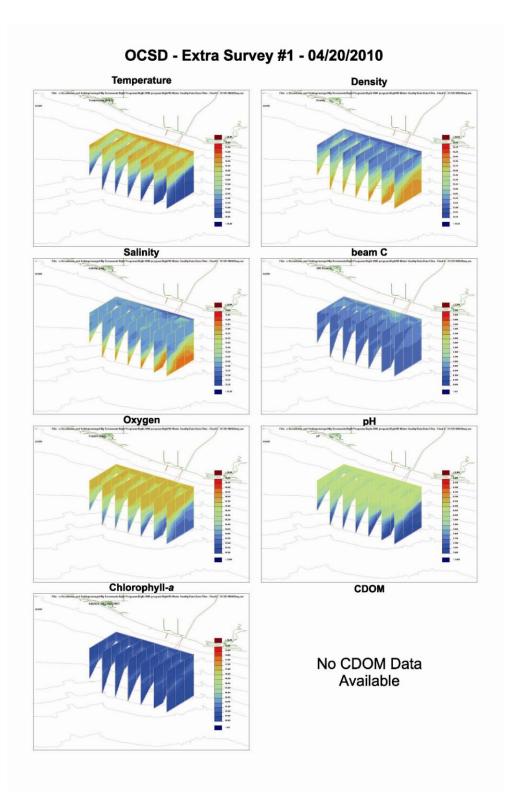
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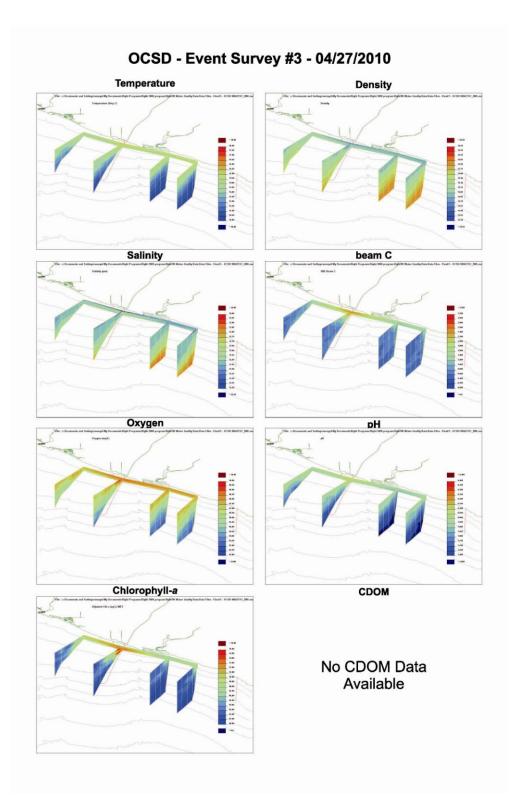
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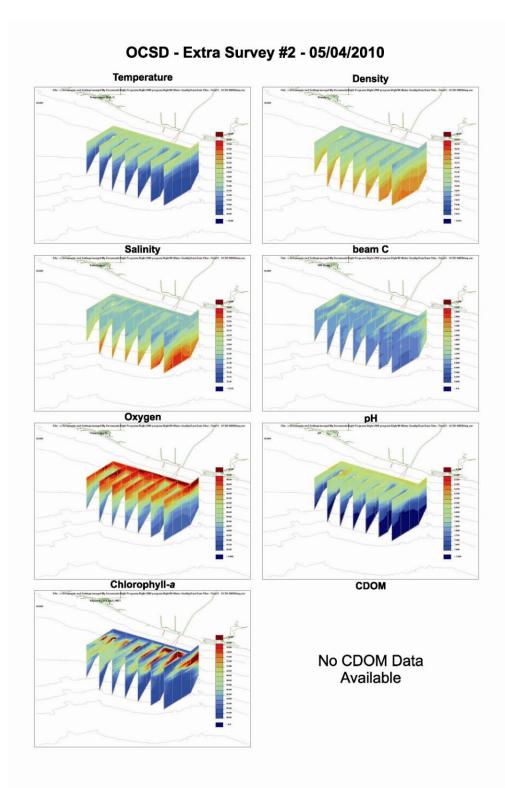


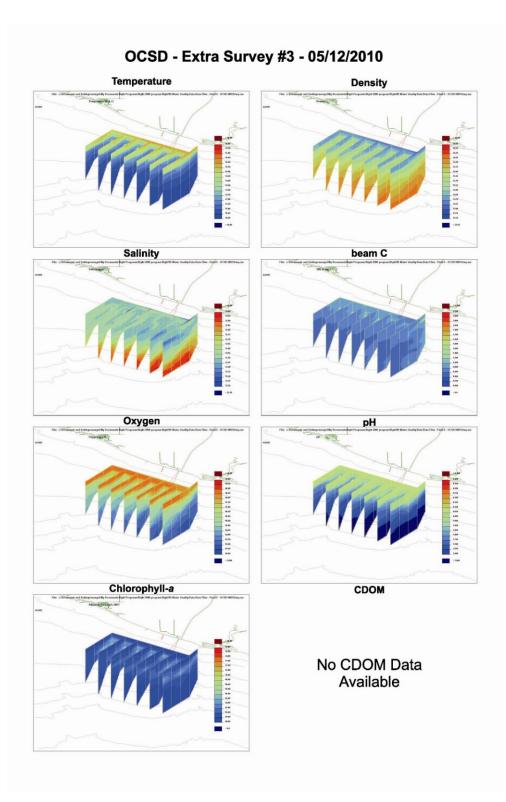


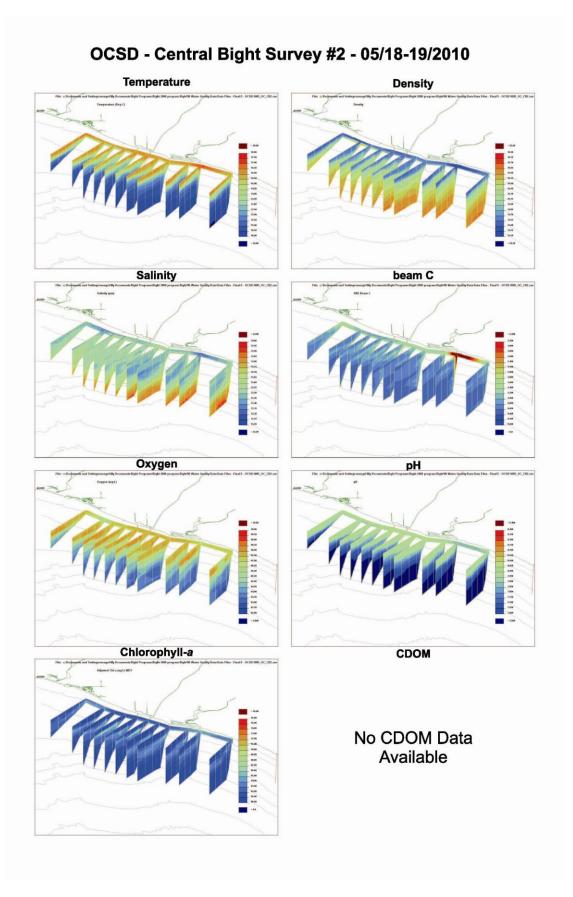






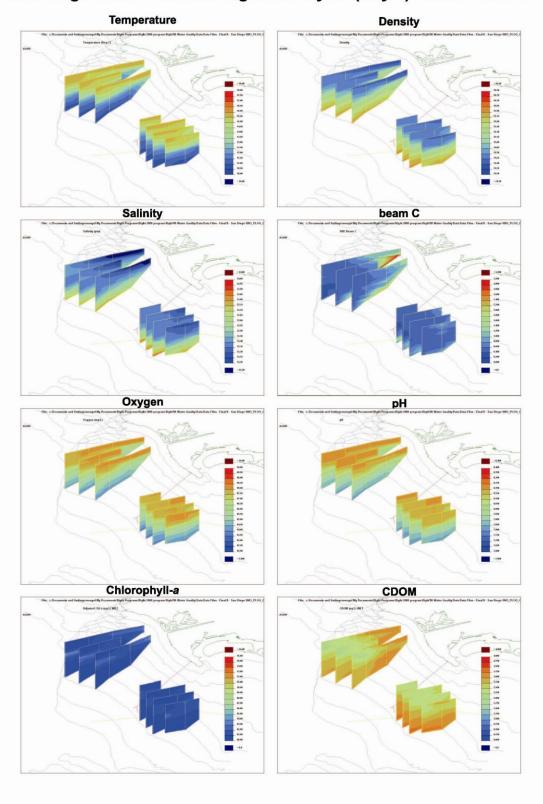




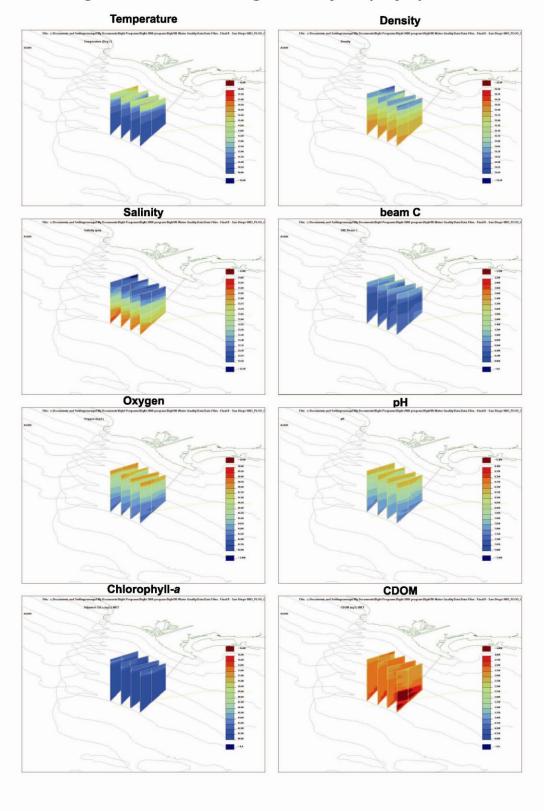


San Diego Ship Survey Data

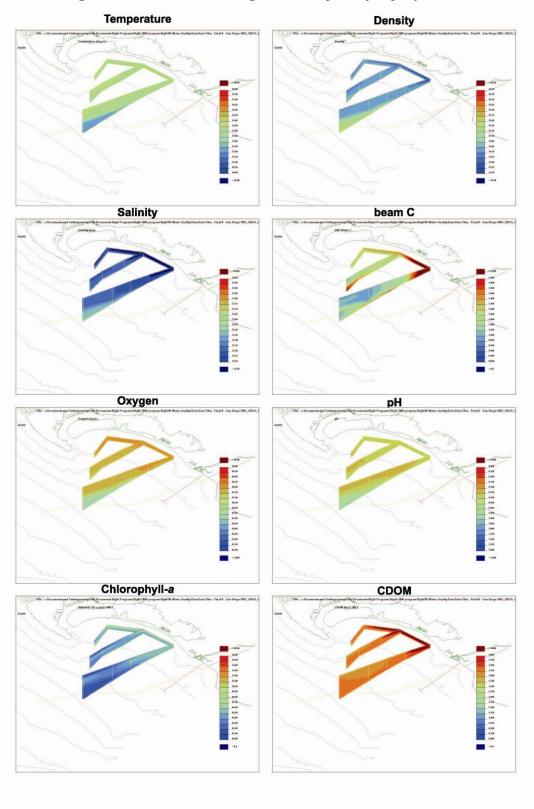
The City of San Diego ship survey CTD data summarized for each survey.



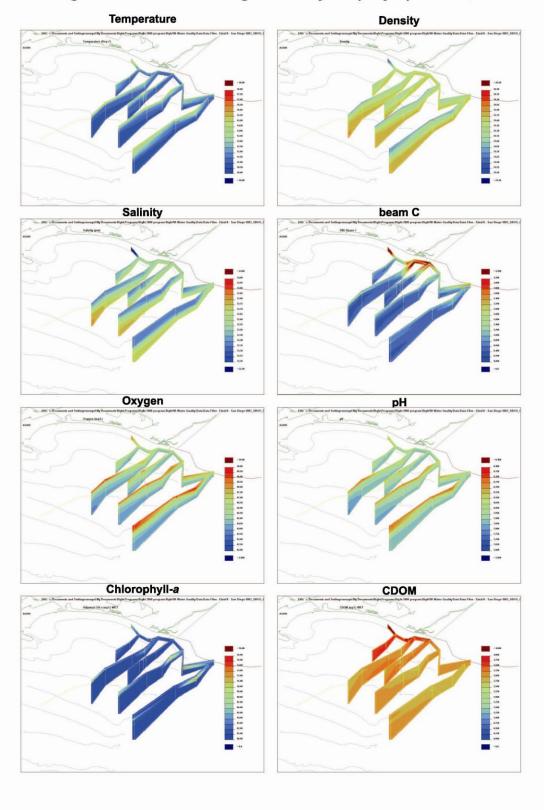
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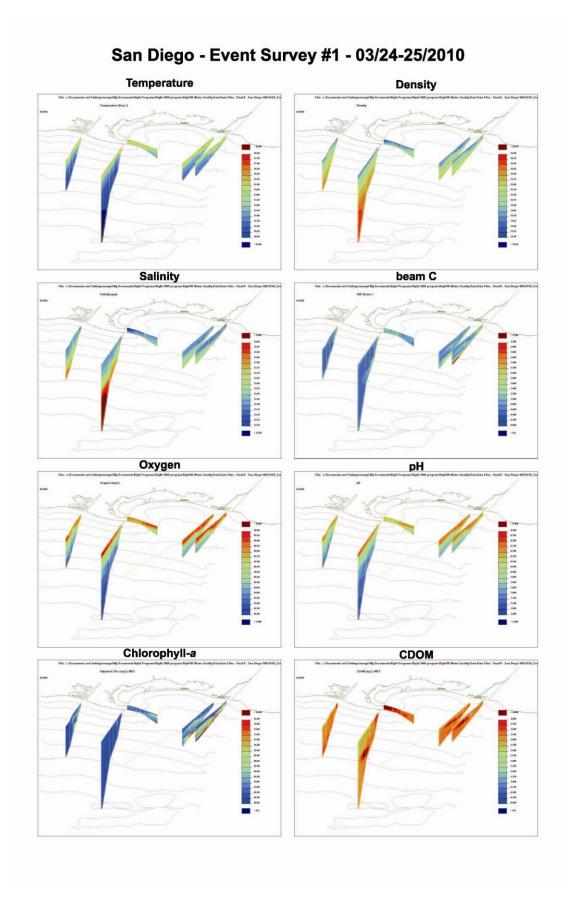
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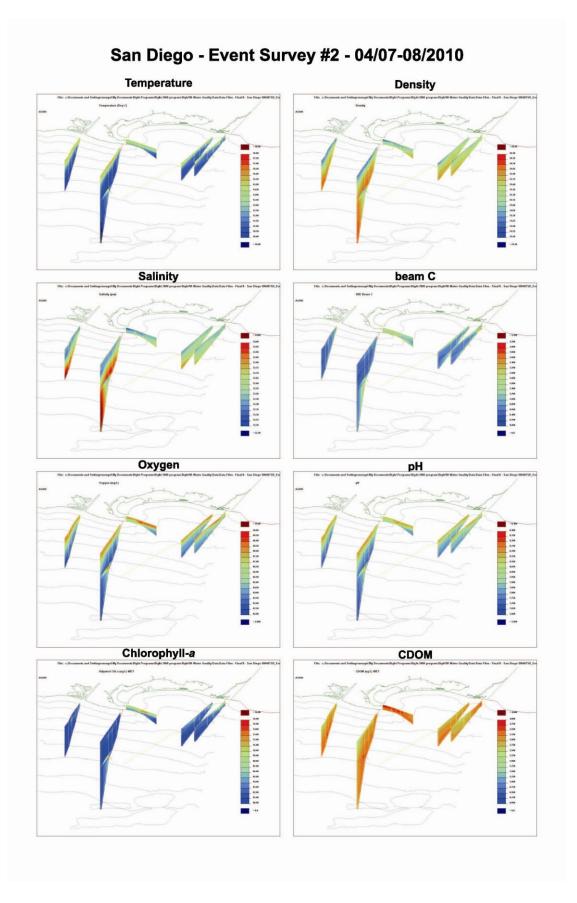


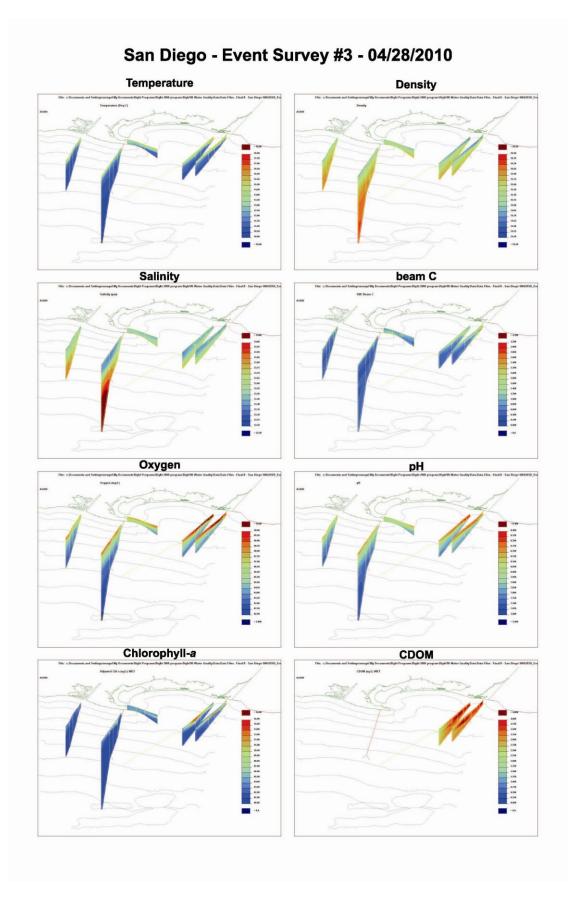
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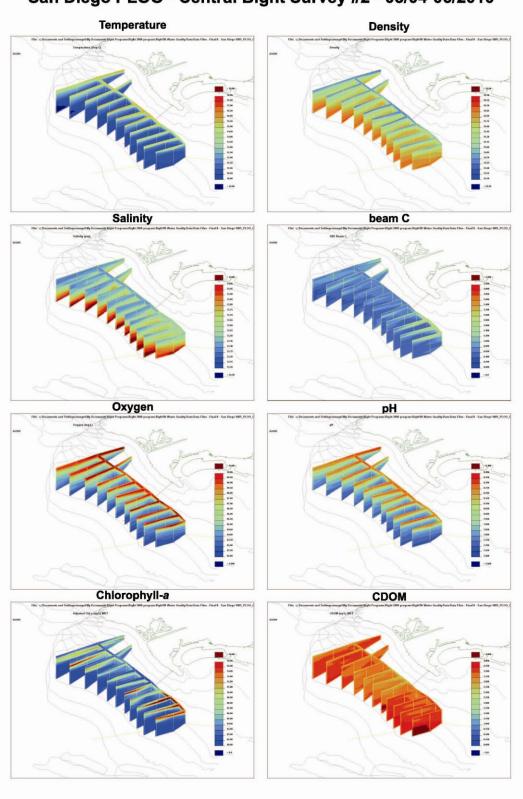


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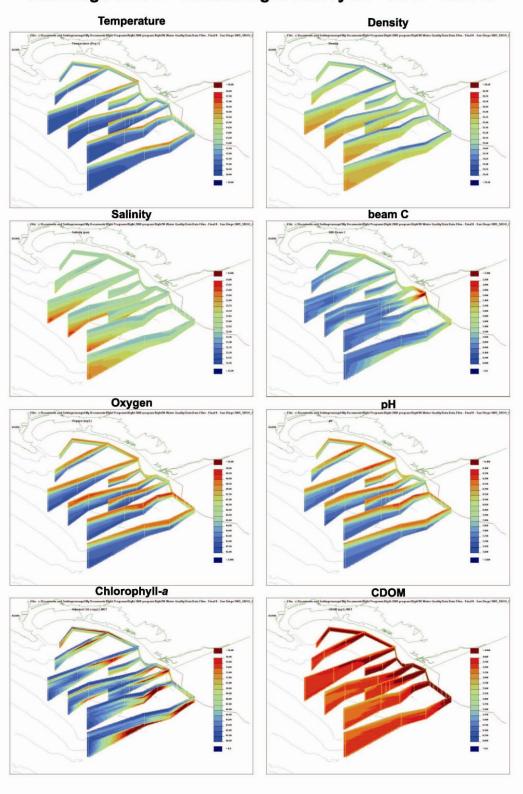








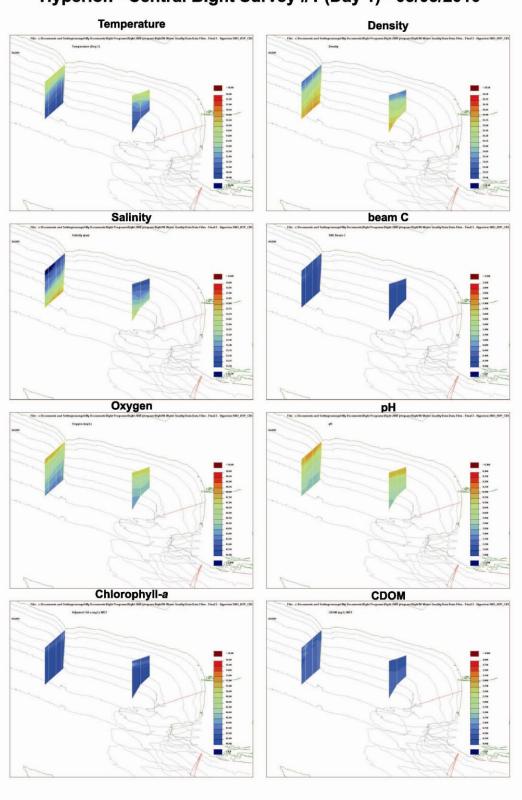
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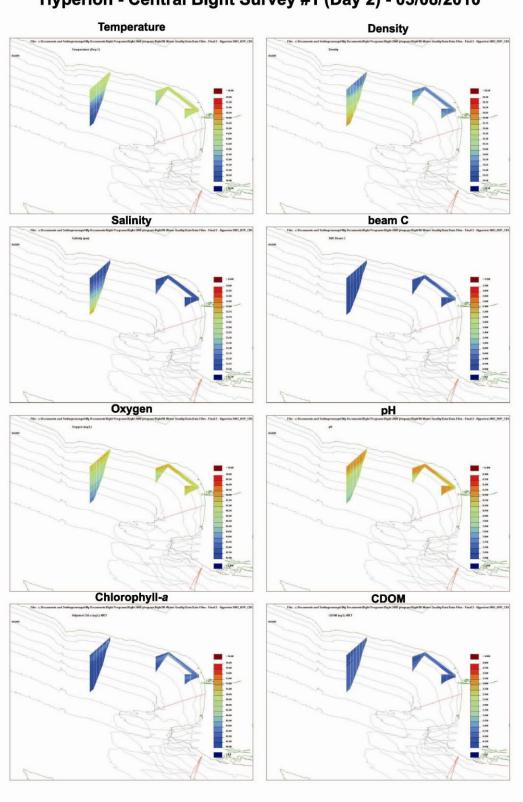
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Santa Monica Bay Ship Survey Data

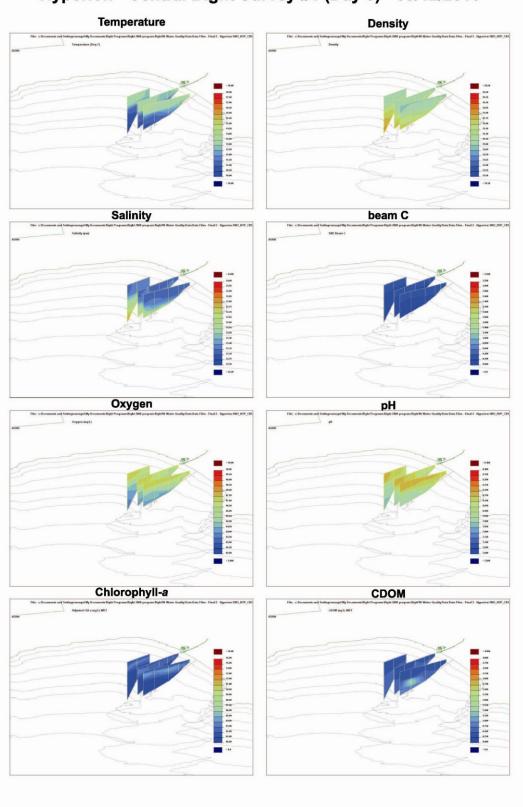
The City of Los Angeles ship survey CTD data summarized for each survey.



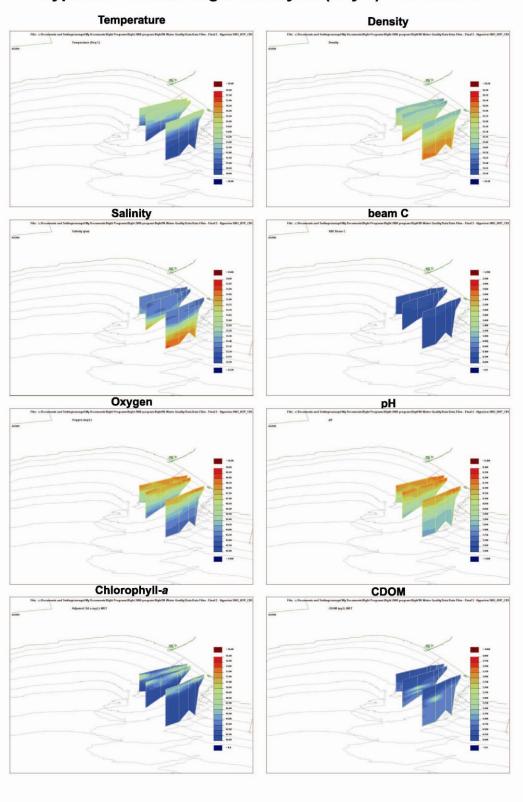
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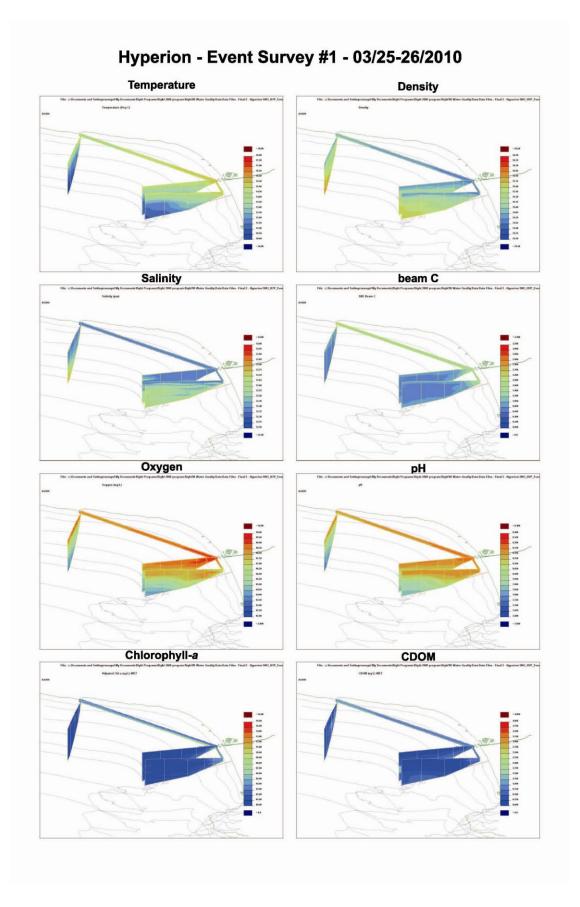
Hyperion - Central Bight Survey #1 (Day 2) - 03/08/2010

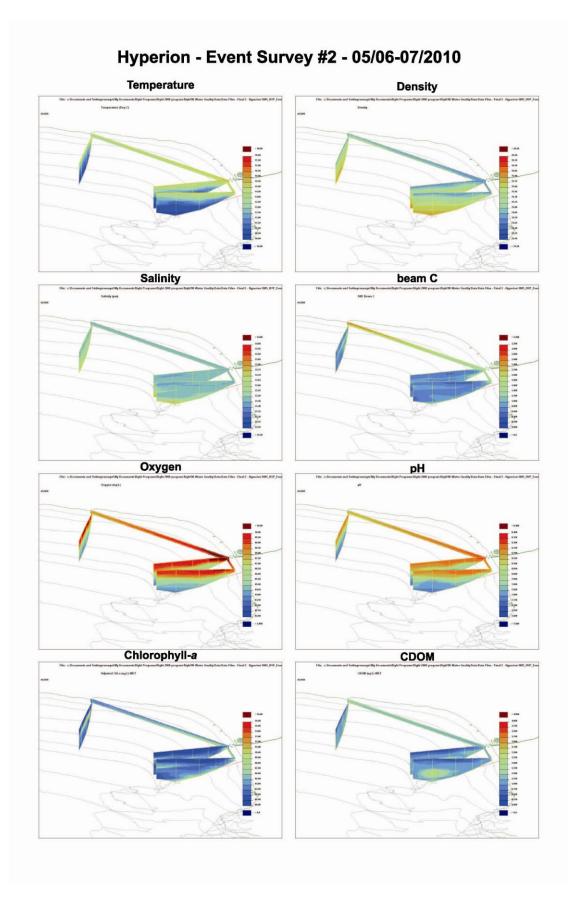


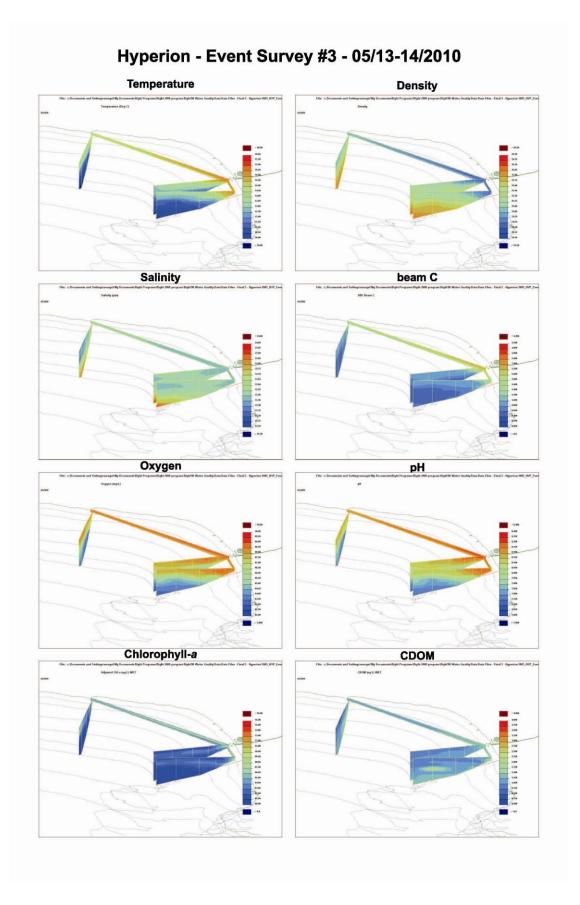
Hyperion - Central Bight Survey #1 (Day 3) - 03/12/2010

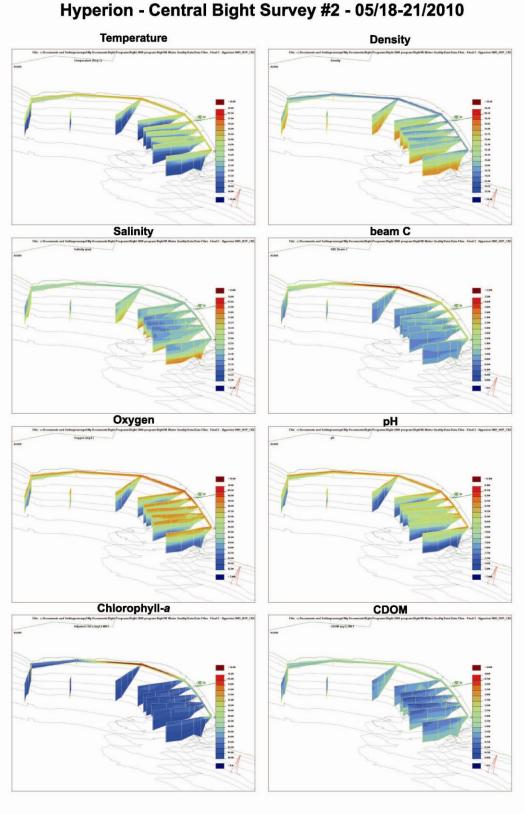


Hyperion - Central Bight Survey #1 (Day 4) - 03/15/2010





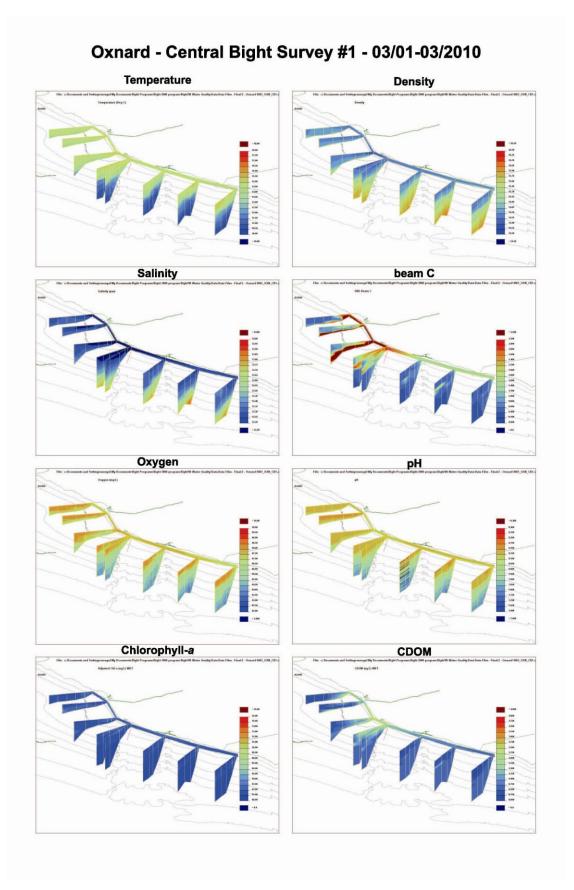


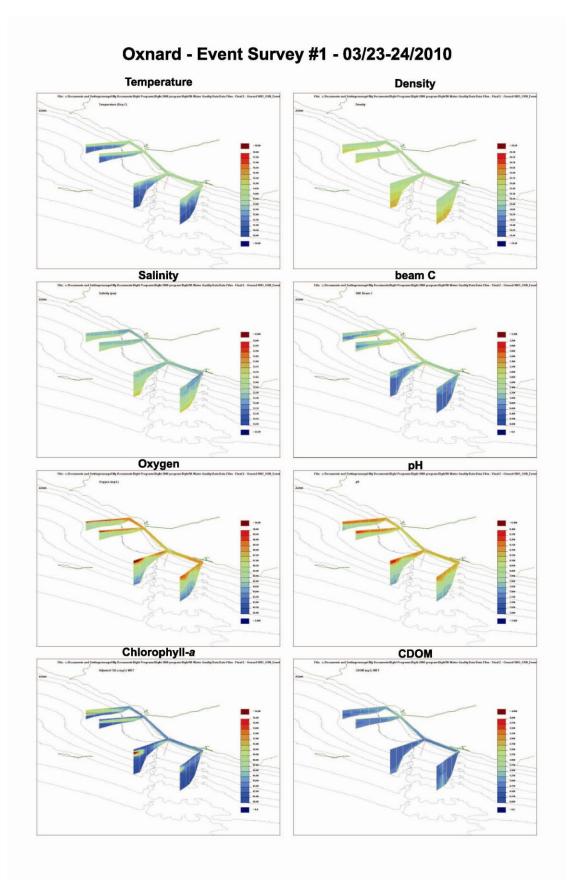


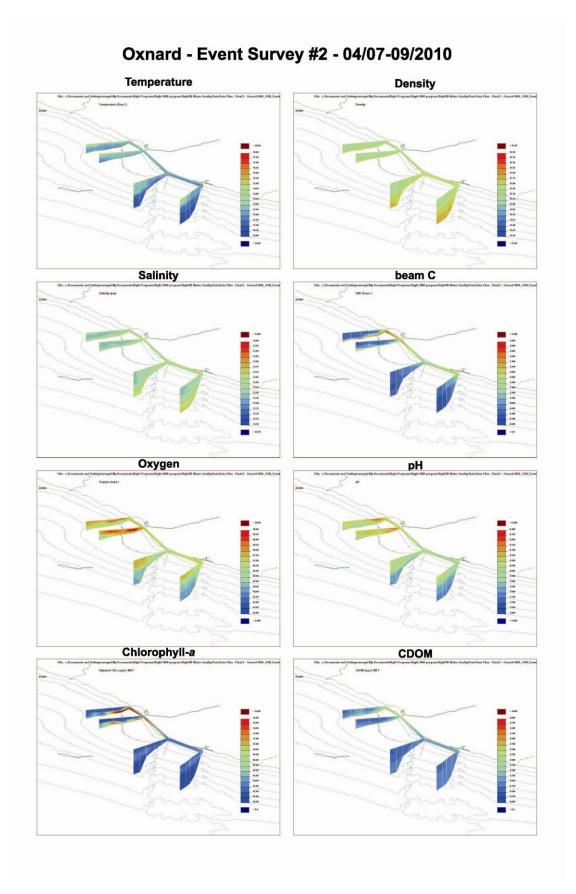
Hyperion - Central Bight Survey #2 - 05/18-21/2010

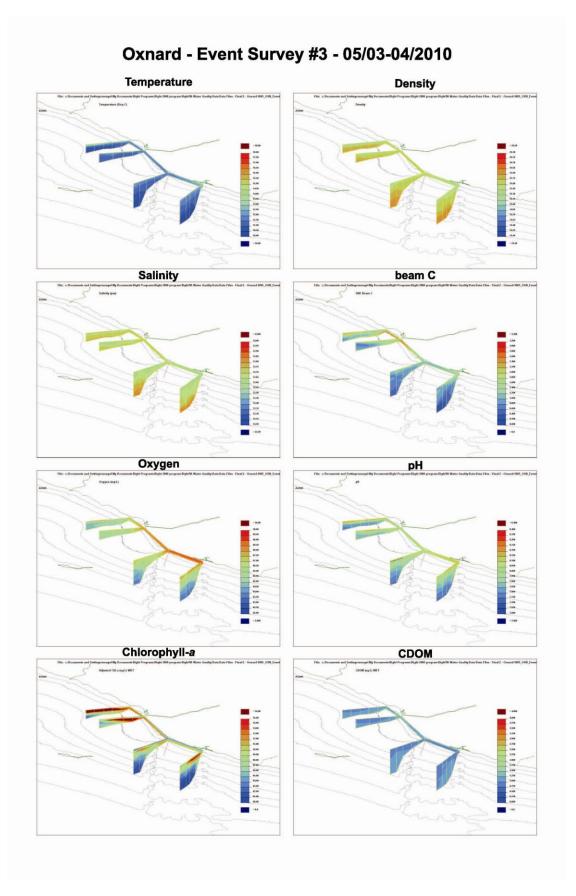
Ventura Ship Survey Data

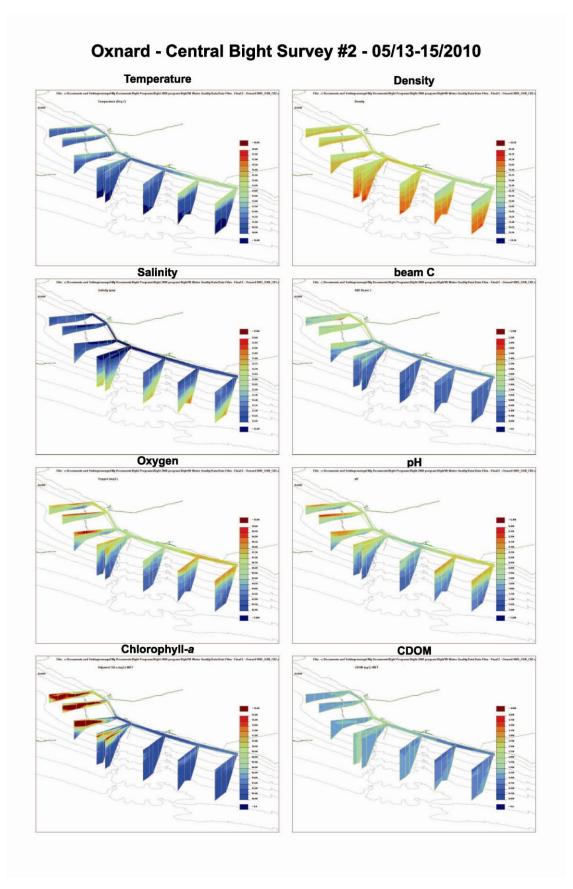
The City of Oxnard ship survey CTD data summarized for each survey.











North San Diego Ship Survey Data

The ship survey CTD data summarized for the one survey conducted by Weston Solutions.

