PERFORMANCE VERIFICATION STATEMENT for the Chelsea UviLux Hydrocarbon and CDOM Fluorometers

TECHNOLOGY TYPE:	Hydrocarbon and CDOM sensors
APPLICATION:	In situ estimates of hydrocarbons (oil-in-water) for coastal moored and profiled deployments
PARAMETERS EVALUATED:	Response range, accuracy, precision and reliability
TYPE OF EVALUATION:	Laboratory and Field Performance Verification
DATE OF EVALUATION:	Testing conducted from May 2011 through January 2012
EVALUATION PERSONNEL:	T. Johengen, G.J. Smith, H. Purcell, S. Loranger, S. Gilbert, T. Maurer, K. Gundersen, C. Robertson, and M. Tamburri

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EXECUTIVE SUMMARY

Instrument performance verification is necessary so that effective existing technologies can be recognized, and so that promising new technologies can become available to support coastal science, resource management, and ocean observing systems. The Alliance for Coastal Technologies (ACT) has therefore completed an evaluation of commercially available in situ hydrocarbon sensors. This verification included test applications for: (1) controlled laboratory tanks with additions of various organic, fluorescent compounds, (2) experimental wave tank with additions of two sources of crude oils with and without dispersants, (3) a moored deployment in Baltimore Harbor, and (4) hydrocast surveys in the Gulf of Mexico near a leaking oil barge.

In this Verification Statement, we present the performance results of the Chelsea Technologies Group (CTG) UviLux Hydrocarbon (UV-HC) and CDOM (UV-CDOM) fluorometers. Quality assurance (QA) oversight of the verification was provided by an ACT QA specialist, who conducted technical systems audits and a data quality audit of the test data. Response specificity of the two UviLux fluorometers to a range of organic compounds was evaluated in a series of lab tests. The CTG UV-CDOM and UV-HC instruments incorporate a linear response photodetector behind the emission optical filters and are configured to provide a 0-5V analog or RS232 digital output over its detection range and ambient signal overload protection above this range. Instrument response with respect to challenge compound concentration varied with respect to the inherent fluorescence properties of the challenge compound as well as sensor optics. The UV-CDOM version exhibited robust linear voltage response to concentration for both quinine sulfate (R^2=0.9998) and carbazole (R^2=0.9999) over a 0 – 1000 ppb and 0-100 ppb concentration range respectively. Diesel Fuel #2 was detected with a 7,750x lower sensitivity (R^2=0.8461). This sensor configuration was also generally insensitive to naphthalene disulfonic acid (NSDA, R^2=0.8672) and Basic Blue 3 (R^2=0.8137) except at challenge concentrations > 500 ppb. In contrast the UV-HC configuration exhibited 9-1000x higher sensitivities to carbazole (R^2=0.0.9997), NSDA (R^2=0.9975) and #2 Diesel Fuel (R^2=0.9865) relative to QS (R^2=0.9789) and sensor output was quenched in presence of BB3. Similar performance was observed in the Bedford Institute of Oceanography – COOGER wave tank test using exposures to Arabian Light and Alaskan North Slope crude oils in the presence of Corexit 9500 with the CTG UV-HC exhibit over 10x higher detection sensitivity than the UV-CDOM configuration. Instrument responses to various challenge compounds linearly scaled with standardized EEMs fluorescence intensity estimated to correspond to the instruments emission optics.

Field deployments in Baltimore Harbor and northern Gulf of Mexico were equivocal as all field reference samples were at or below the limit of detection for total petroleum hydrocarbons (≤ 25 ppb), yet the UviLux-HC and -CDOM output was above the baseline response in deionized water. Instrument response was consistent with environmental background fluorescence as determined by EEMs analysis for both moored and hydrocast surveys, indicating that ambient fluorescence properties need to be accounted for to make quantitative hydrocarbon estimates from these sensors.

During this evaluation, no problems were encountered with the provided software, set-up functions, or data extraction at any of the test sites. Only storm induced damage to an ACT supplied communication cable resulted in premature termination of the UviLux-CDOM deployment at the Baltimore Harbor deployment. One hundred percent of the data was recovered from the instrument and no outlier values were observed for any of the laboratory tests, field deployment tests, or tank exposure tests. We encourage readers to review the entire document for a comprehensive understanding of instrument performance.

BACKGROUND AND OBJECTIVES

Instrument performance verification is necessary so that effective existing technologies can be recognized and so that promising new technologies can be made available to support coastal science, resource management and ocean observing systems. To this end, the NOAA-funded Alliance for Coastal Technologies (ACT) serves as an unbiased, third party testbed for evaluating sensors and sensor platforms for use in coastal environments. ACT also serves as a comprehensive data and information clearinghouse on coastal technologies and a forum for capacity building through workshops on specific technology topics (visit www.act-us.info).

As part of our service to the coastal community, ACT conducted a performance verification of commercially available, in situ hydrocarbon sensors through the evaluation of objective and quality assured data. The goal of ACT's evaluation program is to provide technology users with an independent and credible assessment of instrument performance in a variety of environments and applications. Therefore, the data and information on performance characteristics was focused on the types of information that users most need. ACT surveyed the broader community to define the data and operational parameters that are valuable in guiding instrument purchase and deployment decisions.

As oil remains one of the world's most important energy sources, permissible and unintended release of hydrocarbons into the environment becomes inevitable as oil is explored, extracted, refined, transported, and consumed. There are a number of challenges in assessing hydrocarbon concentrations in coastal aquatic systems that point to the value of sustained in situ observations. This ACT Technology Evaluation examines individual sensor performance both in the laboratory and across different field conditions in moored and vertically profiled applications.

The fundamental objectives of this Performance Verification are to: (1) highlight the potential capabilities of hydrocarbon sensors by demonstrating their utility in a range of coastal environments, (2) verify manufacturer claims on the performance characteristics of commercially available hydrocarbon sensors when tested in a controlled laboratory setting, and (3) verify performance characteristics of commercially available hydrocarbon sensors when applied in real world applications in a diverse range of coastal environments.

In response to the results of ACT's Customer Needs and Use Assessment Survey the performance verification focused on both moored and profiling applications. It was also clear from the user survey that range (i.e., detection limits), reliability, accuracy, and precision are the most important parameters guiding instrument selection decisions. Given that the majority of instruments submitted to the verification utilize fluorometry, and that in situ fluorometry is a relative measurement with no absolute "true value" reference, accuracy cannot be measured directly. As an alternative to the direct measurement of accuracy, this Performance Verification will determine response linearity, or stability of the response/calibration factor, to a defined reference.

INSTRUMENT TECHNOLOGY TESTED

The UviLux is a compact, sensitive, low cost, in-situ UV fluorometer with digital and 0-5V analog output designed for UV excited fluorescence-based real-time monitoring of the concentration of refined polyaromatic hydrocarbons (emission 365 +/- 25 nm), crude hydrocarbons, Coloured Dissolved Organic Matter (CDOM) (emission 455 +/- 27.5 nm), and Tryptophan (a surrogate marker for Biological Oxygen Demand and bacterial contamination in waste, recycled and natural water supplies). The UviLux can be deployed in a wide range of coastal, oceanographic and fresh water applications.

The UviLux has an acetal C resin housing (70mm diameter x 149mm), rated to 600 meters, suitable for deploying independently as well as from submersible vehicles, moored or profiling systems. It utilizes a novel UV LED light source (excitation 255 nm) and a miniature 8-stage photomultiplier coupled to a low noise preamp, which combined with phase sensitive detection, provides highly sensitive measurement capabilities. The sophisticated electronic signal processing, combined with cowl design enables the UviLux to operate successfully in high levels of ambient light. There is no requirement to pump sample through a dark measurement chamber, thus obviating the need for water flow corrections or the cost, inconvenience and power drain of a pump.

The UviLux can be supplied with either recessed or flush windows depending upon the required application and a flow through manifold is available for deck and laboratory use. Ease of use and installation is further enhanced by the provision of galvanic isolation between the power and signal 0V rails, thereby avoiding the possibility of ‘ground loops’. The highly efficient, proprietary, d.c. converter can also handle an unusually wide supply voltage range of 9V to 36V without significant change to the power consumption of 1.3W.

Two signals can be obtained from the standard UviLux: the first is a digital RS232 output in engineering units, the second a 0V to 5V volt calibrated analogue output derived from the digital signal. Single RS422 and SDI-12 outputs are also available as options. This flexibility makes the UviLux suitable for integrating into many different systems and platforms. The standard measuring range is 0 to 10 µg/l with a resolution of 0.02 µg/l. The UviLux-HC is supplied with a calibration against Carbazole and the UviLux-CDOM with a calibration against Perylene.

A Windows based Graphical User Interface (GUI) is provided that allows the user to both plot and record time stamped data when operating the UviLux directly from a PC and gives control over many instrument parameters, including: sampling rate, detector sensitivity, and calibration factors.

SUMMARY of VERIFICATION PROTOCOLS

The protocols used for this performance verification were developed in conference with ACT personnel, the participating instrument manufacturers and a technical advisory committee. The protocols were refined through direct discussions between all parties during a Hydrocarbon Sensor Performance Verification Protocol Workshop held on 2-4 February, 2011 in Moss Landing, CA. All ACT personnel involved in this Verification were trained on use of instruments by manufacturer representatives and on standardized water sampling, storage, analysis and shipping methods during a training workshop held on 18-20 May 2011 in Moss Landing, CA. The manufacturer representative and the ACT Chief Scientist verified that all staff were trained in both instrument and sample collection protocols.

This performance verification report presents instrument output voltage, relative florescence units or derived hydrocarbon values reported over time, position, or depth as directly downloaded from the test instruments or captured through independent dataloggers. The report includes means, standard deviations, and number of replicates of laboratory determined Diesel Range Organics, Volatile Hydrocarbons, EEMS, Absorbance, CDOM and Chlorophyll values for corresponding reference samples at the same time, position, or depth of the instrument measurements. The report also includes turbidity values for each sample measured on site using a Hach Turbidimeter which was used for all laboratory and field tests. Instruments were tested under four different applications, including: (1) laboratory tests with known additions of variance hydrocarbons; (2) a wave tank test with known additions of crude oil with and without dispersant; (3) a moored deployment in Baltimore Harbor; and (4) vertical profiling deployments in the Gulf of Mexico at a site with known leaking bunker oil. A summary of the testing protocols is provided below. A complete description of the testing protocols is available in the report, *Protocols for the ACT Verification of In Situ Hydrocarbon Sensors* (ACT PV11-01) and can be downloaded from the ACT website (www.act-us.info/evaluations.php).

Analysis of Reference Samples

Hydrocarbon concentrations

Diesel range hydrocarbons (C10 to C36) and volatile organic hydrocarbons were analyzed by using GC-FID by the contract laboratory, Test America (West Sacramento Lab), following their internal SOP's based on EPA SW846 Method 8015B, C. The Laboratory provides reporting limits of 50 ppb for this hydrocarbon range. Reference samples were collected in certified pre-cleaned amber glass bottles supplied by Test America. Bottles were filled, stored and shipped according to their SOP's. Reference samples, along with sampling blanks, were shipped to the contract lab not more than three days after collection to meet their holding time requirements.

Excitation Emission Matrix Spectroscopy (EEMS)

A SPEX ISA Fluoromax-2 scanning spectrofluorometer, operated in ratio mode, was used to generate room temperature (22 ± 1°C) EEM fluorescence spectra for all reference samples. To optimize sample throughput, fluorescence spectra were determined over an excitation range of 230-500 nm at 5 nm intervals and an emission range of 300 – 600 nm at 3 nm intervals. For each scan, an integration time of 1 second was used, and bandpass widths were set to 5 nm for both excitation and emission spectrometers. Xenon lamp intensity as well as emission monochrometer performance were verified and recalibrated once per day according to the instrument manual.

For all generated EEM's, dark counts were subtracted and spectra were subsequently corrected for wavelength-dependent instrument effects using ISA-supplied and user-generated correction files. Fluorescence spectra intensities were then normalized to the area under the Raman peak, determined daily using MilliQ water (Murphy, 2011; Murphy et al. 2010). This value exhibited less than 2% variation over the length of the study period. In addition to daily Raman scans, daily EEM's of MilliQ water were generated as background blanks and were subtracted from all subsequent sample EEM's. At the beginning and end of each analytical batch a four-point calibration curve (0-50 ppb) of Quinine Sulfate (QS) in 50 mM H_2SO_4 was run to track drift in fluorometer response over time. The QS response factor was used to standardize emission intensities across each analytical batch (Coble et al. 1993). Finally, all sample EEM's were corrected for Raman and Rayleigh scattering peaks, following Zepp et al. 2004.

Excitation and emission windows for each instrument (based on the reported FWHM for the filter sets as provided by manufacturers) were mapped onto each reference sample EEM space and corresponding integrated quinine sulfate normalized fluorescence intensities obtained for direct comparison to instrument output under the various challenge concentrations.

Colored Dissolved Organic Matter (CDOM)

Approximately 50 ml of the CDOM designated subsample were filtered using 47 mm GF/F filters with low vacuum pressure and poured into an acid-cleaned, combusted, 60 ml amber glass bottle. All samples were stored in the dark at 4° C until analysis, within approximately one month of collection. A dual-beam spectrophotometer was blanked with MilliQ water in cuvettes in both the sample and reference positions. Matched 10 cm quartz or optical glass cells were used for a dual-beam spectrophotometer. MilliQ samples were run intermittently during each analytical batch to assess instrument baseline drift. Scans were run between 200 and 800 nm and electronic files were saved for each sample.

MilliQ blank and turbidity (750 nm) corrected spectra were used to estimate CDOM abundance by non-linear regression of the absorption spectra over 400 – 575 nm.

$$a[\lambda] = a[400]e^{(-S\lambda)} \qquad (1)$$

Where $a[\lambda]$ is absorption (m^{-1}) at wavelength λ, $a[400]$ is absorption (m^{-1}) at the anchor wavelength of 400 nm, and S is the spectral slope (nm^{-1}). Note that wavelength must be expressed as $\lambda - 400$ before fitting for the anchor value to be at 400 nm. *A[400]* is used as a proxy for CDOM abundance in reference samples.

Chlorophyll a

Chlorophyll grab samples were analyzed on a Turner Designs 10AU fluorometer from samples filtered on 2.5 cm GF/F filters and frozen at -20 ^{o}C until analyzed according to Parsons, et al. 1984. Optimum filtration volumes were determined on site. All chlorophyll analyses were performed by the Chesapeake Biological Laboratory according to their existing SOPs. The laboratory is a State of Maryland certified lab and has undergone previous audits by ACT during prior evaluations. Samples were shipped to CBL in liquid nitrogen dry shippers to ensure they remained frozen at the required temperature.

Turbidity

Turbidity concentrations of reference grab samples were determined by a Hach 1100AN benchtop turbidity sensor in NTU. The lab analyzer was calibrated with certified standards prior to use and a QA check of the standards were run during each analytical batch. Samples were run immediately upon collection. The same instrument was used at each test site.

Laboratory Tests

Performance against surrogate standards and challenge environmental variables

Laboratory tests of response factor, precision, range, and reliability were conducted at Moss Landing Marine Lab. Challenge compounds utilized in laboratory characterizations of instrument performance are listed in Table 1 and cover the range of optical detection windows utilized by participating hydrocarbon sensors. Laboratory challenges were performed in insulated 500 L, black acrylic tanks in a dark room using filtered deionized water (DI) as the background medium. Test tanks have been preconditioned by several years of use with deionized and seawater exposures and cleanings. Temperature was maintained at 15 ± 1 ^{o}C with Nestlab recirculating chillers and copper heat exchange tubing. Water was continuously circulated with submersible pumps (*ca* 10 L/min) placed at opposite ends of the tank. Temperature was monitored at opposite ends of the tank at sensor detector level by two calibrated RBR 1060 recording thermometers. Each test level began with a 30 minute equilibration, and reference water samples were collected at 10 minute intervals over the following 30 minute exposure. Instrument response, reported as the average of 5 minutes of readings encompassing reference sample times, was used to characterize instrument response at each challenge level. Instrument response factors are calculated by regression of mean instrument output against challenge compound concentrations. Precision tests were conducted by monitoring the variance of instrument response over the 5 minute periods. Originally proposed turbidity and CDOM interference tests, and temperature response factors, were not conducted.

Table 1. Challenge compounds for laboratory evaluations of hydrocarbon sensors.

Compound	***Ex***	***Em***	***Carrier***	***Stock (ppm)***
Carbazole	270	342, 358	methanol	5000
1,5-Naphthalene Disulfonic Acid	270	380	0.05 M H_2SO_4	5000
Quinine Sulfate	350	450	0.05 M H_2SO_4	5000
Basic Blue 3	250, 320	430	water	5000
Diesel Fuel SPEX CRM	250-300	350-500	methanol	5000

Performance against crude oils and dispersants in a Wave Tank Test

A test application of instrument response against crude oil compounds, with and without addition of dispersant, was performed in a simulated water column using the DFO/US EPA Wave Tank Facility located at the Bedford Institute of Oceanography, Dartmouth, Nova Scotia, Canada. Reference samples were collected from the tank to allow real-time characterization of the sample water with three-dimensional Excitation/Emission spectroscopy (EEMS).

Instrument performance was examined against two types of crude oil, Arabian Light (weathered 7%) and Alaskan North Slope, run under two conditions including the pure oil compounds and with compounds at oil to dispersant ratio of 25:1. The test materials were created experimentally at the Bedford Institute of Oceanography (Department of Fisheries and Oceans, Halifax, Nova Scotia) using the two source oils and Corexit 9500 dispersant. Experiments were performed in a step-up addition batch mode with breaking waves to physically disperse the oil throughout the tank. Oil additions were cumulative to the same batch of water and were made at six timepoints at approximately one hour intervals. The amount of oil added ranged between 0 to 360 grams and produced oil concentrations of 0, 0.3, 0.6, 1.5, 3.0, 6.0, and 12.0 ppm, respectively, at each consecutive step. Reference sampling occurred 50 minutes after each new oil addition. Continuous instrument records were sub-sampled to the last 10 minutes of equilibrated conditions of the exposure period and corresponding reference sampling times.

A Seabird SBE26+ CTD, SeaPoint chlorophyll fluorometer and LISST particle analyzer were deployed to provide ancillary time-series data on water quality and to confirm degree of physical dispersion of added oil. Reference samples were collected from three sampling inlet ports distributed across the width of the tank and located adjacent to the sensor window. An aggregate sample was produced for the reference sample analyses, except for hydrocarbon subsamples which were taken and analyzed independently. Hydrocarbon analysis was conducted on-site by certified COOGER BIO facility personnel using either gas chromatography with a flame graphite detector (GCFID) or gas chromatography with a mass spectrometer (GCMS) depending on concentration levels.

Field Tests

Moored Deployment

A moored application test was conducted at the Maritime Environmental Resource Center barge facility located within Winans Cove, Baltimore Harbor, MD (39.2614N, 76.6008W). The moored test was planned for a duration of four continuous weeks; however, the test was cut short after 18 days due to the passage of Hurricane Irene. In addition, the deployment was interrupted after day two due to a breakage in the mooring structure. The mooring was re-established on August 18^{th} and operated for 9 days prior to retrieval.

Instrument Setup – The test instruments were programmed to record data at the highest frequency that the instruments' battery would maintain over the deployment period. The internal clock was set to local time and synchronized against the time standard provided by www.time.gov. A photograph of each individual instrument and the entire instrument rack was taken just prior to deployment and just after recovery to provide a qualitative estimate of biofouling during the field tests. Prior to deployment, the test instrument was exposed to freshly prepared reference solutions (QS and NDSA) made up in DI water both before and after deployment as an estimate of instrument reliability. The post-deployment reading was taken after the instruments were cleaned according to manufacturer specifications.

Reference samples were collected on three days of each week at four separate times spaced at one-hour intervals. Reference field samples were collected within 1 m from the sensor window. The water samplers were soaked at sampling depth for 1 minute prior to sampling. All sampling times were recorded on logsheets and entered into a database for final data comparisons. Two standard 4L Van Dorn trace metal compatible water samplers were used to collect duplicate water samples for reference measurements. The standard reference sample suite was processed, stored, and shipped as described above. Once per week, Type I lab water was loaded into the clean Van Dorn sampler, taken to the sampling locale and a corresponding set of field blank sample bottles filled to provide monitoring for potential environmental contamination.

Vertical Profiling

The vertical profiling application was conducted at two test sites in the northern Gulf of Mexico onboard the R/V *Acadiana* (Louisiana Universities Marine Consortium). One profile was conducted just outside Terrebonne Bay (29.0465N, 90.5568W) to provide a contrast of high CDOM coastal waters. Five profiles were conducted at a second site that was located near a known shipwreck leaking oil (28.5675N, 90.9813W). To avoid contamination between casts, the CTD rosette was cleaned with a dilute, non-fluorescent, surfactant solution between profiles. For each profile, reference samples were collected during the upcast at five discrete depths spaced throughout the water column. On each cast, one of the five discrete depths was sampled in replicate with two independent water collection bottles. At each of the selected depths, the rosette was paused for 1 minute to ensure that the test instrument had stabilized prior to water sampling. The rosette and test instrument assembly were lowered and raised at a standard rate of approximately 0.25 m/sec. All test instrument and reference sample data are shown for the upcast only to match up sampling times. Temperature and salinity profiles are taken from the undisturbed, continuous downcast. If the test instrument was not internally logging, it was connected to a common WET Labs DH4 datalogger powered with an external battery package. The reference water sample data were matched up with the hydrocarbon sensor data by averaging the instrument readings for 10 seconds before and after the specific time the water bottle was fired.

Ancillary In Situ Environmental Data

In-situ measurements were generated every 15 minutes over the duration of the moored field tests. A calibrated YSI sonde and three RBR 1060 temperature loggers were attached to the mooring. In conjunction with each water sample collection, technicians recorded basic site-specific conditions on standardized log sheets including: date and time, weather conditions (e.g., haze, % cloud cover, rain, windspeed/direction), recent large weather events or other potential natural or anthropogenic disturbances, tidal state and distance from bottom of sensor rack, and any obvious problems or failures with instruments.

Ancillary data is presented to provide a general history of weather patterns and changes in ambient water quality conditions. These data were not used for any direct calibration, correction, or statistical comparison to the reported test data.

Quality Assurance/Quality Control

This Performance Verification was implemented according to the QA test plans and technical documents prepared during planning workshops and approved by the manufacturer and the ACT hydrocarbon sensor advisory committee. Technical procedures included methods to assure proper handling and use of test instruments, laboratory analysis, reference sample collections, and data. Performance evaluation, technical system, and data quality audits were performed by QA personnel independent of direct responsibility for the verification test. All implementation activities were documented and are traceable to the Test/QA plan and to test personnel.

The main component to the QA plan included technical systems audits (TSA) conducted by an ACT Quality Assurance Manager of the laboratory tests at MLML and of the field test in Baltimore Harbor to ensure that the verification tests were performed in accordance with the test protocols and the ACT *Quality Assurance Guidelines*. All analytical measurements were performed using materials and/or processes that are traceable to a Standard Reference Material. Standard Operating Procedures were utilized to trace all quantitative and qualitative determinations to certified reference materials. Lastly, ACT's QA Manager audited approximately 10% of the verification data acquired in the verification test to assure that the reported data and data reduction procedures accurately represented the data generated during the test.

RESULTS of LABORATORY TEST

Laboratory tests of response factor, precision, range, and reliability were conducted at Moss Landing Marine Lab utilizing five different challenge compounds covering a range of fluorescent properties (see Table 1 above) to facilitate comparisons against the range of optical detection windows utilized by participating hydrocarbon sensors. Tests were performed in insulated 500 L, black acrylic tanks in a dark room using filtered deionized water (DI) as the background medium (Photo 1). Reference samples of these challenge compounds were characterized and quantified using EEMS on a FluorMax-2 (photo 2) over a range of concentrations from 0 – 5000 ppb (nominally 0, 1, 5, 10, 50, 100, 500, 1000, 5000 ppb).

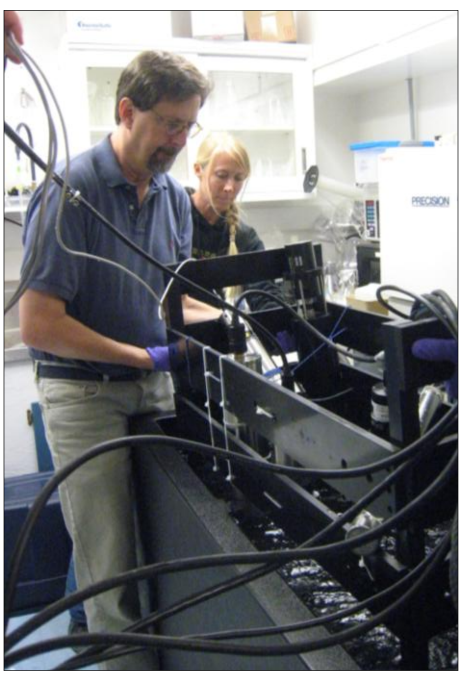


Photo 1. Instrument Rack and tank.

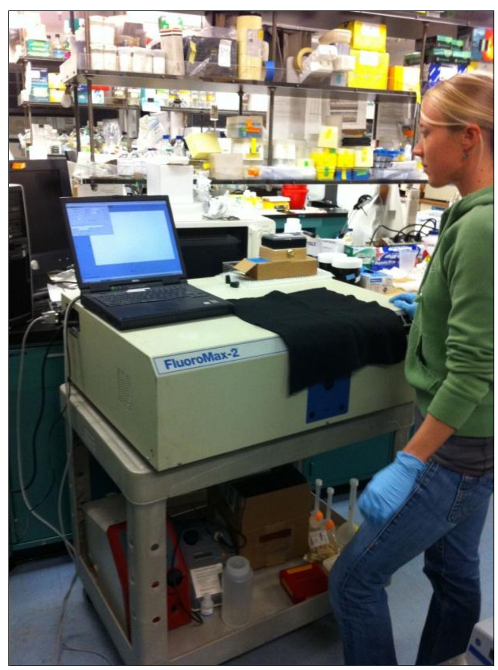
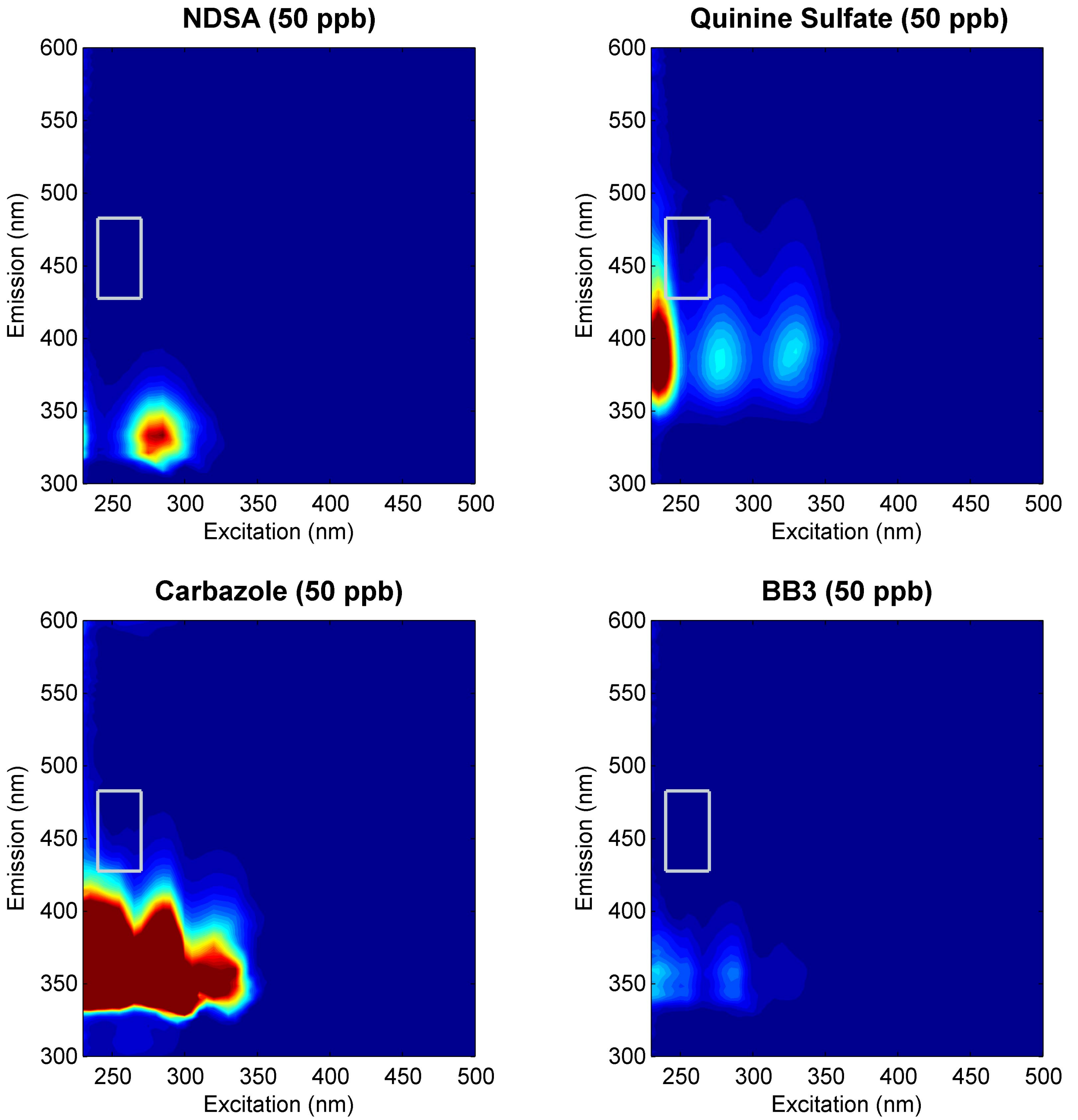
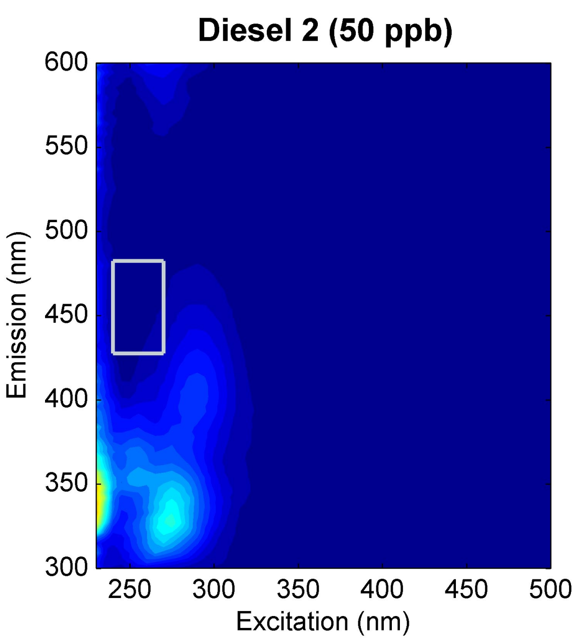


Photo 2. EEM's Generation

EEM fluorescence maps of each of the five challenge compounds, dosed at a concentration of 50 ppb, are presented along with the region of the optical window of the UviLux-CDOM (Figure 1) and UviLux-HC (Figure 2) filter sets. Excitation and Emission maximums of the challenge compounds varied by over 100 nm, with Quinine Sulfate mapping most closely with the optical window UviLux-CDOM filter set and Carbazole mapping most closely with the optical window of the UviLux-HC filter set. Response curves for both fluorometers were generated against each of the challenge compounds over concentrations ranging from 1 – 1000 ppb (see Fig. 3 for the UviLux-CDOM and Fig. 4 for the UviLux-HC). Results show instrument response (in mV) presented against both concentration and estimated EEM_{QSE} (Quinine Sulfate equivalent) fluorescence intensity for each challenge compound. Baseline signal in deionized water was approximately 375 mV for the UviLux-CDOM and HC units. As expected by factory design, the voltage output response to increasing concentration (panel A) was linear up to detector saturation where ambient overload rejection circuitry produced an apparent quenching in maximum signal output (see Fig 4, responses to Carbazole and NSDA). The instrument response to increasing concentration was significantly less for those compounds with fluorescence maxima outside the optical window wavelength centers of the instruments filter set and hence lower predicted EMM_{QSE} intensities (panel B).

Ancillary water quality conditions for turbidity and CDOM are presented for each challenge compound at each of the concentrations tested. In general the challenge compounds, except for BB3, had little effect on turbidity levels and the instrument response reflected the fluorescence properties of the challenge compound. BB3 additions increased measured turbidity, but since neither of the UviLux fluorometers were responsive to this particular challenge compound no obvious quenching effects were evident at the higher challenge concentration levels. Spectroscopically derived CDOM levels were compound specific (Fig. 5, panel B) and reached asymptotes above 10 ppb additions, well below the instruments' response capacity (>100 ppb for QS and #2 diesel fuel oil).





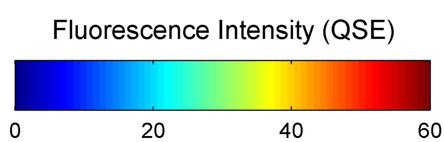


Figure 1. Excitation-Emission Matrix (EEM) fluorescence maps of challenge compounds used in the laboratory trials at Moss Landing Marine Laboratories. Reported fluorescence intensities (cps) are normalized to Quinine Sulfate Equivalents (QSE). Boxes denote optical window for the CTG UviLux CDOM (2141-008-PL-C) based on full width half maximum (FWHM) ranges described for the instrument filter set. This region is used to generate integrated fluorescence intensities (EEM_{QSE}) observed by the corresponding instrument.

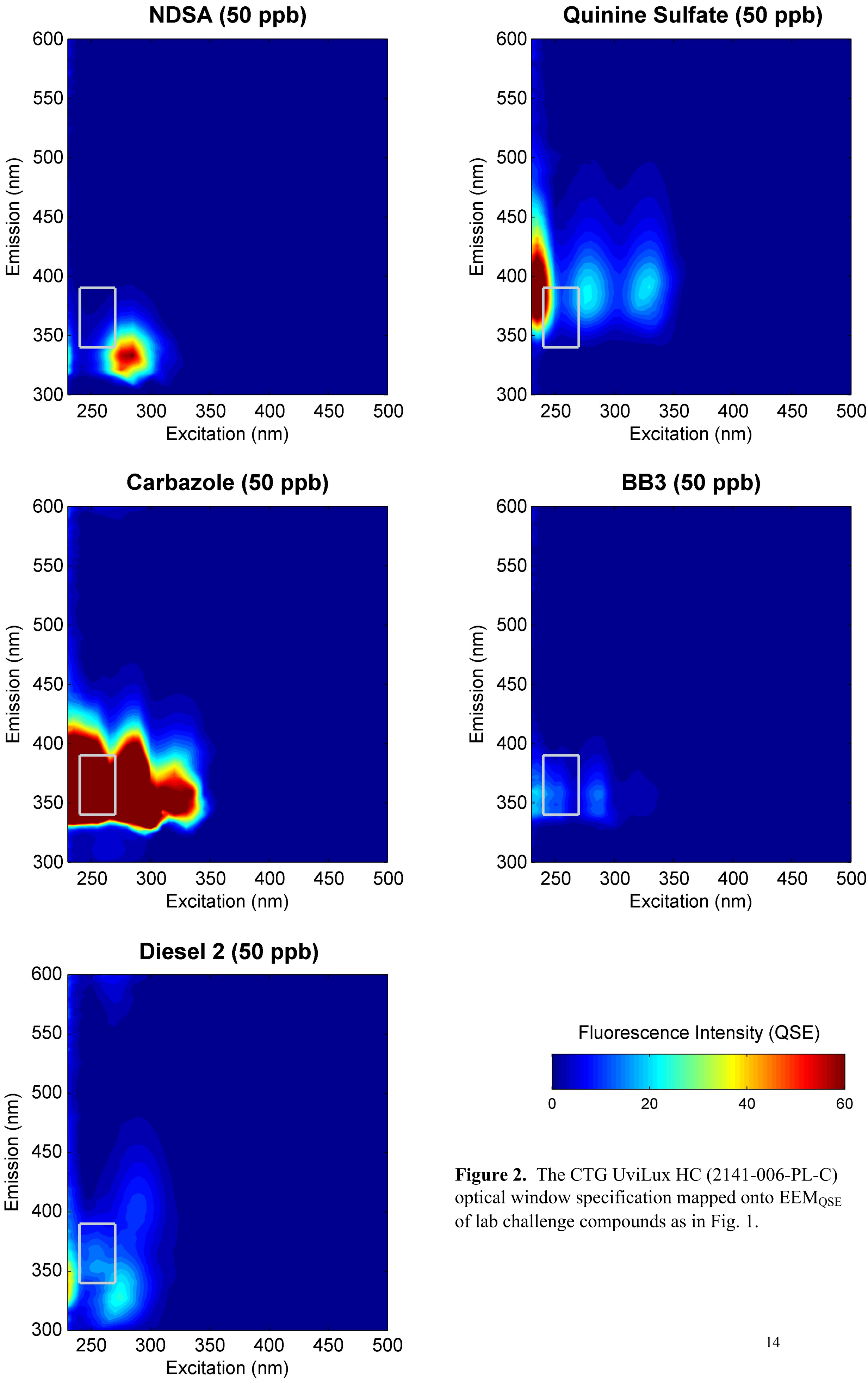


Figure 2. The CTG UviLux HC (2141-006-PL-C) optical window specification mapped onto EEM_{QSE} of lab challenge compounds as in Fig. 1.

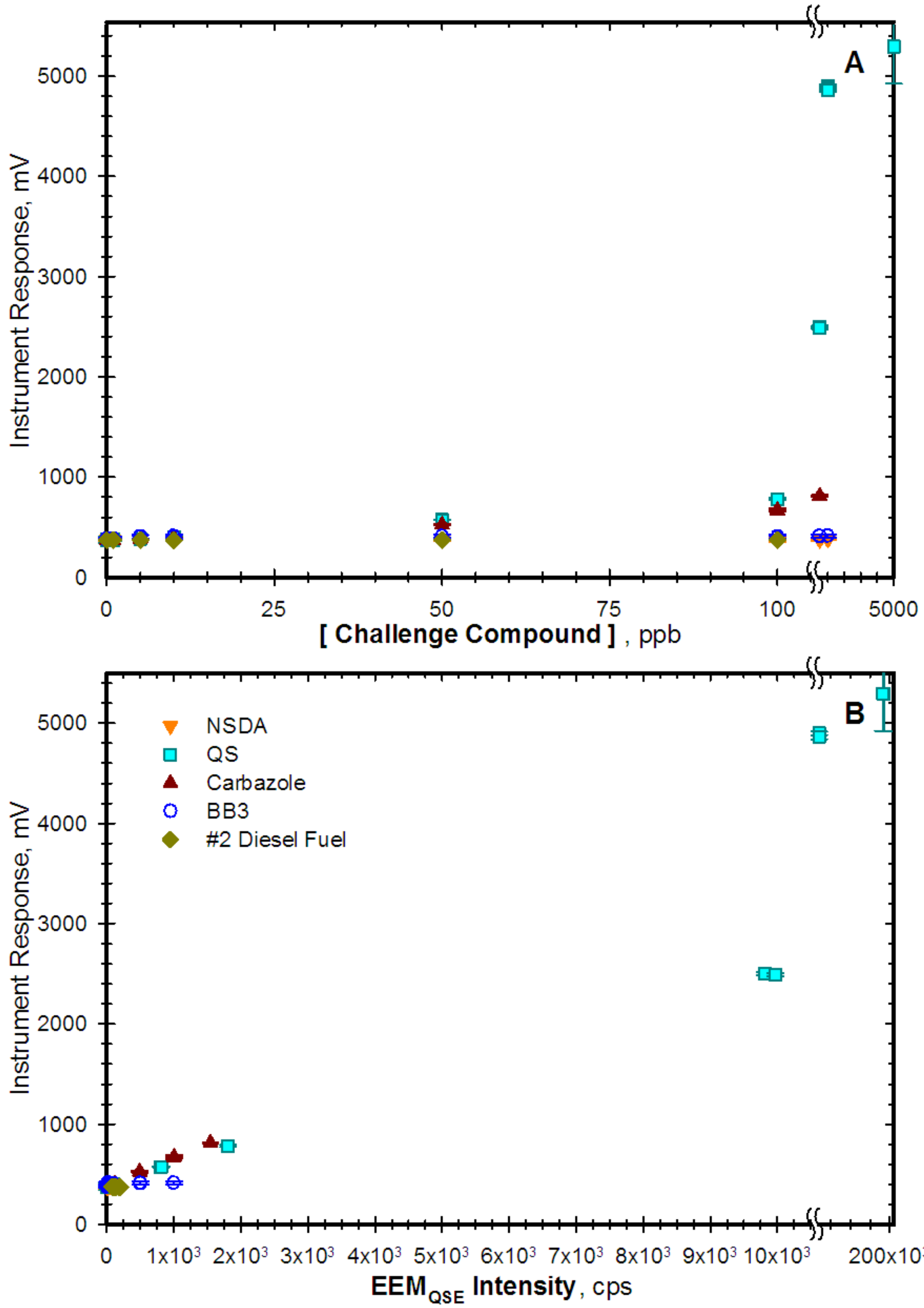


Figure 3. Response of CTG UviLux CDOM to challenge compound additions. Reported measurements represent the average (+/- S.D.) instrument response over 10 minute windows starting 30 minutes after addition of the challenge compound. (A) Instrument response to concentration of challenge compound in de-ionized water. Scaling changes to 1000 ppb per tick after axis break. (B) Relationship of instrument response to predicted EEM$_{QSE}$ based on instruments specified optical window. Scaling changes to 50,000 cps per tick after axis break.

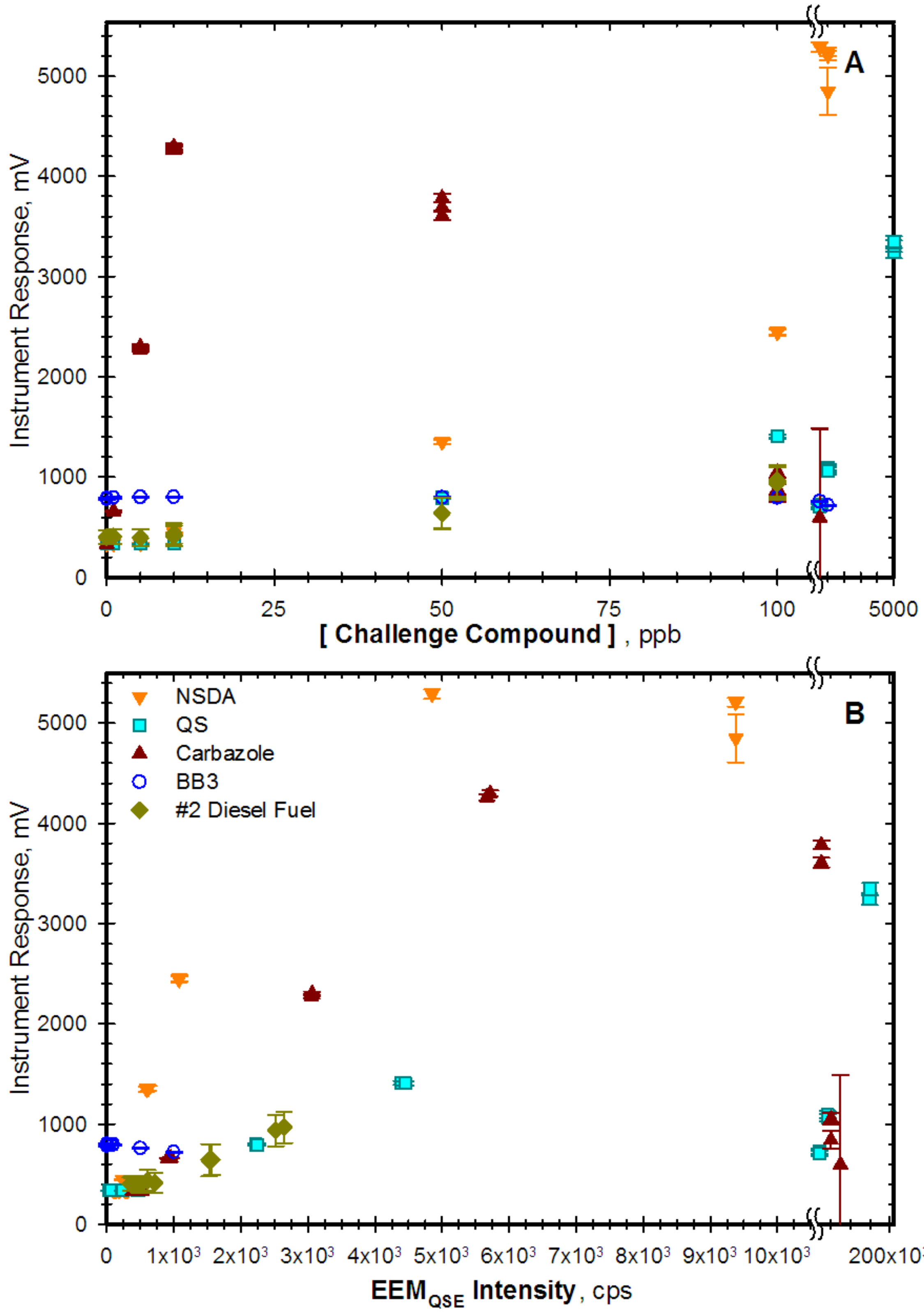


Figure 4. Response of CTG UviLux HC to challenge compound additions. Reported measurements represent the average (+/- S.D.) instrument response over 10 minute windows starting 30 minutes after addition of the challenge compound. (A) Instrument response to concentration of challenge compound in de-ionized water. Scaling changes to 1000 ppb per tick after axis break. (B) Relationship of instrument response to predicted EEM$_{QSE}$ based on instruments specified optical window. Scaling changes to 50,000 cps per tick after axis break.

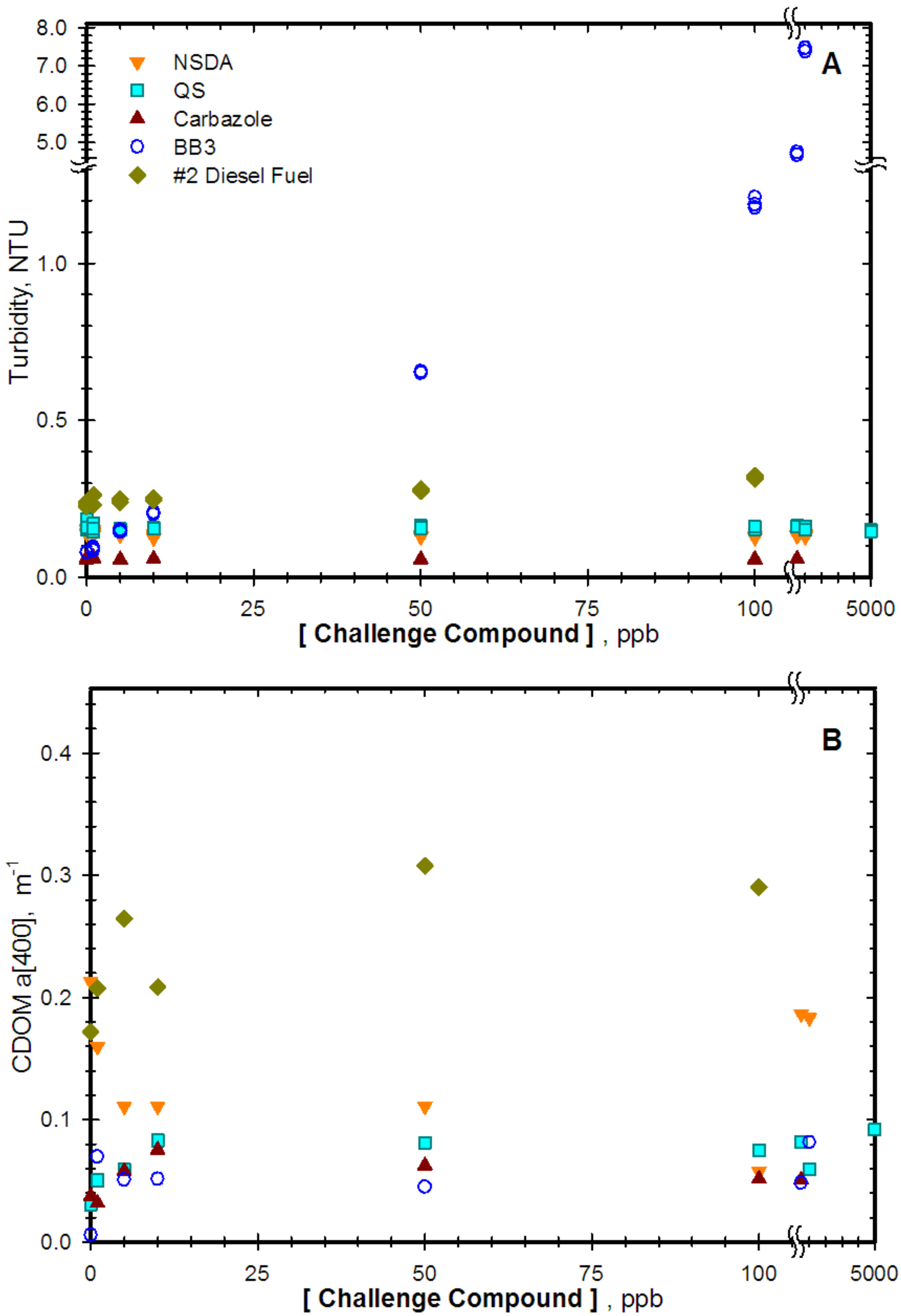


Figure 5. Variation in Turbidity and CDOM measurements with respect to challenge compound and challenge concentration during laboratory trials. (A) Turbidity (NTU) measured with a calibrated Hach 2100 AN. (B) Relative CDOM (chromophoric dissolved organic matter) concentration estimated as the absorbance at 400 nm, predicted from an exponential fit of sample absorbance spectra over 400-600 nm as described in text.

RESULTS of WAVE TANK TEST

Tests were conducted at the Bedford Institute of Oceanography's (BIO) Center for Offshore Oil, Gas and Energy Research (COOGER) in their 32 m wave tank facility. This wave tank was constructed at the BIO in collaboration between Fisheries and Oceans Canada (DFO) and the U.S. Environmental Protection Agency (EPA) for controlled oil dispersion studies (Photo 3). The wave tank is able to continually produce breaking waves at precise locations in the tank (Photo 4) and is fully equipped to enable measurements of dispersed oil in the water column. The tank is equipped with a flap-type wave maker that generates waves with periods varying from about 0.5 to 1.5 seconds. On the opposite end of the tank, a series of inclined screens is in place to absorb wave energy and minimize reflection.

Photo 3. Bedford Institute of Oceanography's Wave Tank

Oil additions were performed by BIO research staff using established protocols. In brief, oil was first added to two liters of the ambient bay water and mixed on a shaker plate for approximately 15 minutes, with or without dispersant depending on the test. The oil slurry was then poured slowly into the wave generating end of the tank (Photo 5 and 6) and allowed to be mixed by wave motion. Oil concentrations become uniformly distributed throughout the tank after approximately 30 minutes of mixing.

A summary of the test conditions and background water quality concentrations of the seawater used during oil additions are provided in Table 2. Temperature and salinity conditions were consistent over the test period and chlorophyll and CDOM levels were relatively low.

Table 2. Comparison of ancillary physical and water quality conditions for hydrocarbon sensor verification tests conducted in the wave test tank at the Bedford Institute of Oceanography, Halifax, Nova Scotia.

Site		Temperature (^oC)	Salinity	Chlorophyll (µg/L)	CDOM A_{400}, m^{-1}	Turbidity (NTU)
BIO Wave Tank	Min	8.3	14.5	0.1	0.61	0.3
	Max	9.5	15.8	0.9	1.33	5.0
	Mean	8.9	15.6	0.5	0.95	1.5

Photo 4. BIO Wave Tank prior to Hydro Carbon addition

Photo 5. Addition of oil and Corexit 9500

Photo 6. Addition of oil

The time series response of the UviLux-CDOM and UviLux-HC fluorometers to the stepwise addition of crude oil and dispersant are plotted in figures 6 and 7, respectively. Each lettered panel represents a day-long test of specific source oil and dispersant ratio at seven different concentrations including ambient background. The highest concentration was not tested on day 1, but this whole experiment was repeated on day 3 during which the highest concentration level was included. The background fluorescence of the incoming seawater varied with each trial, averaging between 400 – 800 mV for both sensors. The elevated ambient background fluorescence is also evident in the non-zero EEM_{QSE} values at the start of each trial series (Fig. 10 & 11, panel B). Representative EEM maps from reference samples collected after the fourth oil addition (mass added ca. 85 grams; concentration ca. 3 ppm) are presented in figures 8 and 9. The optical windows used for estimating the integrated fluorescent intensities of the UviLux-CDOM and UviLux-HC units are overlaid on the maps in figure 8 and 9, respectively. The overlap of the optical window of the UV-HC unit to the region of maximum fluorescence of the oil mixtures was clearly greater than that for the UV-CDOM unit.

The response of the UviLux CDOM unit to the oil additions was minimal but linear only in trials conducted with the chemical dispersant Corexit 9500 (Fig 10, panel A). In all cases the background reading in seawater was not subtracted from the instrument response during oil additions. The response of the UviLux-HC unit was significantly greater (Fig 11 panel A) than the UviLux-CDOM unit for all oil exposures, and this response difference was greatest for the three tests in which chemical dispersant was used in addition to physical wave dispersion.. Chemical dispersion resulted in an approximate doubling of the fluorescence detected by the UviLux instrument. The UviLux-CDOM also reported a similar relative signal enhancement but greatly dampened absolute signal response.

Cross plots of instrument response versus oil concentration and estimated EEM_{QSE} intensity clearly reveal differences in the detection capabilities of these two UViLux configurations (Fig 10, CDOM unit and Fig. 11, HC unit). The response of the CDOM unit to increased oil concentration was much dampened and the overall range of EEM intensity within the optical window was greatly compressed (Fig. 10). The response of the UviLux-HC unit was much greater but was highly dependent on both the type of oil and most importantly to whether or not chemical dispersant was added to the crude oil (Fig. 11). The instrument response was roughly 3-5 times greater when the crude oil had been dispersed by the Corexit. The instrument response of the HC unit to oil additions was linear through 3000 ppb added oil but showed a significant response above background to even the lowest oil addition of *ca* 300 ppb TPH in the presence of Corexit 9500. There was good agreement between instrument response versus oil concentration for independent trials with the same source crude oil (Fig. 11A; ALC, DOR 1:25).

Figure 12 summarizes various water quality parameters over the course of the five tests. Concentrations of chlorophyll, CDOM, and turbidity were conducted on discrete reference samples, while particle concentration estimates were generated in situ with a LISST. Although levels of chlorophyll, CDOM and turbidity varied at the start of each day, their effect on the initial background fluorescence of the seawater was relatively small. Changes in chlorophyll and CDOM concentrations during the step-up oil additions were relatively small. Turbidity increased almost linearly when dispersant was present with the oil, but showed little change to increasing oil concentrations above 1.5 ppm without dispersant. Similarly, the increase in mean particle concentrations was much greater in the presence of dispersant than without, indicating a physical repacking of the oil is also taking place, which would likely account for much of the differences in fluorescent response of the test mixtures.

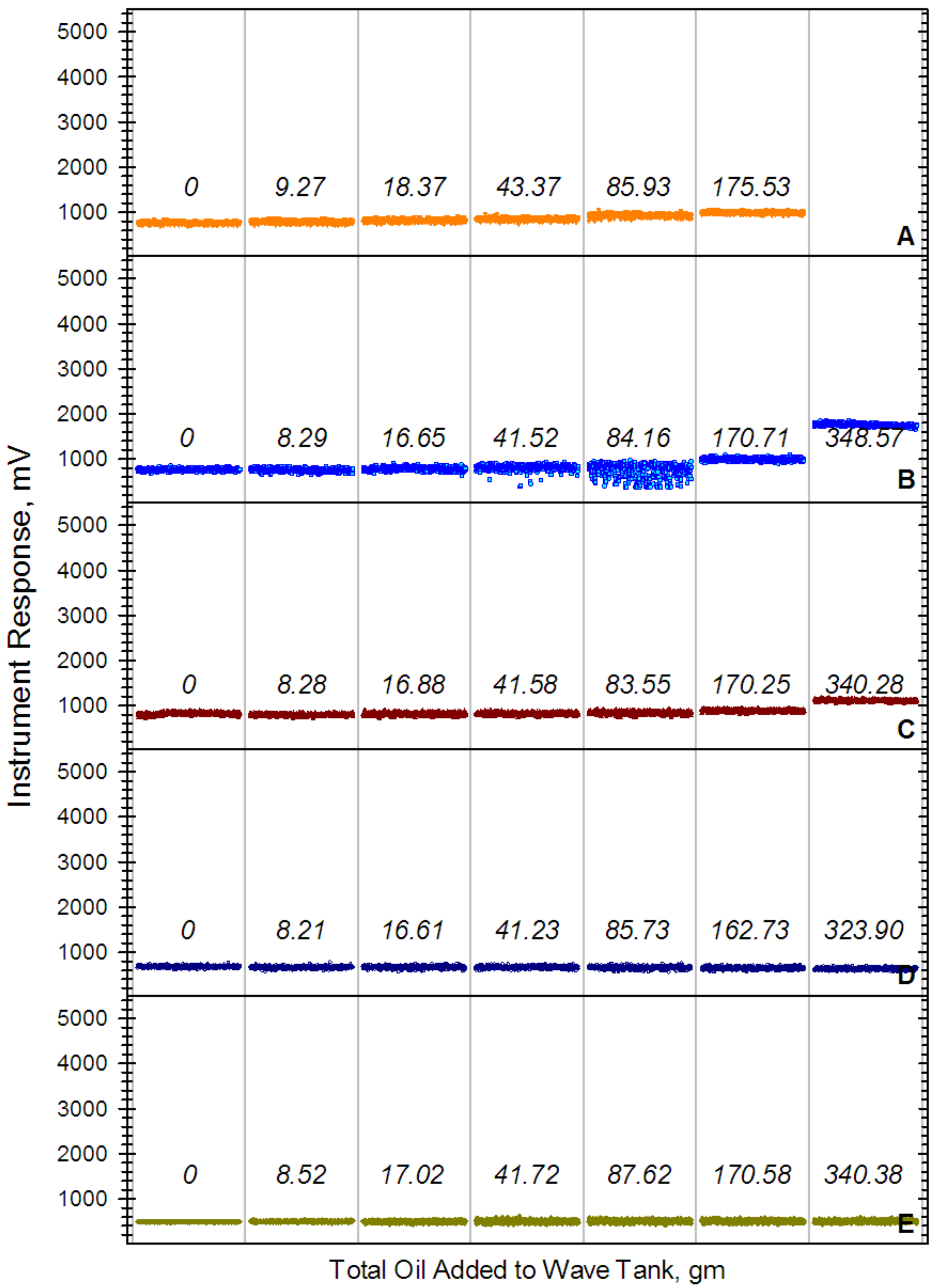


Figure 6. Time-series response of the CTG UviLux CDOM to crude oil additions in the COOGER wave tank at the Bedford Institute of Oceanography, Bedford, NS. Instrument response reported over the 10 minute interval starting 40 minutes after addition of the indicated challenge compound level. Each tank trial was conducted as step-up oil additions to a fixed seawater parcel subject to mixing by breaking waves. Numeric legends indicate cumulative grams of corresponding crude oil added to test tank. (A) Arabian Light Crude (ALC, 7% weathered) + Corexit 9500 at a detergent to oil ratio (DOR) of 1:25. (B) Alaskan North Slope (ANS) DOR 1:25. (C) ALC, DOR 1:25. (D) ALC, DOR 0. (E) ANS, DOR 0.

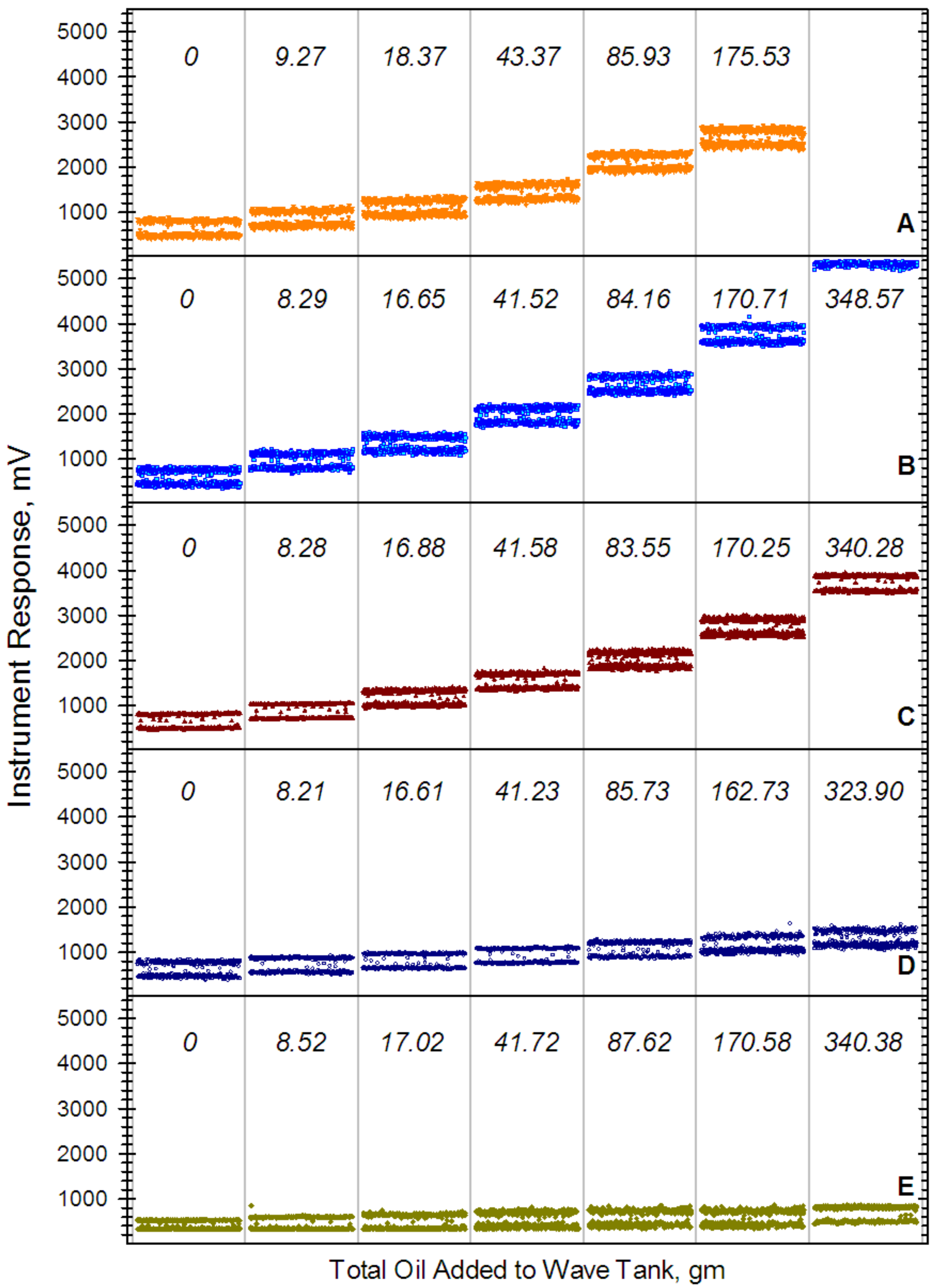


Figure 7. Time-series response of the CTG UviLux HC to crude oil additions in the COOGER wave tank at the Bedford Institute of Oceanography, Bedford, NS. Instrument response reported over the 10 minute interval starting 40 minutes after addition of the indicated challenge compound level. Each tank trial was conducted as step-up oil additions to a fixed seawater parcel subject to mixing by breaking waves. Numeric legends indicate cumulative grams of corresponding crude oil added to test tank. (A) Arabian Light Crude (ALC, 7% weathered) + Corexit 9500 at a detergent to oil ratio (DOR) of 1:25. (B) Alaskan North Slope (ANS) DOR 1:25. (C) ALC, DOR 1:25. (D) ALC, DOR 0. (E) ANS, DOR 0. The underlying cause of the bimodal signal output is unresolved and not observed in other deployments.

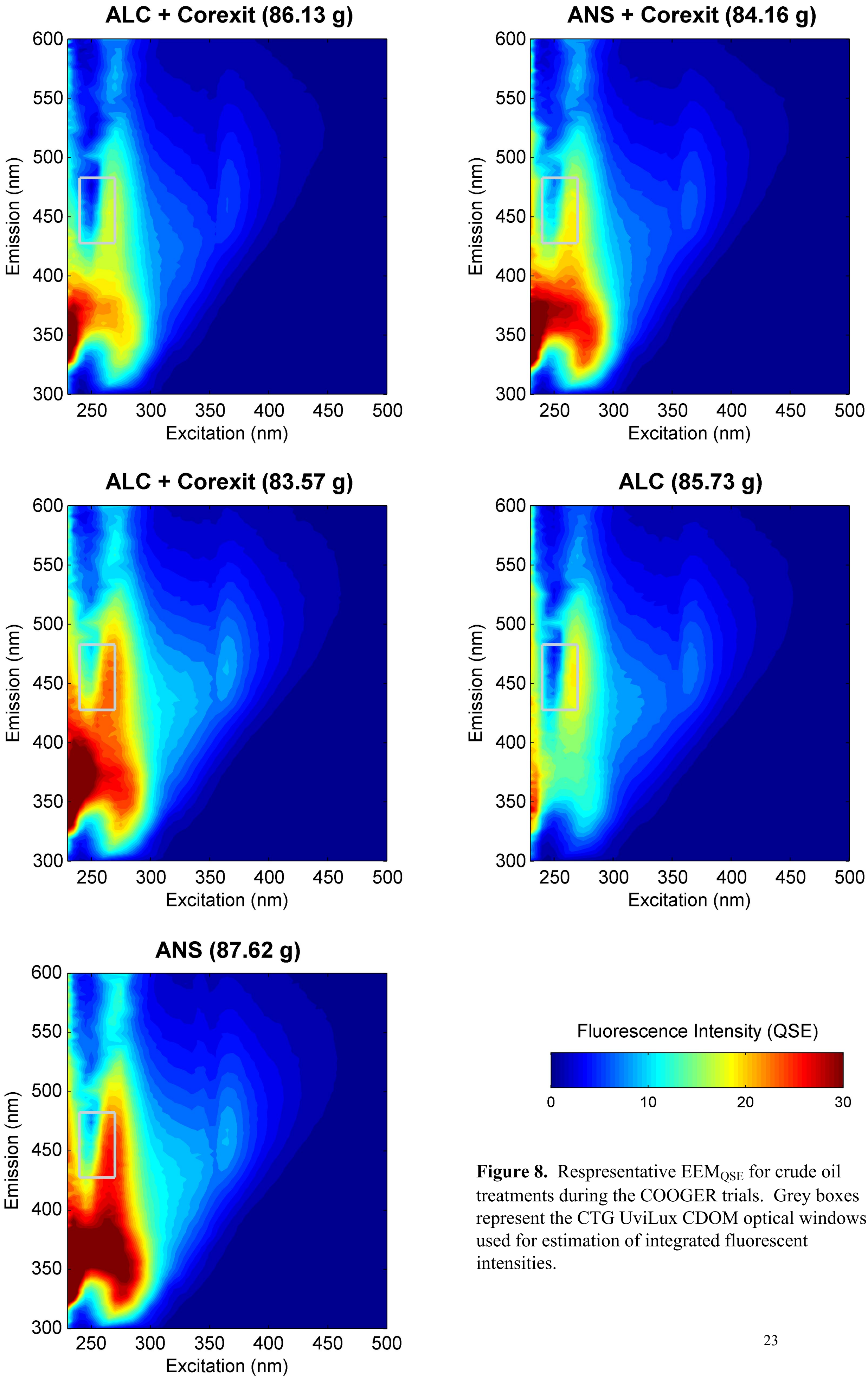


Figure 8. Rrespresentative EEM_{QSE} for crude oil treatments during the COOGER trials. Grey boxes represent the CTG UviLux CDOM optical windows used for estimation of integrated fluorescent intensities.

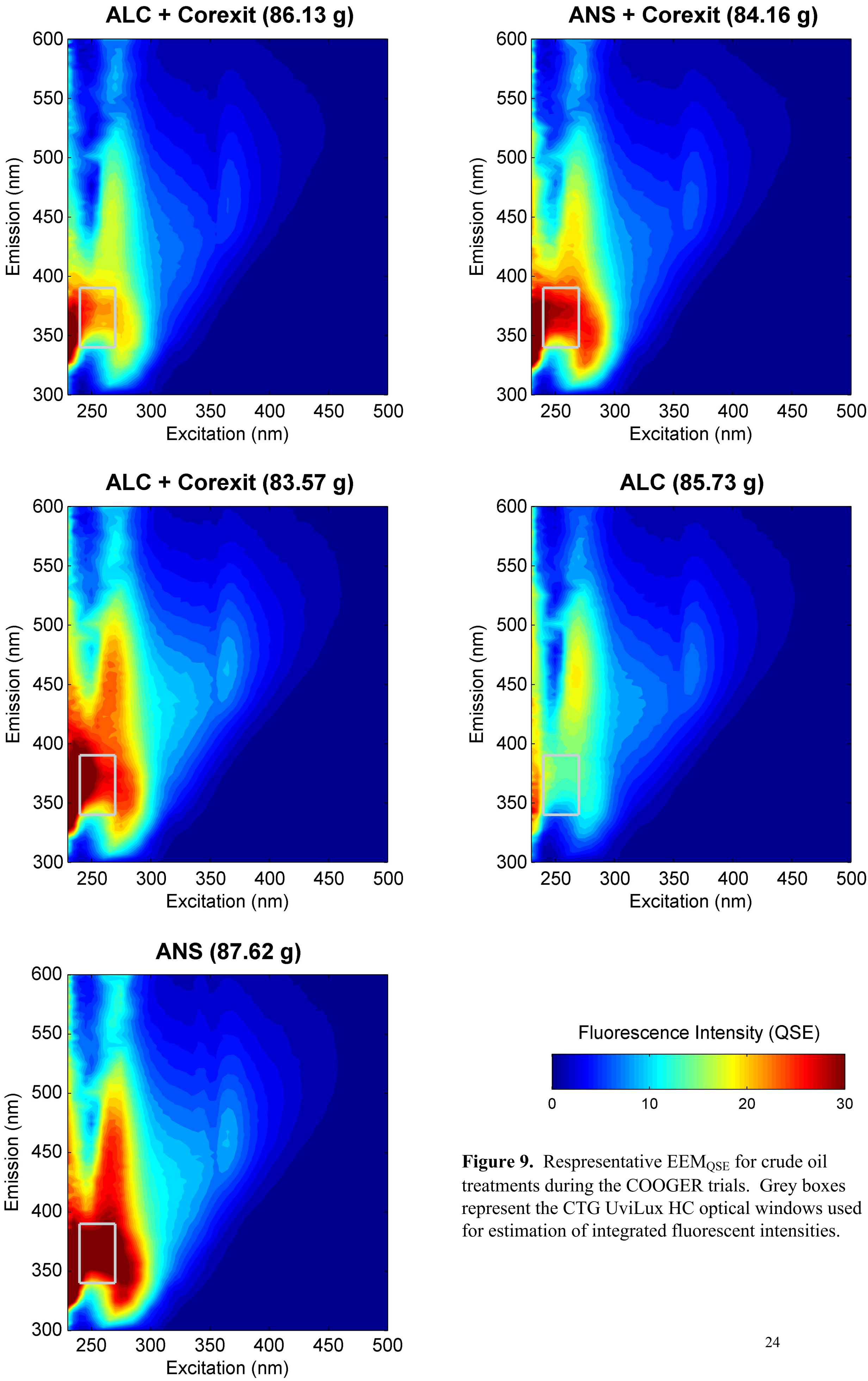


Figure 9. Respresentative EEM_{QSE} for crude oil treatments during the COOGER trials. Grey boxes represent the CTG UviLux HC optical windows used for estimation of integrated fluorescent intensities.

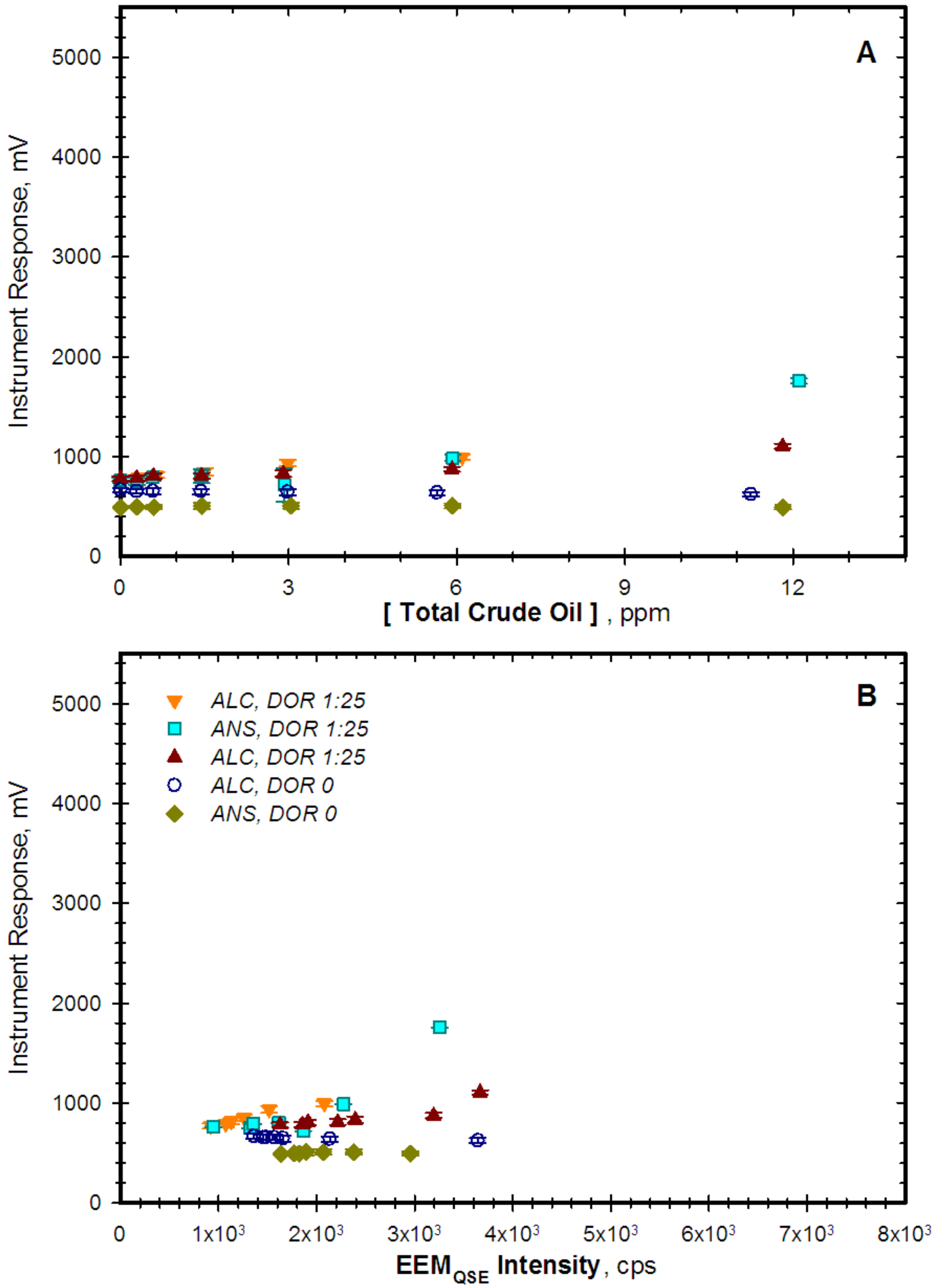


Figure 10. Response of CTG UviLux CDOM to total crude oil concentration in presence/absence of the chemical dispersant Corexit 9500. All exposures experienced breaking waves. Reported measurements represent the average (+/- S.D.) instrument response over 10 minute windows starting 40 minutes after addition of the challenge compound derived from data in Fig. 5. (A) Instrument response to total oil concentration (ppm) in Bedford Basin seawater (B) Relationship of instrument response to predicted EEM_{QSE} based on instrument's optical window. Note variable offsets in initial EEM_{QSE} indicative of daily variation in ambient CDOM loads in tank source waters.

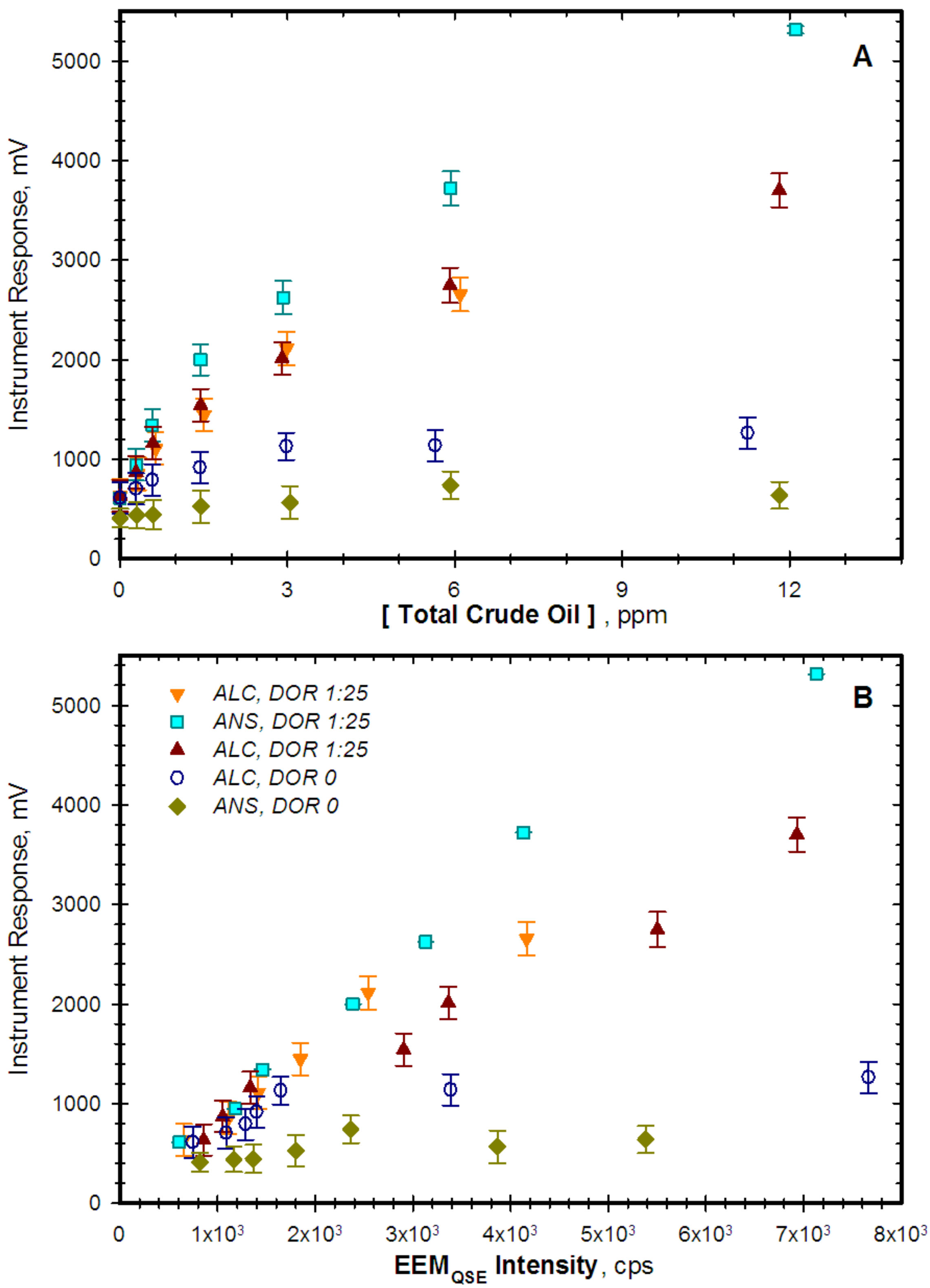


Figure 11. Response of CTG UviLux HC to total crude oil concentration in presence/absence of the chemical dispersant Corexit 9500. All exposures experienced breaking waves. Reported measurements represent the average (+/- S.D.) instrument response over 10 minute windows starting 40 minutes after addition of the challenge compound derived from data in Fig. 5. (A) Instrument response to total oil concentration (ppm) in Bedford Basin seawater (B) Relationship of instrument response to predicted EEM_{QSE} based on instrument's optical window. Note variable offsets of initial EEMQSE levels indicative of changes in ambient CDOM/HC loads in tank source waters.

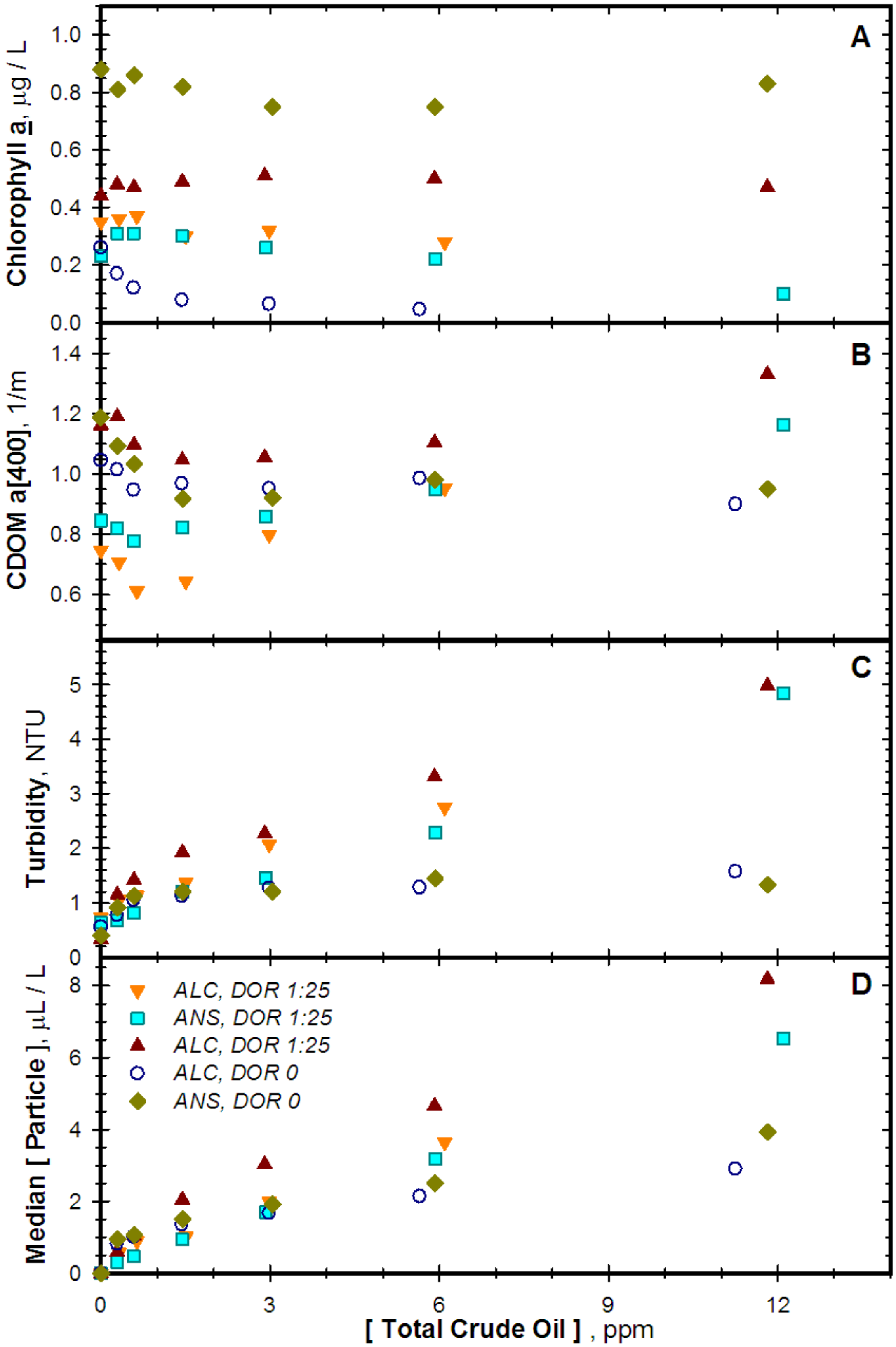


Figure 12. Variation of ancillary water quality parameters with crude oil additions during the COOGER trials. (A) Extracted chlorophyll a . (B) Absorbance at 400 nm as a proxy for CDOM. (C) Turbidity measured with a Hach 2100 AN. (D) Median particle concentration measured in situ using a LISST (Sequoia Inst).

RESULTS of MOORED FIELD TEST

Moored Deployment in Baltimore Harbor Maryland

The moored deployment field test occurred in Winans Cove, Baltimore Harbor, MD (Photo 7). The port of Baltimore is highly industrialized, especially in the area surrounding Winans Cove. Runoff from industry and nearby Interstate 95 directly impacts the test area, especially during rains. The instruments were deployed at a depth of 1 meter on a deployment system attached to a research barge at the end of a US Government pier. The pier was behind a locked gate, guarded and only accessible to authorized personnel.

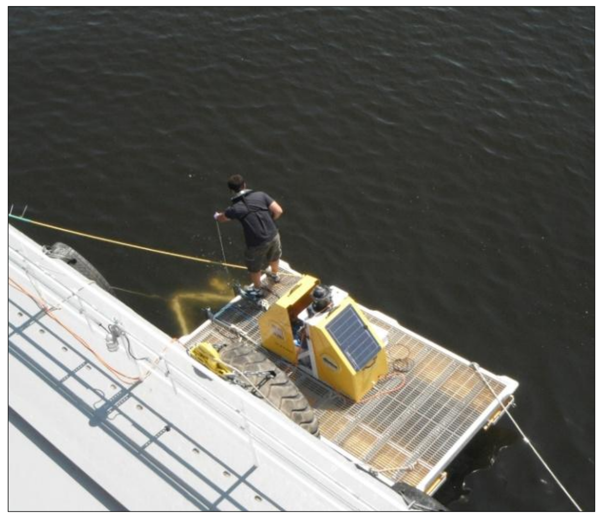


Photo 7. Site map and photo of the field test site located in Winans Cove, Baltimore Harbor MD.

The original mooring was damaged by storm waves after only four days and had to be re-established after instruments were checked and repaired. The initial deployment occurred at 21:00 local on August 11, 2011. Samples were collected on August 11th and 12th. The deployment rack and set-up was checked daily on the days ACT staff were not available on site for sampling. At some point between the visual inspection at 17:00 on August 14th and arrival of ACT staff on site at 10:30 on August 15th, the supports for the mooring rack were damaged due to a series of strong overnight storms. The mooring rack was found suspended in the water by two safety lines but lacking any support to the floating platform. This separation from the platform caused several of the data cables leading from the instruments to the dataloggers to either be severed or pulled free. The deployment rack and instruments were removed from the water and cleaned. The instruments were tested using quinine sulfate (QS) and naphthalene disulfonic (NDSA) acid to verify that they were working correctly. The cable for UviLux-CDOM unit could not be repaired properly and only the UviLux-HC unit was redeployed at 13:00 on August 18, 2011. A modified mooring set-up was designed to better handle the motion caused by waves reflecting off the barge hull. On August 26, 2011, the instruments had to be removed as the barge was relocated due to the approach of Hurricane Irene.

Instrument Photographs

Before and after photos were taken of the instrument to examine the extent and possible impacts of bio-fouling (Photo 8.)

UviLux HC Prior to 1st Deployment

UviLux HC Prior to 2nd Deployment

UviLux CDOM Prior to 1st Deployment

UviLux HC after Deployment

Photo 8. Chelsea UviLux HC and CDOM instrument photos from Baltimore Harbor test site before and after deployments. The UviLux CDOM was damaged beyond repair and was not redeployed.

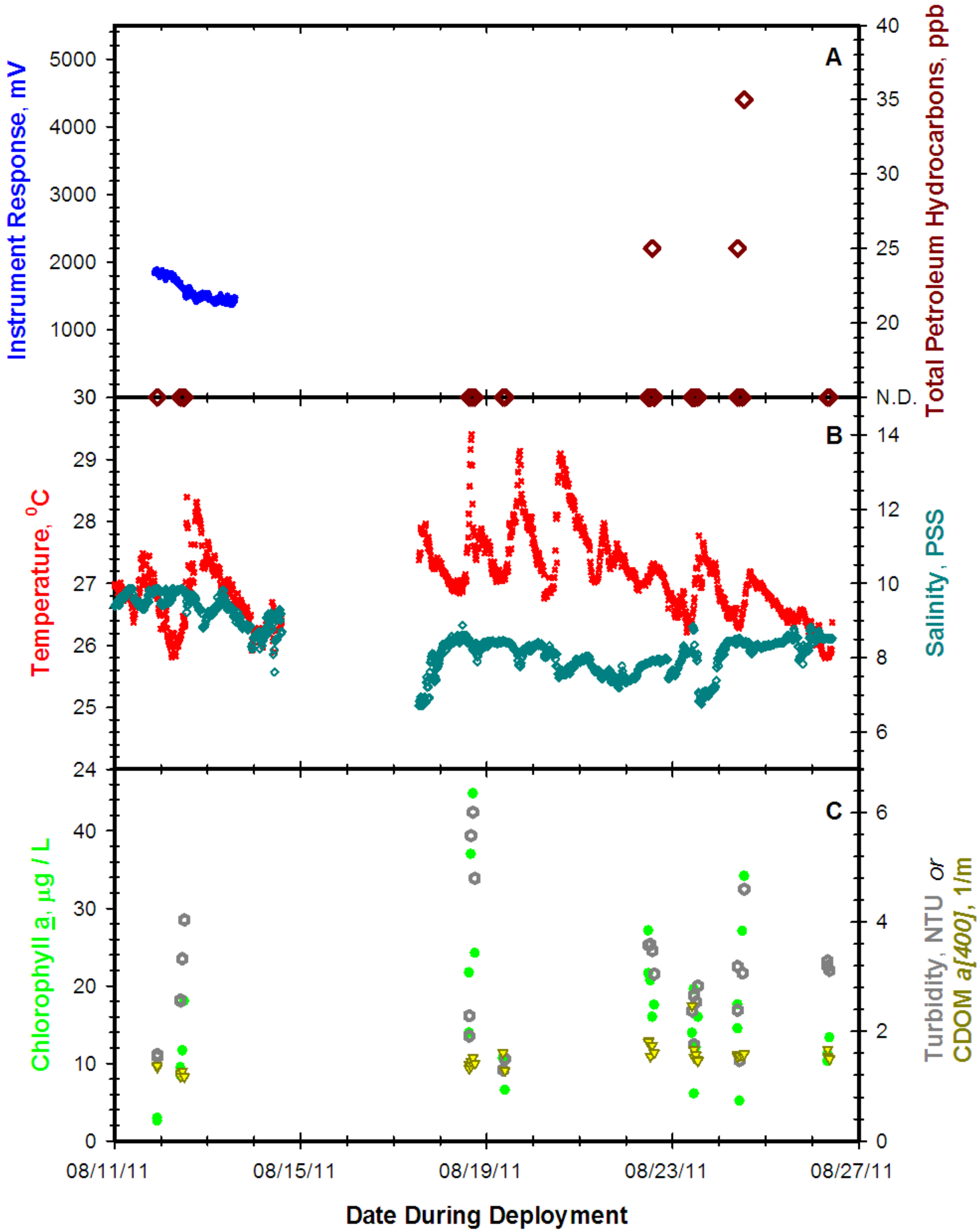


Figure 13. Summary of field deployment results for the CTG UviLux CDOM at the MERC facility in Baltimore, MD. (A) Instrument time series response during deployment along with GC-FID measurements of total petroleum hydrocarbons (TPH, Test America- Sacramento) in grab samples taken at the time of instrument sampling. (B) Variation in temperature and salinity at the deployment site measured by a YSI 6600 sonde. (C) Variation in Chlorophyll a, turbidity and CDOM determined from grab samples taken adjacent to the instruments during deployment. Note that shortened data record is due to failure of communications cable damaged by storm surge.

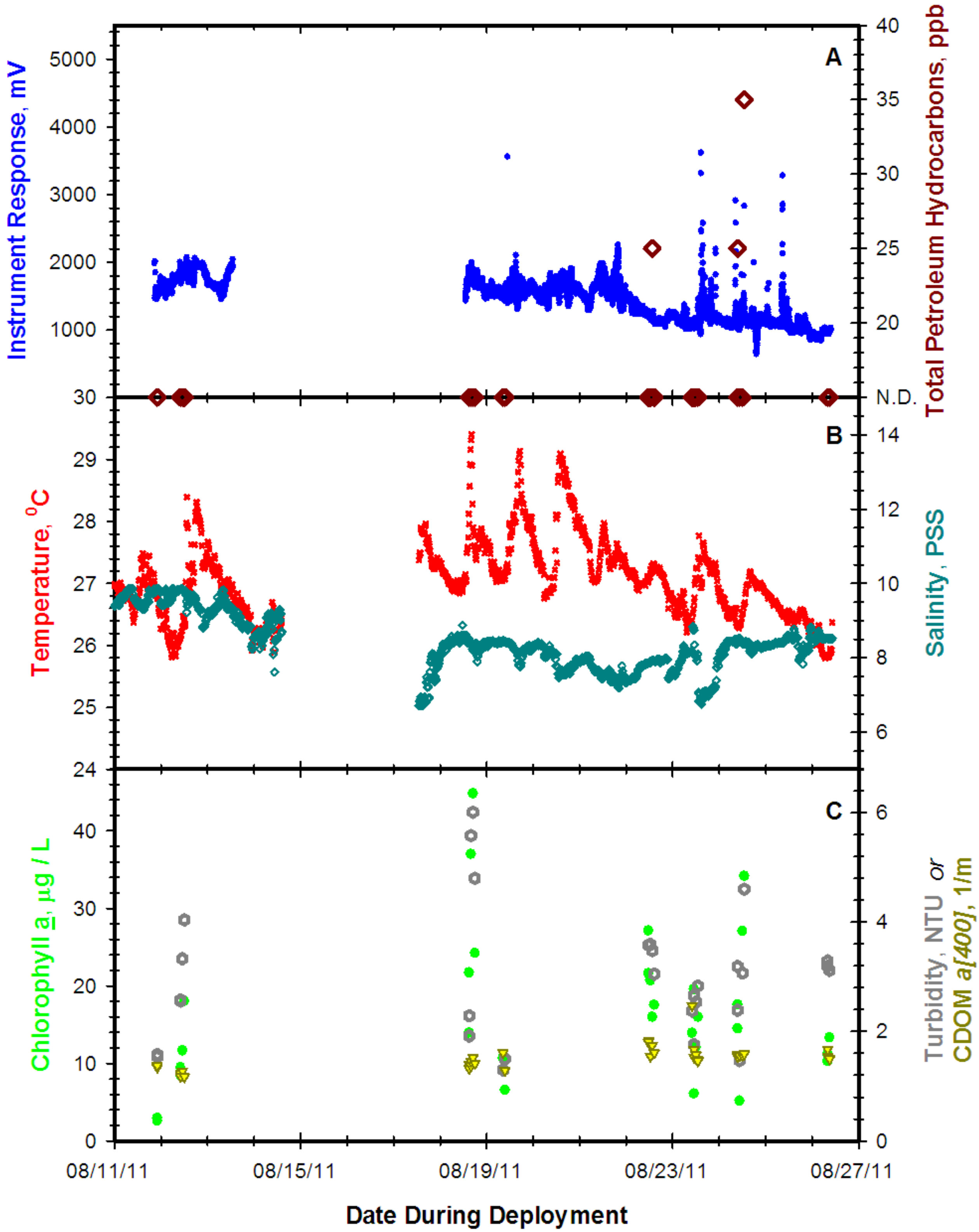
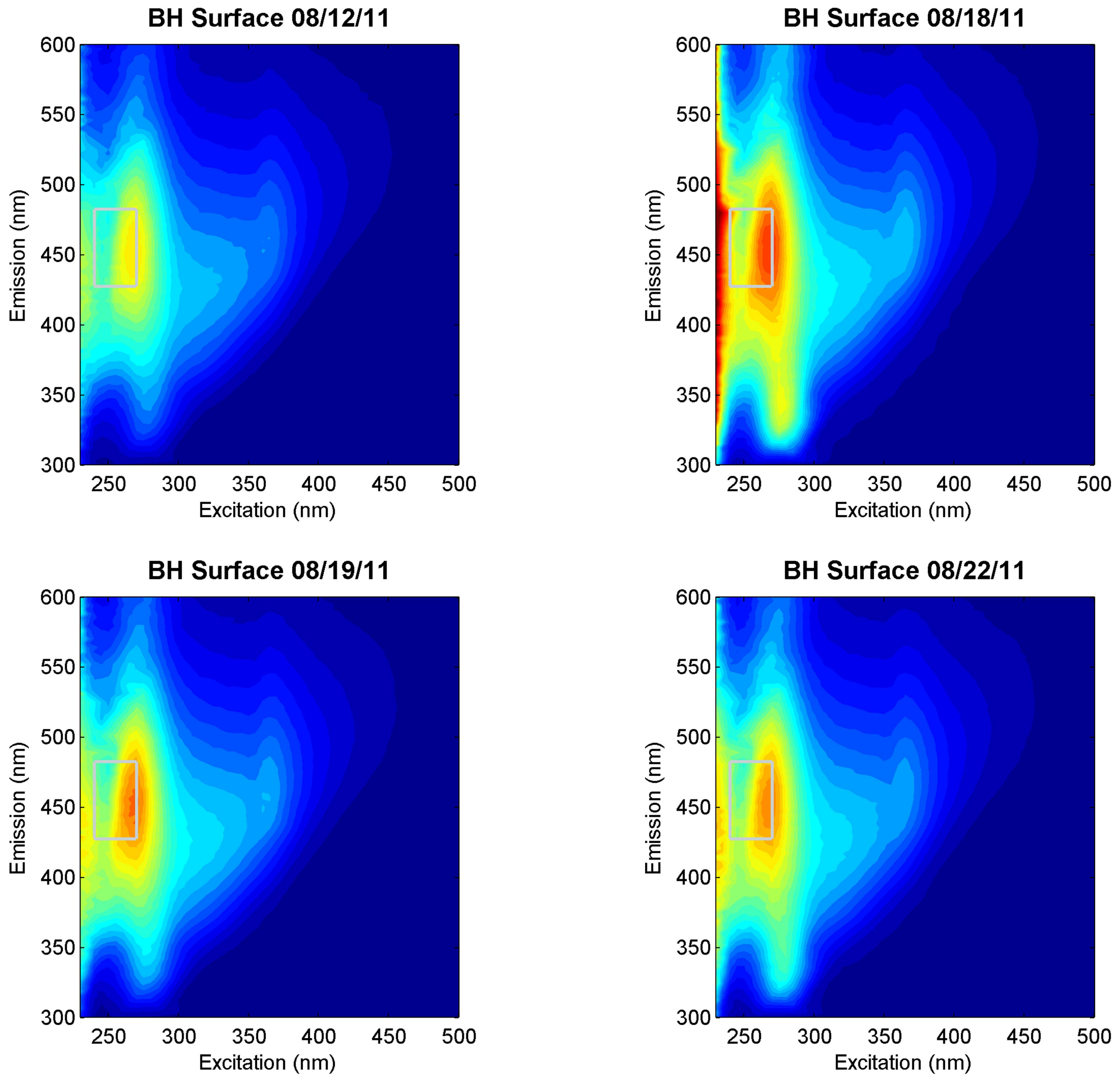
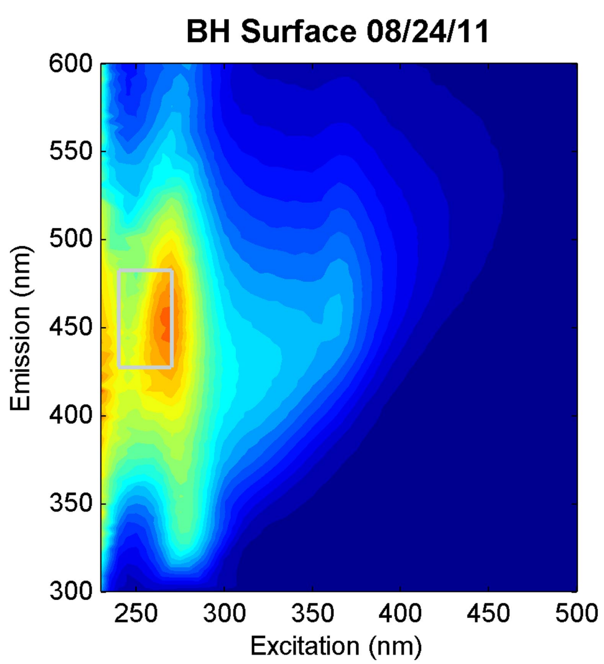


Figure 14. Summary of field deployment results for the CTG UviLux HC at the MERC facility in Baltimore, MD. (A) Instrument time series response during deployment along with GC-FID measurements of total petroleum hydrocarbons (TPH, Test America- Sacramento) in grab samples taken at the time of instrument sampling. (B) Variation in temperature and salinity at the deployment site measured by a YSI 6600 sonde. (C) Variation in Chlorophyll a, turbidity and CDOM determined from grab samples taken adjacent to the instruments during deployment.





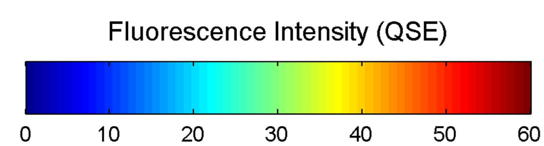
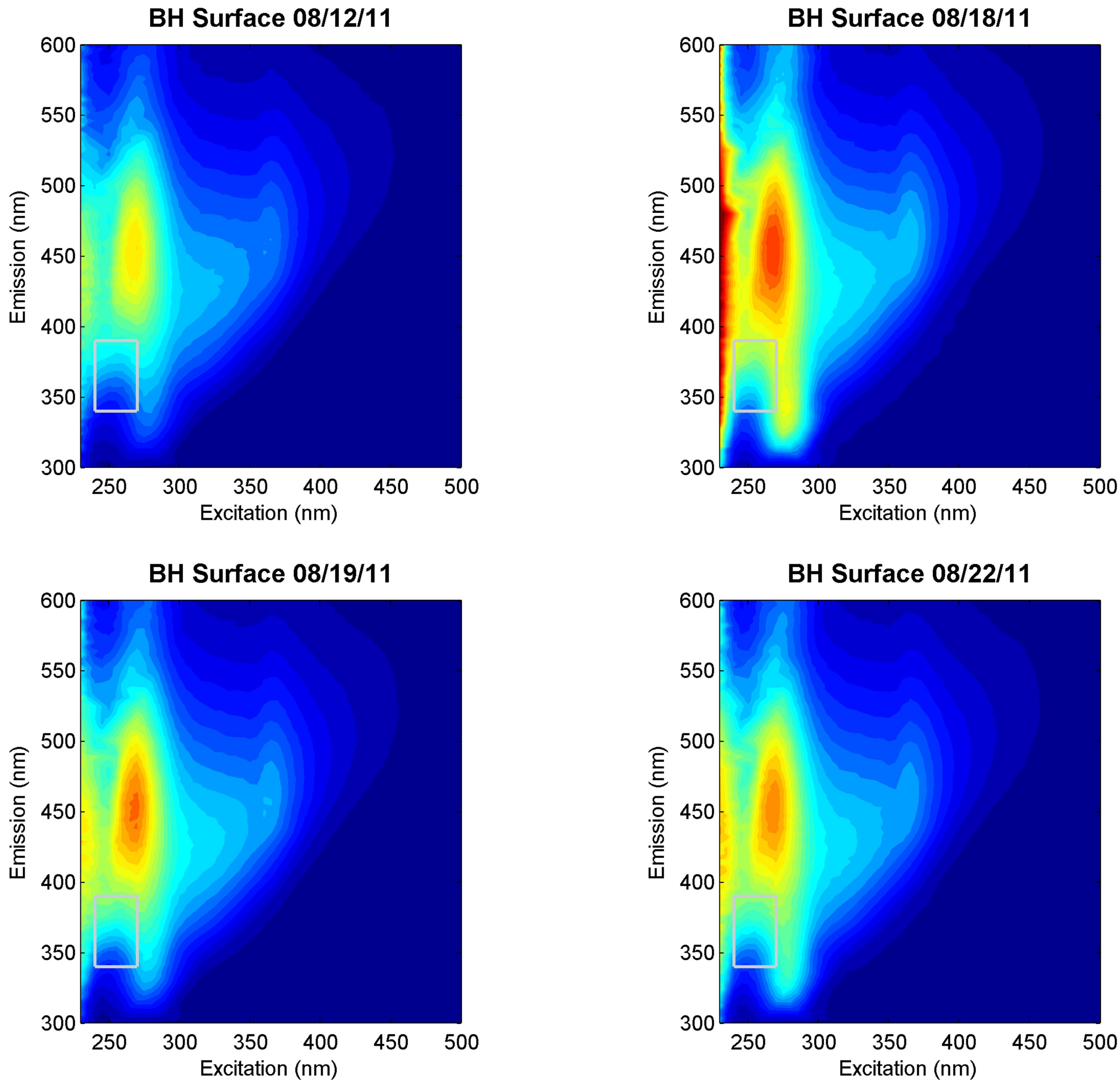
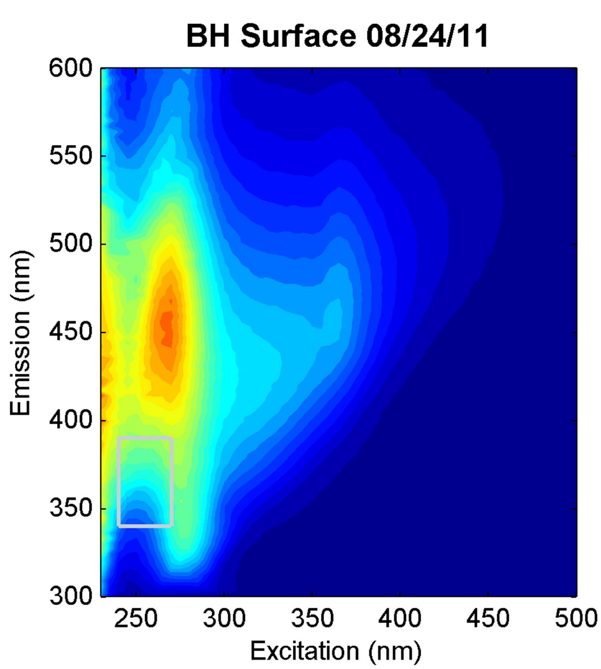


Figure 15. Representative EEM_{QSE} of Baltimore Harbor water from grab samples taken during the deployment. Grey box represents the optical window of the CTG UviLux CDOM used for estimation of integrated fluorescence intensity available to the instrument sensor. The 8/22 and 8/24 samples had significant TPH detections by independent analytical methods.





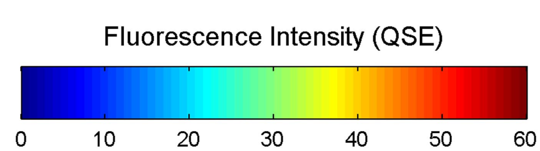


Figure 16. Representative EEM_{QSE} of Baltimore Harbor water from grab samples taken during the deployment. Grey box represents the optical window of the CTG UviLux HC used for estimation of integrated fluorescence intensity available to the instrument sensor. The 8/22 and 8/24 samples had significant TPH detections by independent analytical methods.

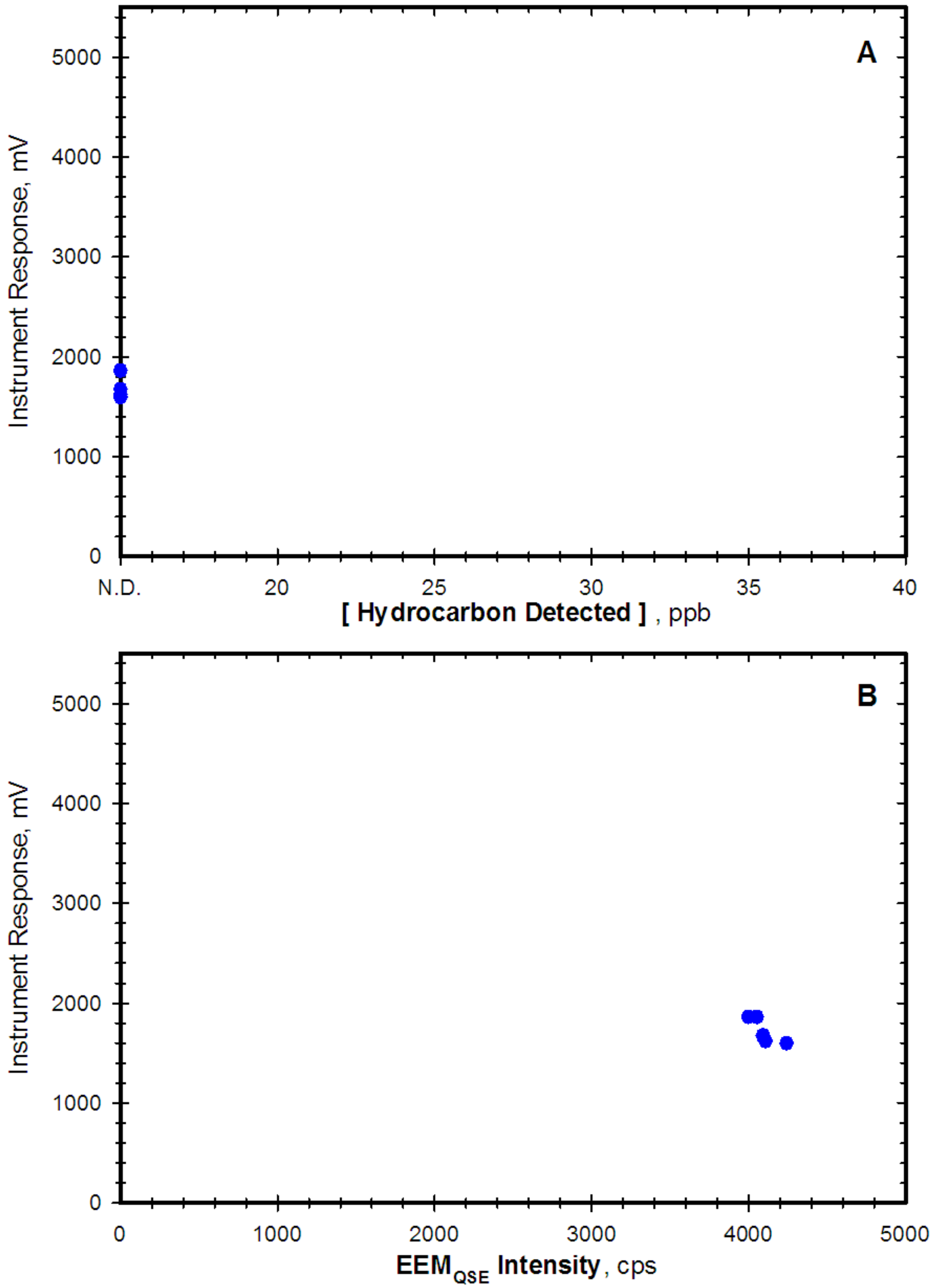


Figure 17. Response of CTG UviLux CDOM to ambient hydrocarbons and water fluorescence properties during the Balitmore Harbor deployment. (A) Instrument response to TPH detected by GC-FID. (B) Instrument response relative to predicted EEM_{QSE} fluorescent intensity. Note deployment dataset truncated due to storm associated communication failure.

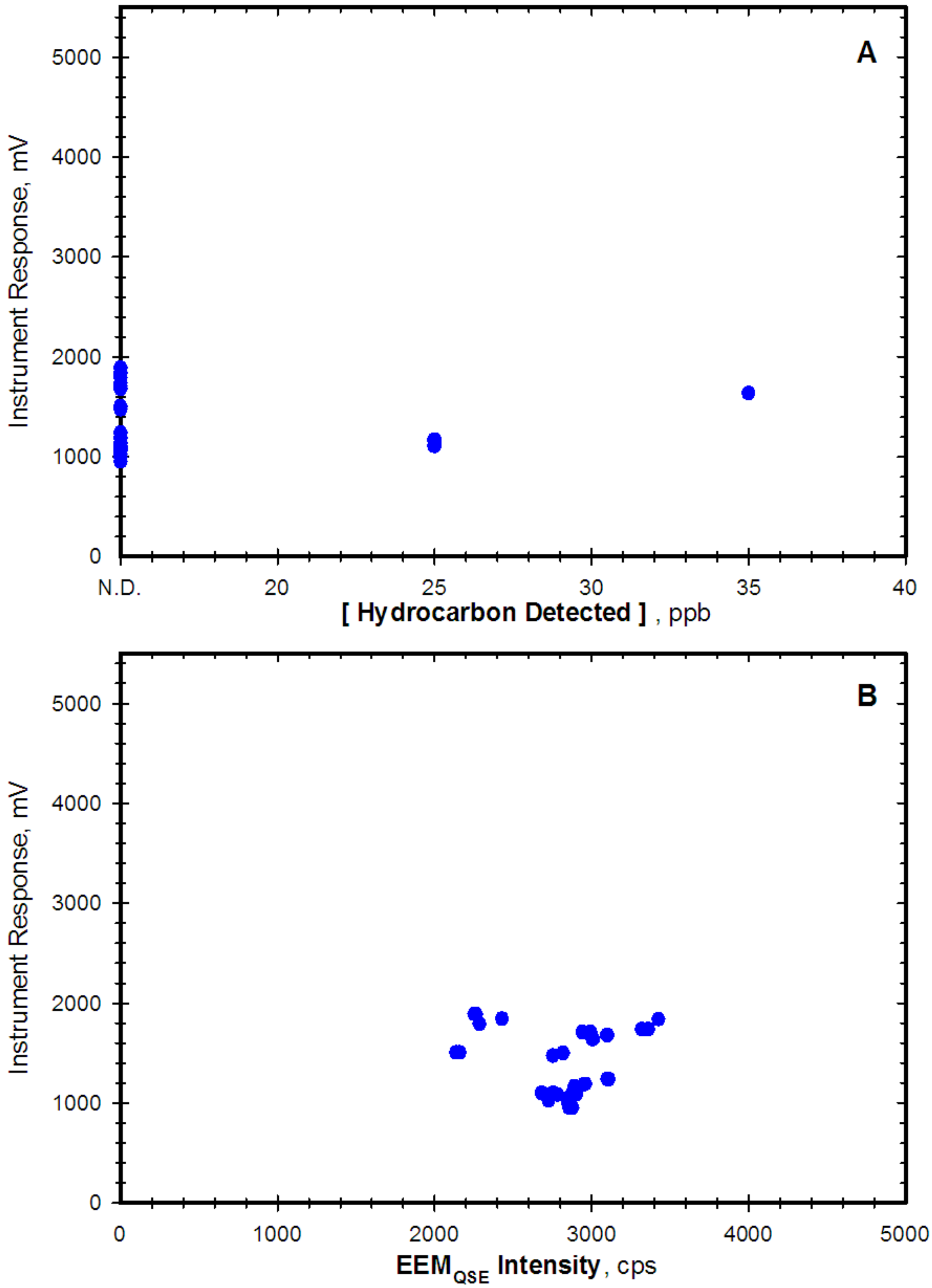


Figure 18. Response of CTG UviLux HC to ambient hydrocarbons and water fluorescence properties during the Balitmore Harbor deployment. (A) Instrument response to TPH detected by GC-FID. (B) Instrument response relative to predicted EEM_{QSE} fluorescent intensity. Clustering of positive trends indicative of detection of HC in distinct water masses.

RESULTS of VERTICAL PROFILING FIELD TEST

The vertical profiling application was conducted at two test sites in the northern Gulf of Mexico onboard the R/V *Acadiana* (Louisiana Universities Marine Consortium) over the course of two days. One profile was conducted just outside Terrebonne Bay (29.0465N, 90.5568W) to provide a contrast of high CDOM coastal waters. Five profiles were conducted at a second site that was located near a known submerged shipwreck presently leaking oil from a depth of approximately 25 m (28.56N, 90.98W; see Photo 9).

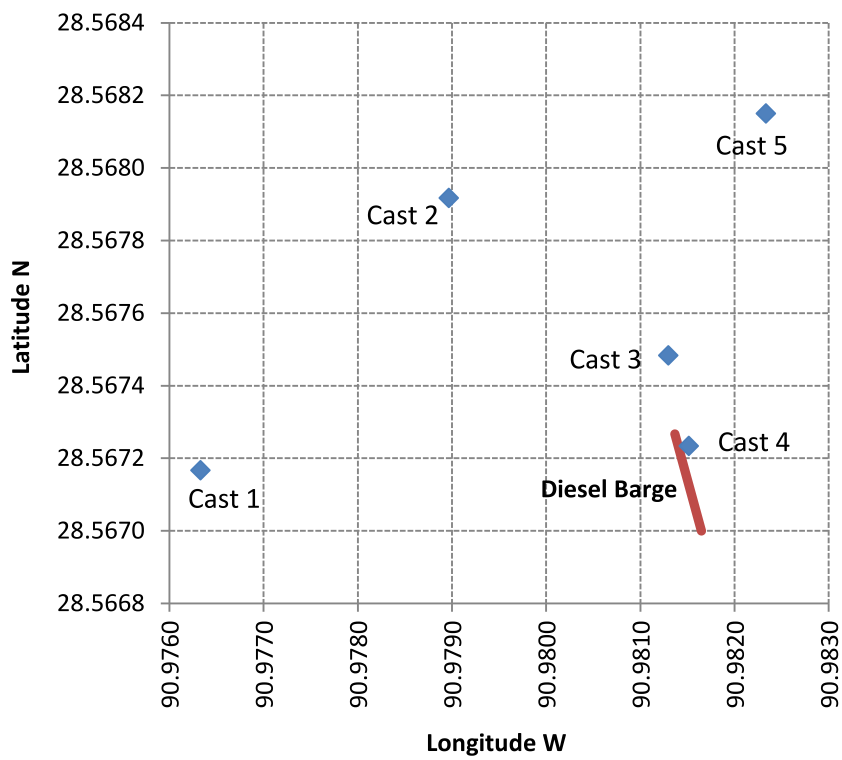


Photo 9. Site 2 for vertical profiles within the Gulf of Mexico located near a submerged, leaking diesel barge.

Ancillary physical-chemical conditions and discrete reference samples were collected with a standard CTD Rosette and Niskin bottles (Photo 10). At site 1 only a single surface depth was sampled. At site 2, five discrete depths were sampled with one depth sampled in duplicate for each cast. At site 2, casts were taken within and immediately surrounding the area with an observable oil slick on the surface of the water (Photo 10).

Photo 10. Vertical Profiling Rosette and visible surface oil slick above location of submerged, leadking barge.

A general summary of the water quality conditions at the two sites are shown in Table 4. Site 1 in Terrebonne Bay had an average salinity of 29.9, with a high level of chlorophyll, turbidity and CDOM. Site 2 was more typical of open-ocean, with an average salinity of 36.2 and mean chlorophyll levels less than 1 µg/L. CDOM and turbidity levels varied significantly at this site, in particular, showing increased levels at depth in regions where oil was present.

Table 4. Comparison of ancillary physical and water quality conditions for hydrocarbon sensor verification tests conducted at two vertical profiling field sites in the Gulf of Mexico.

Site		Temperature (°C)	Salinity	Chlorophyll (µg/L)	CDOM A_{400}, m^{-1}	Turbidity (NTU)
Site 1 Terrebonne Bay	Min	25.3	28.9	--	--	--
	Max	25.9	31.1	--	--	--
	Mean	25.6	29.9	9.7	0.72	3.4
Site 2 Leaking Barge	Min	27.1	36.1	0.3	0.09	0.7
	Max	28.4	36.3	2.0	0.72	15.4
	Mean	27.3	36.2	0.8	0.22	3.4

Vertical profiling results for all six casts are presented in figures 19-24. Results are presented sequentially for the CDOM and HC units. Each panel displays the UviLux response in mV along with a continuous trace of temperature and salinity for that cast. In addition, total hydrocarbon, CDOM, turbidity, and chlorophyll concentrations from the discrete reference samples are plotted on the same graph. It should be noted that despite the presence of a visible sheen of oil on the surface of the water above the leaking barge, all total hydrocarbon concentrations were reported as below detection for all reference samples collected (stated method of detection limit ≤ 25 ppb). EEM characterizations of the reference samples revealed some possible evidence of hydrocarbons in the surface sample of site 2, cast 5 and mid-depth sample for site 2, cast 3 (Fig. 25 and 26), however, even those peaks fell mostly outside the optical windows of both the UviLux-CDOM (Fig. 25) and the UviLux-HC (Fig. 26) fluorometers. Only minimum levels of fluorescent signal was observed throughout the EEM maps of the other samples.

In general, there was very little difference in instrument response between the CDOM and HC units among all profiles. Minor variability (noise) is seen among different casts at this fine resolution scaling, but this likely reflects water motion effects as the variance is often greatest at sampling depths during which the rosette was paused for few minutes to collect a water sample. Additionally, minimal difference in instrument response was observed between the nearshore (site 1) and offshore (site 2) sites for both units tested, despite significant differences in CDOM and chlorophyll levels. Cross plots of instrument response versus TPH concentration and estimated EEM_{QSE} intensity are presented in figure 27 for the UviLux-CDOM unit and in figure 28 for the UviLux-HC unit. As previously noted there were no detectable hydrocarbons within the collected reference water samples. Both units showed a similar non-variant response (approximately 375 mV) to the small range (roughly 1500 cps) of estimated EEM_{QSE} intensity from the reference samples (Fig. 27 and 28, panel B).

SUMMARY of INSTRUMENT RESPONSE ACROSS ALL TEST APPLICATIONS

The CTG UViLux-CDOM and UViLux-HC in situ fluorometers exhibited the expected linear voltage responses to challenge analyte concentration with the response slope (sensitivity) mapping to the overlap of the compounds' fluorescence properties to the optical configuration of the instrument package (Figs 29 & 30). The UViLux HC detected polyaromatic hydrocarbons with high sensitivity with detector saturation occurring above 10 ppb carbazole and 100 ppb NDSA. Chemically dispersed crude oils were detected through 12,000 ppb. The UViLux CDOM instrument exhibited lower sensitivity to these compounds only exhibiting detector saturation above 5000 ppb QS and higher thresholds for detection (>30 ppb). This instrument configuration did reveal differences in source waters and since it shares excitation optical configuration, may aid in interpretation of refined hydrocarbon detect signals by UViLux HC instruments. In general ***care should be taken in specific interpretation of environmental fluorescence signals in absence of analytical reference samples.***

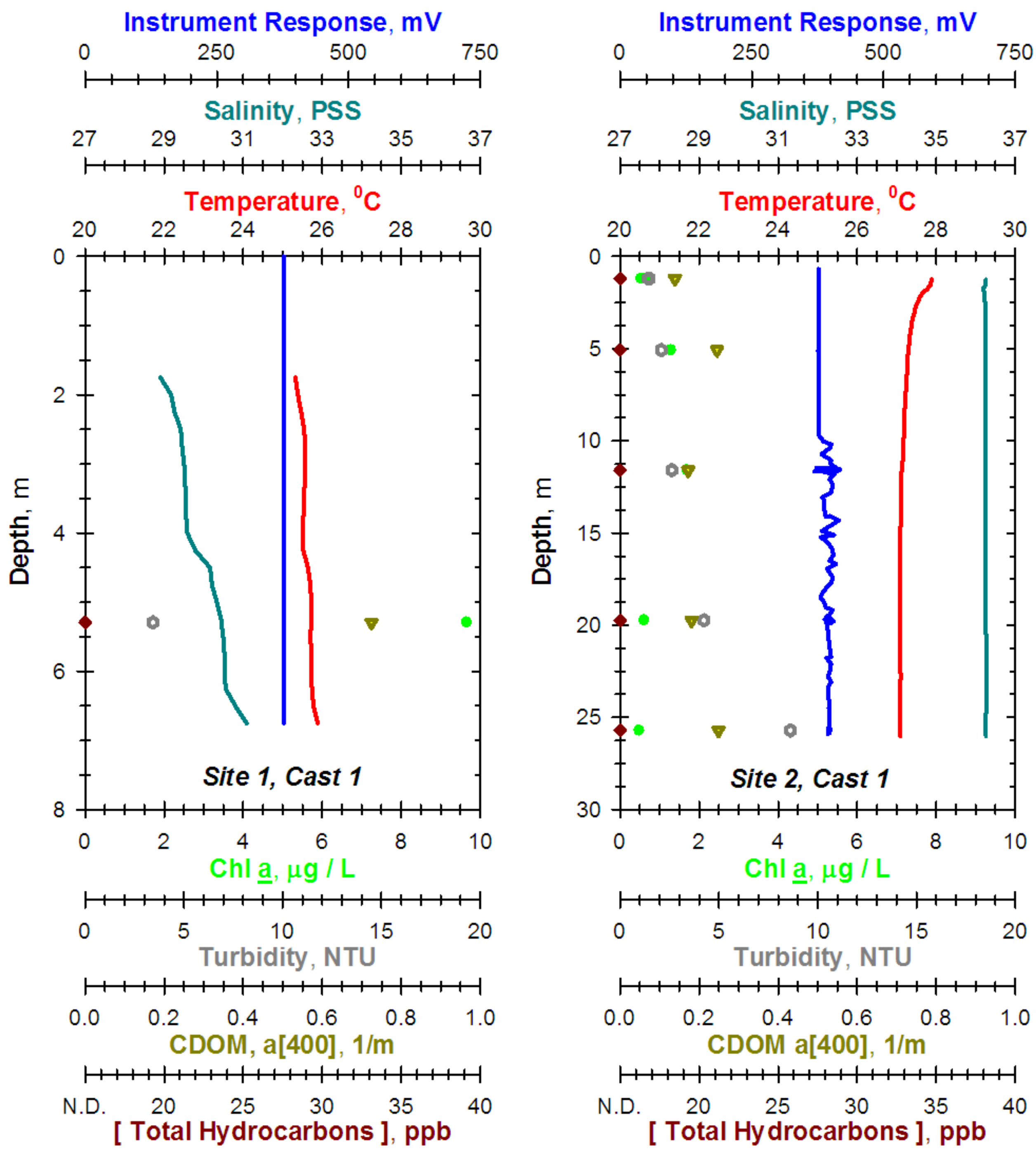


Figure 19. Gulf of Mexico field trials with the CTG UviLux CDOM, Site 1 and Site 2, Cast 1. Hydrocast profiles in a nearshore and offshore environment. Left panel: Site 1; Terrebone Bay (29.02.791N. 90.33.410W). Right Panel: Site 2, Cast1; 1000' due East of a sunken fuel oil barge (28.34.03N, 90.58.58W). Note that scale is expanded relative to previous figures to reveal small responses in this environment.

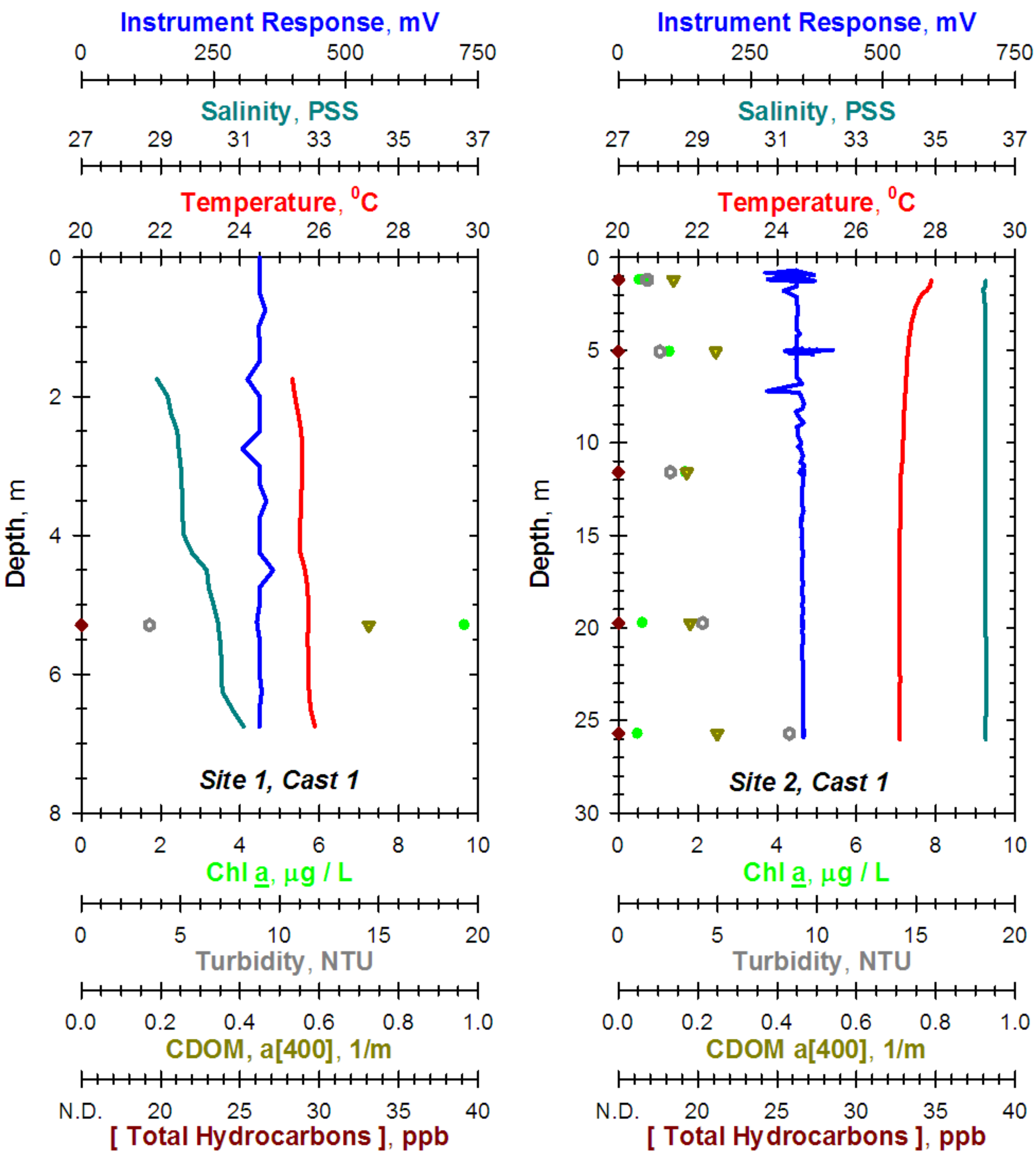


Figure 20. Gulf of Mexico field trials with the CTG UviLux HC, Site 1 and Site 2, Cast 1. Hydrocast profiles in a nearshore and offshore environment. Left panel: Site 1; Terrebone Bay (29.02.791N. 90.33.410W). Right Panel: Site 2, Cast1; 1000' due East of a sunken fuel oil barge (28.34.03N, 90.58.58W). Note that scale is expanded relative to previous figures to reveal small responses in this environment.

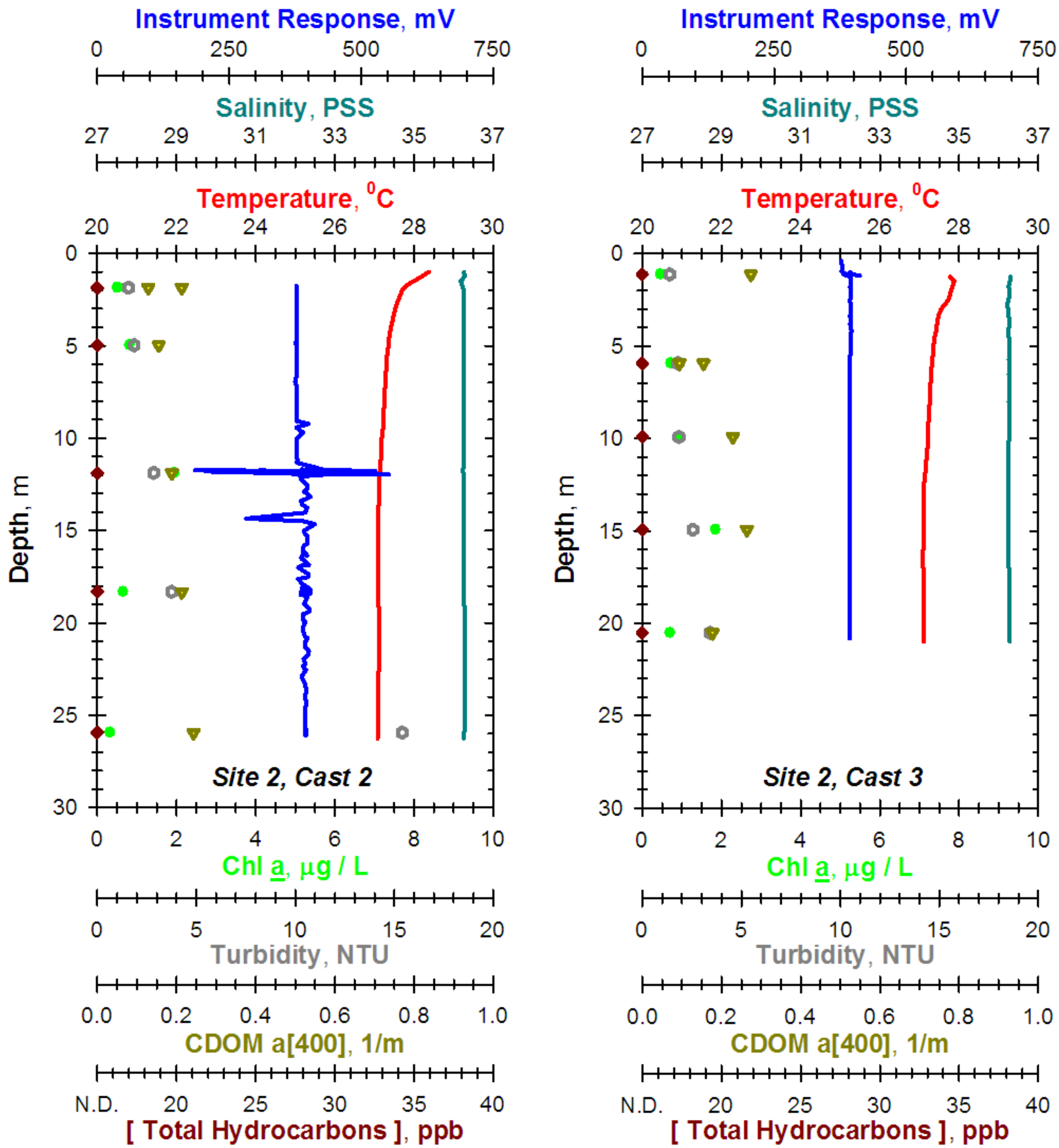


Figure 21. Gulf of Mexico hydrocast profiles from the CTG UviLux CDOM, Site 2, Cast 2 and Cast 3. Left panel: Cast 2; Inside slick from oil barge (28.34.075N, 90.58.738W). Right panel: Cast 3; Inside plume near submerged barge (28.34.049N, 90.58.878W). Refer to Fig. 19 for details.

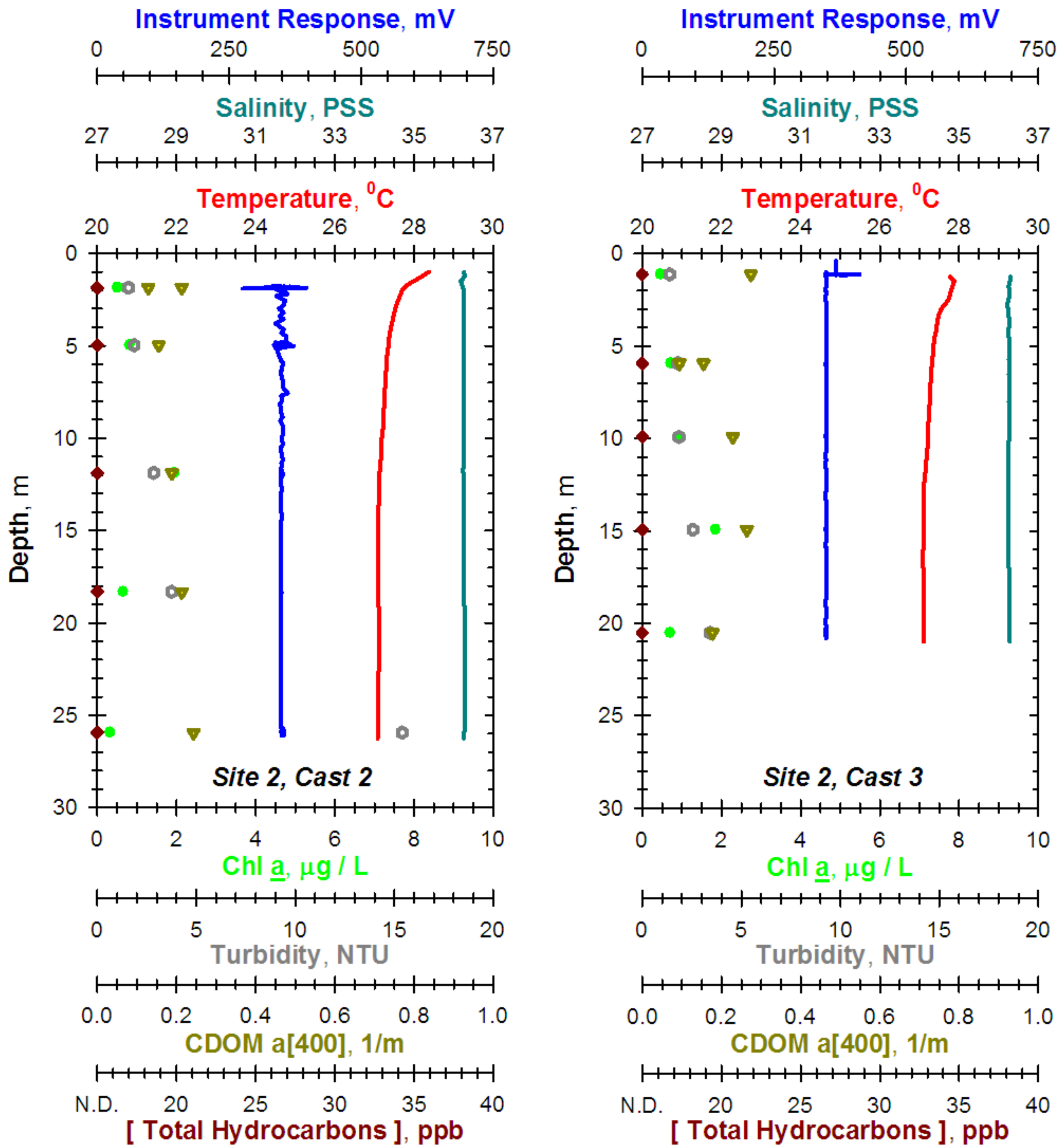


Figure 22. Gulf of Mexico hydrocast profiles from the CTG UviLux HC, Site 2, Cast 2 and Cast 3. Left panel: Cast 2; Inside slick from oil barge (28.34.075N, 90.58.738W). Right panel: Cast 3; Inside plume near submerged barge (28.34.049N, 90.58.878W). Refer to Fig. 19 for details.

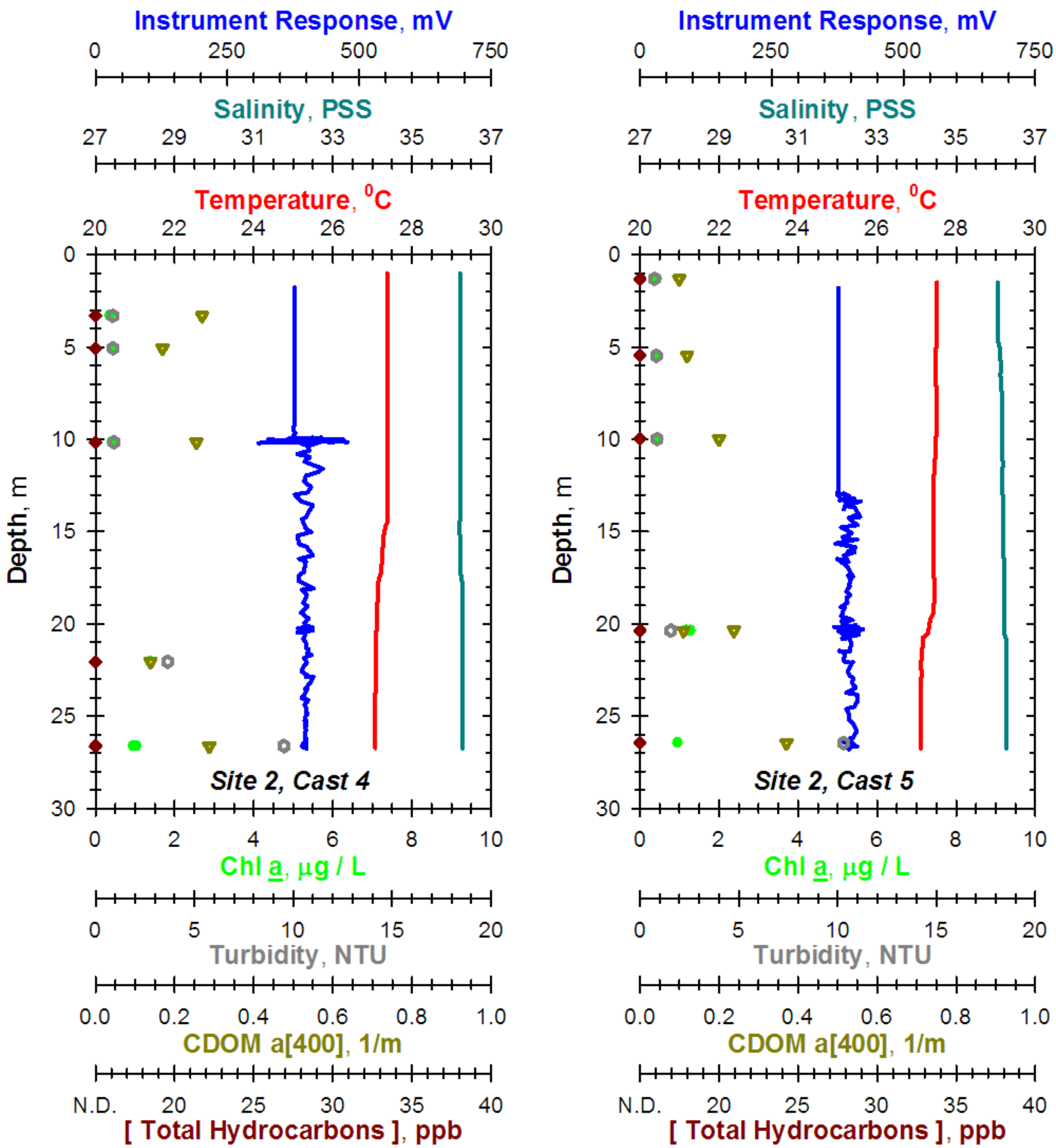


Figure 23. Gulf of Mexico hydrocast profiles from the CTG UviLux CDOM, Site 2, Cast 4 and Cast 5. Left panel: Cast 4; Over sunken barge, drifting with surface slick (28.34.034N, 90.58.891W). Right panel: Cast 5; Down stream of sunken barge (28.34.089N 90.58.940W). Refer to Fig. 19 for details.

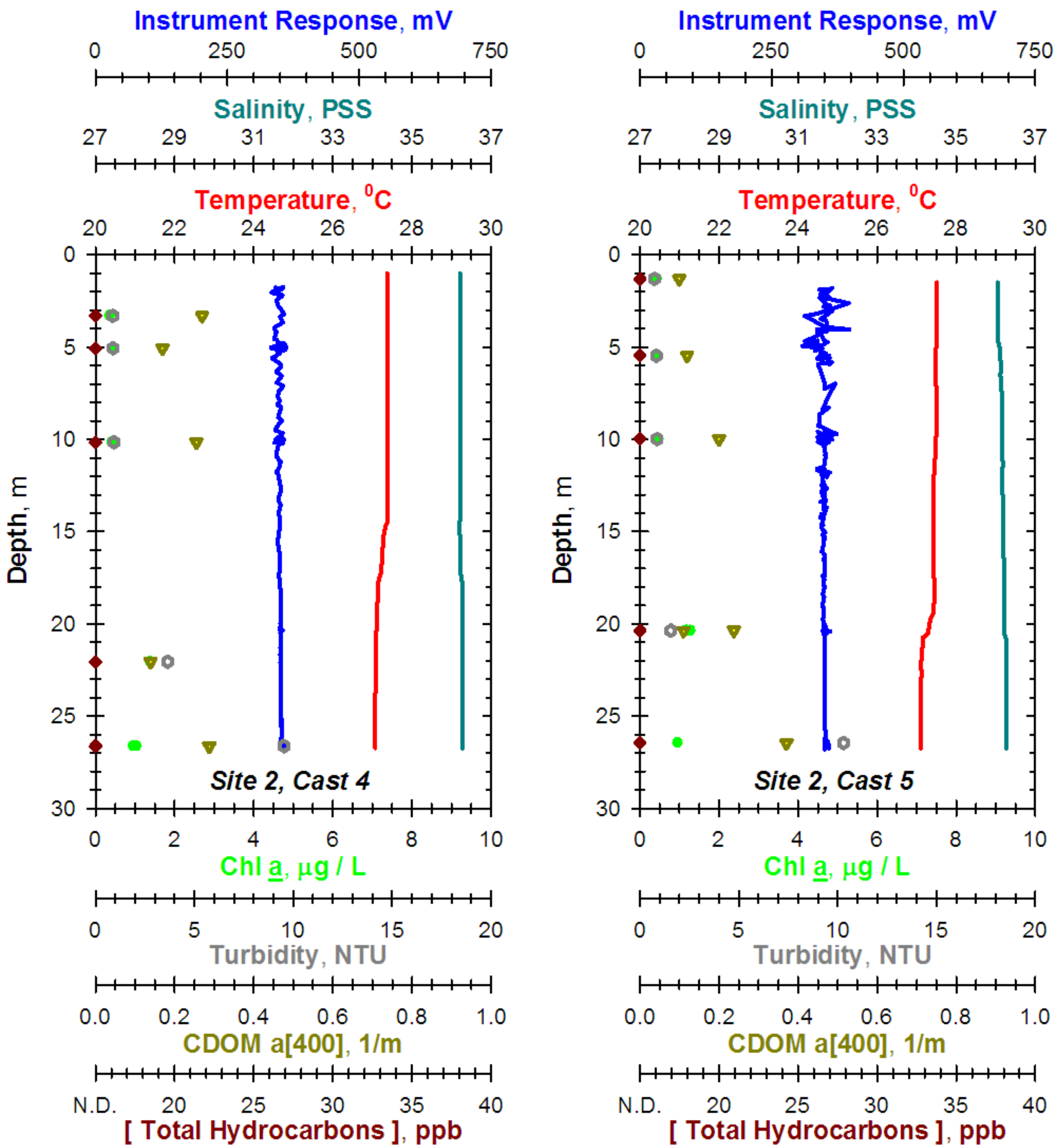
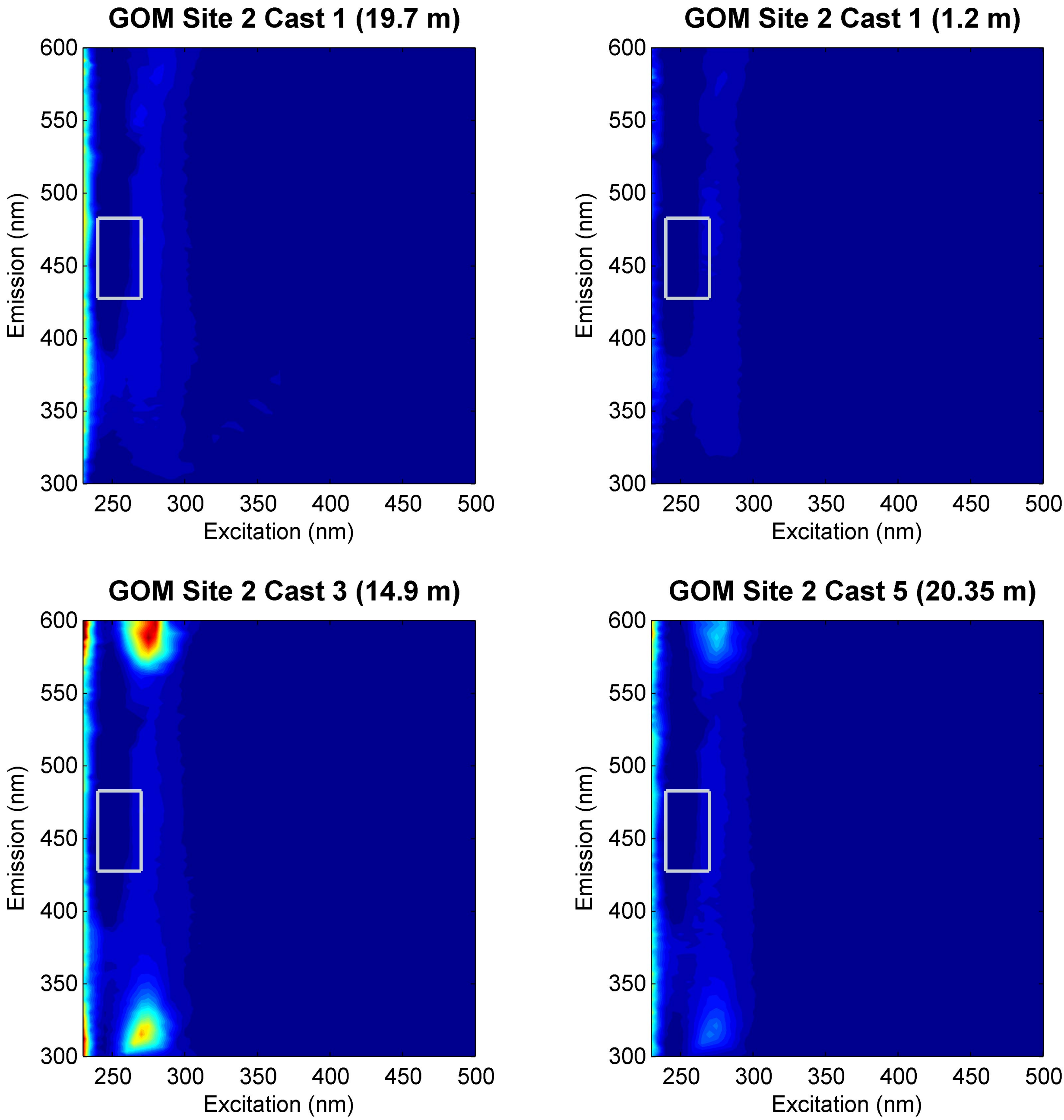
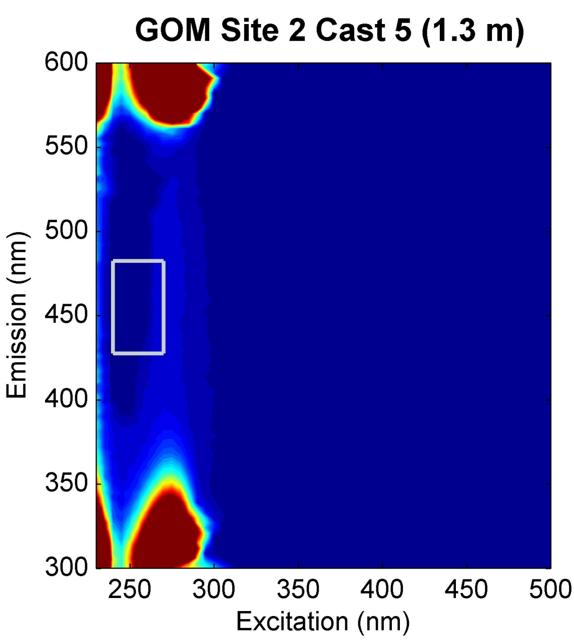


Figure 24. Gulf of Mexico hydrocast profiles from the CTG UviLux HC, Site 2, Cast 4 and Cast 5.
Left panel: Cast 4; Over sunken barge, drifting with surface slick (28.34.034N, 90.58.891W). Right panel: Cast 5; Down stream of sunken barge (28.34.089N 90.58.940W). Refer to Fig. 19 for details.





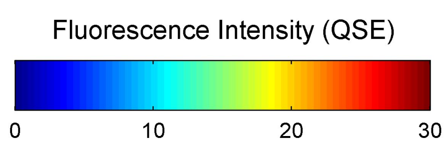
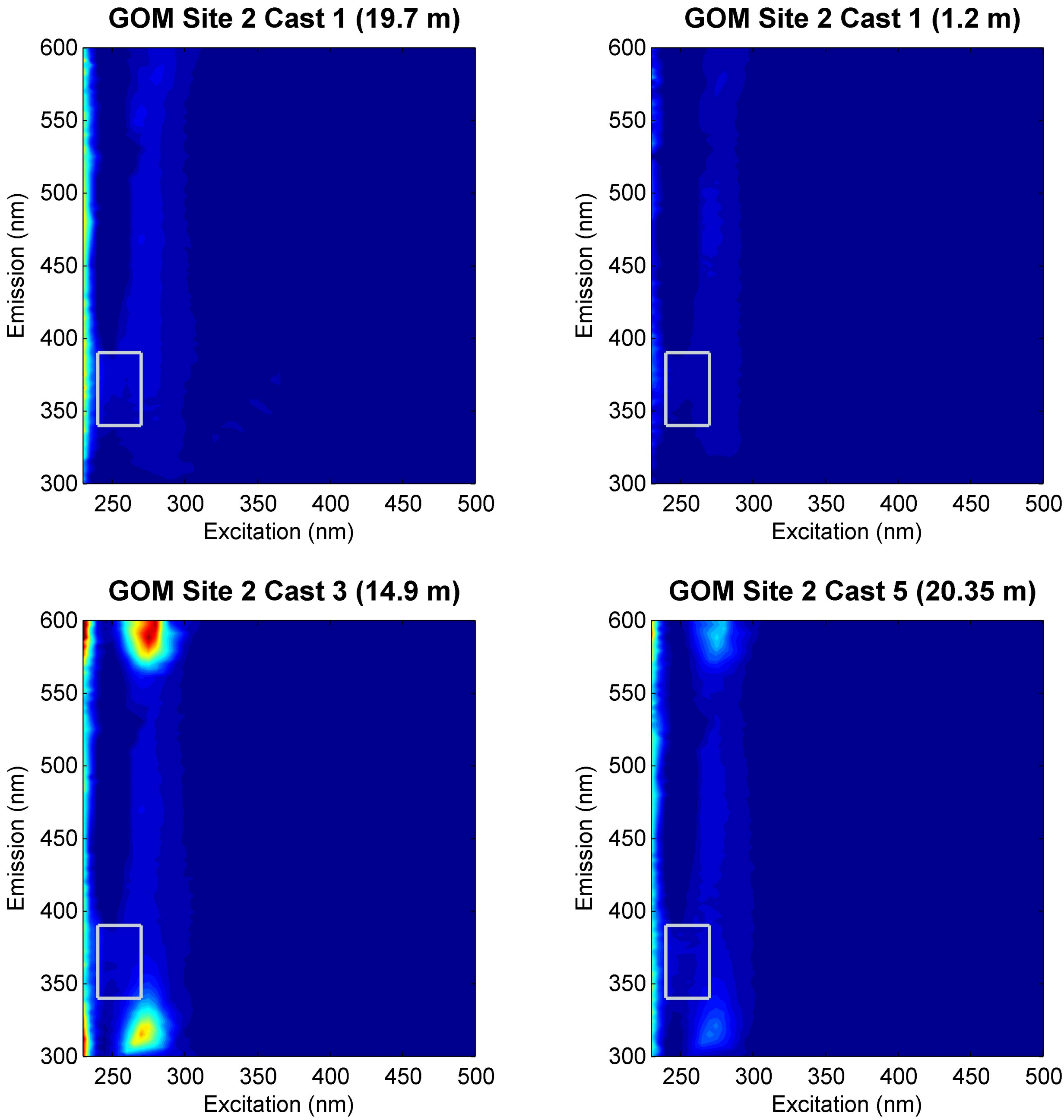
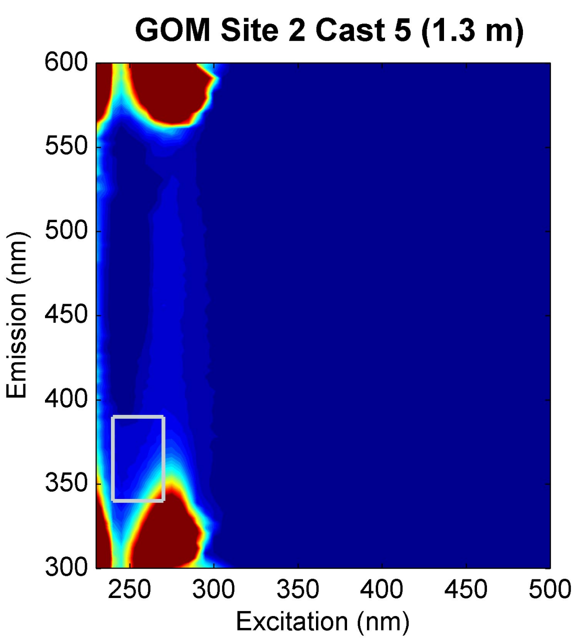


Figure 25. Representative EEM_{QSE} for Gulf of Mexico hydrocast samples with the optical window for the CTG UviLux CDOM represented by the grey outline box. No petroleum hydrocarbons were detected by GC-FID.





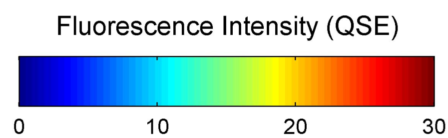


Figure 26. Representative EEM_{QSE} for Gulf of Mexico hydrocast samples with the optical window for the CTG UviLux HC represented by the grey outline box. No petroleum hydrocarbons were detected by GC-FID.

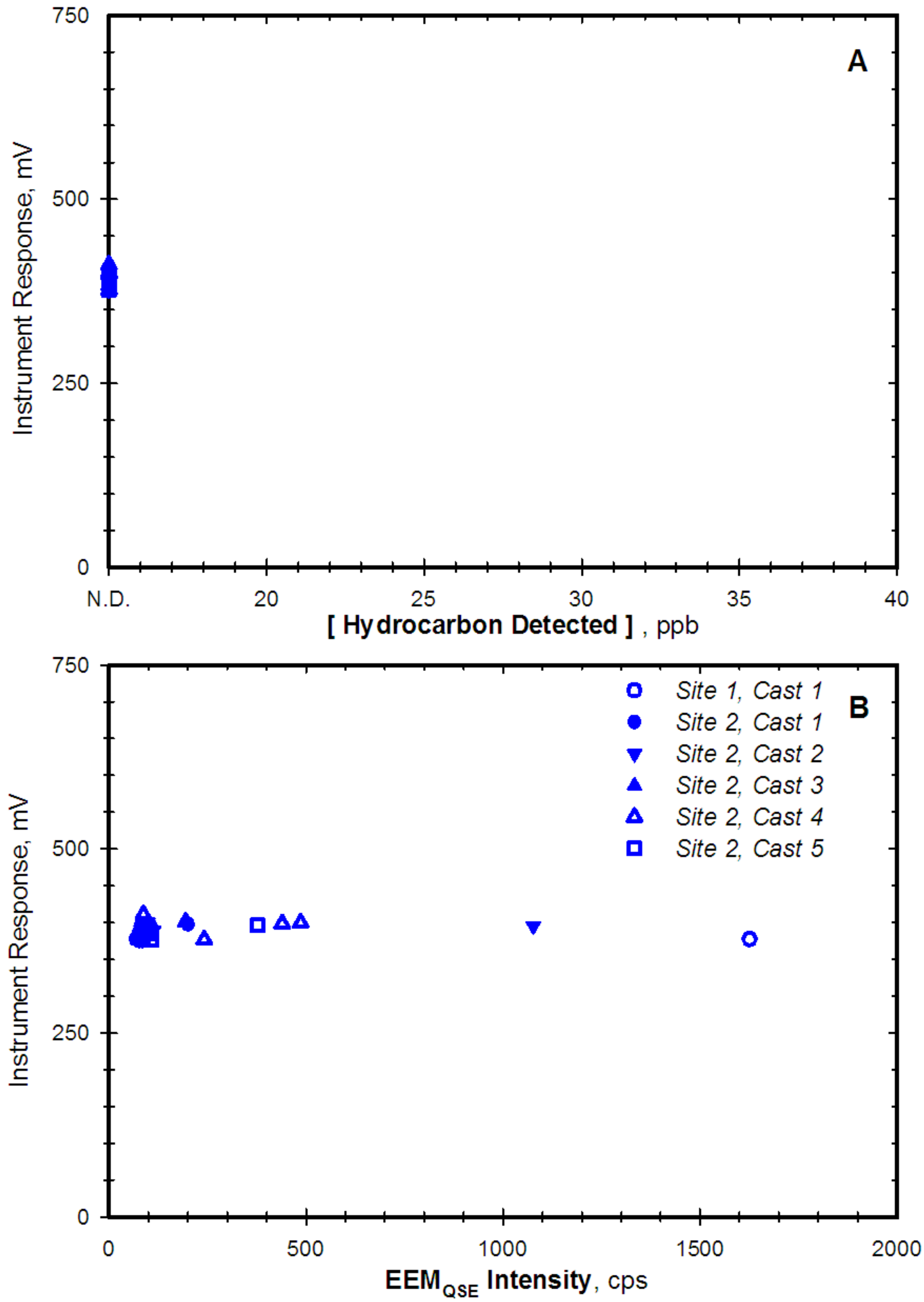


Figure 27. Response of the CTG UviLux CDOM to ambient hydrocarbons and water fluorescence properties at the Gulf of Mexico hydrocast sites. (A) Instrument response to TPH detected by GC-FID, no TPH reported for this same batch. (B) Instrument response relative to predicted EEM$_{QSE}$ fluorescent intensity. Scale has been expanded relative to other figures to help reveal instrument response in this environment. Ambient CDOM levels based on EEM$_{QSE}$ lower than observed in BH or COOGER wave tank.

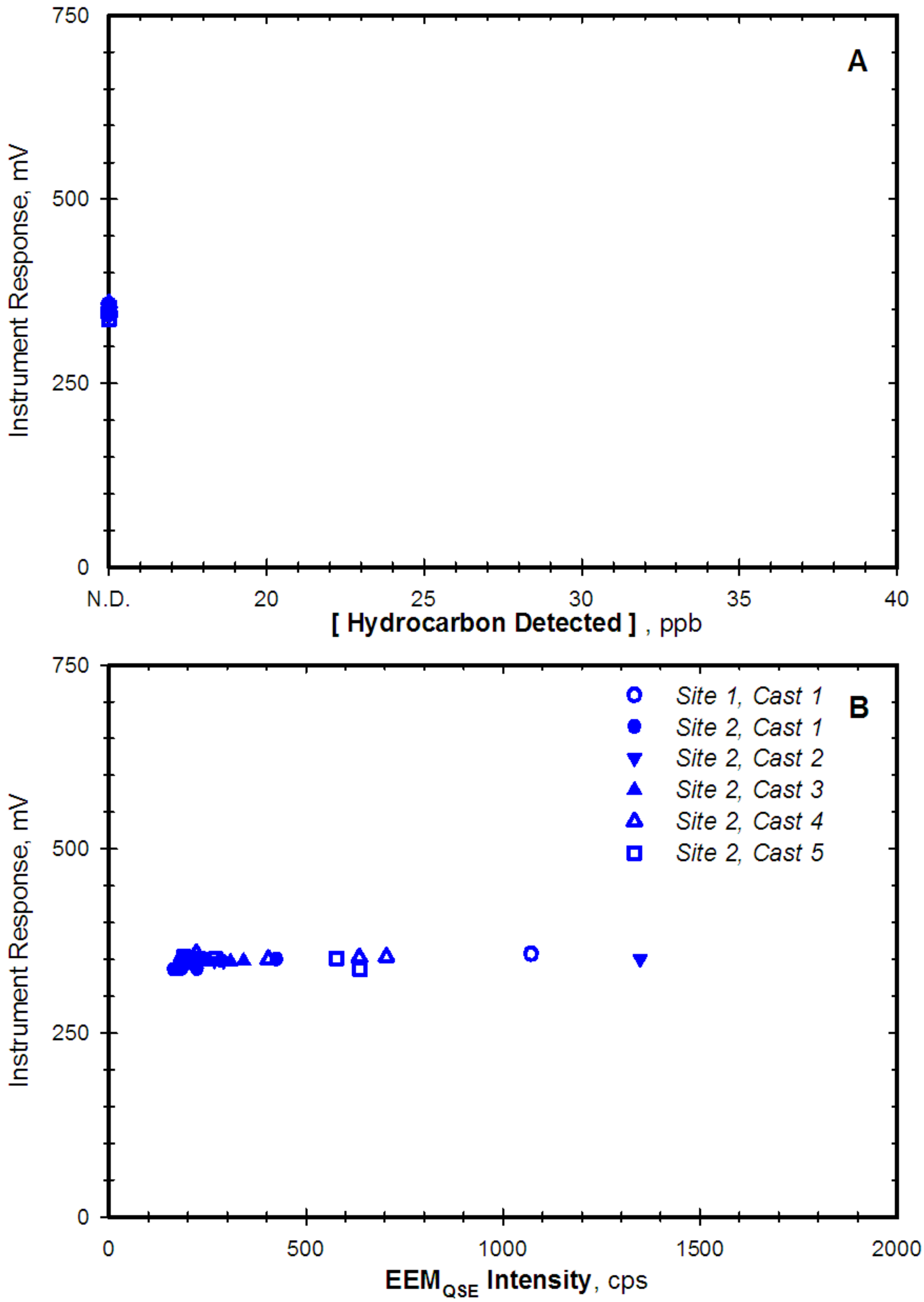


Figure 28. Response of the CTG UviLux HC to ambient hydrocarbons and water fluorescence properties at the Gulf of Mexico hydrocast sites. (A) Instrument response to TPH detected by GC-FID, no TPH reported for this same batch. (B) Instrument response to predicted EEM_{QSE} fluorescent intensity. Scale has been expanded relative to other figures to help reveal instrument response in this environment. Ambient CDOM / HC levels based on EEM_{QSE} generally lower than observed in BH or COOGER wave tank.

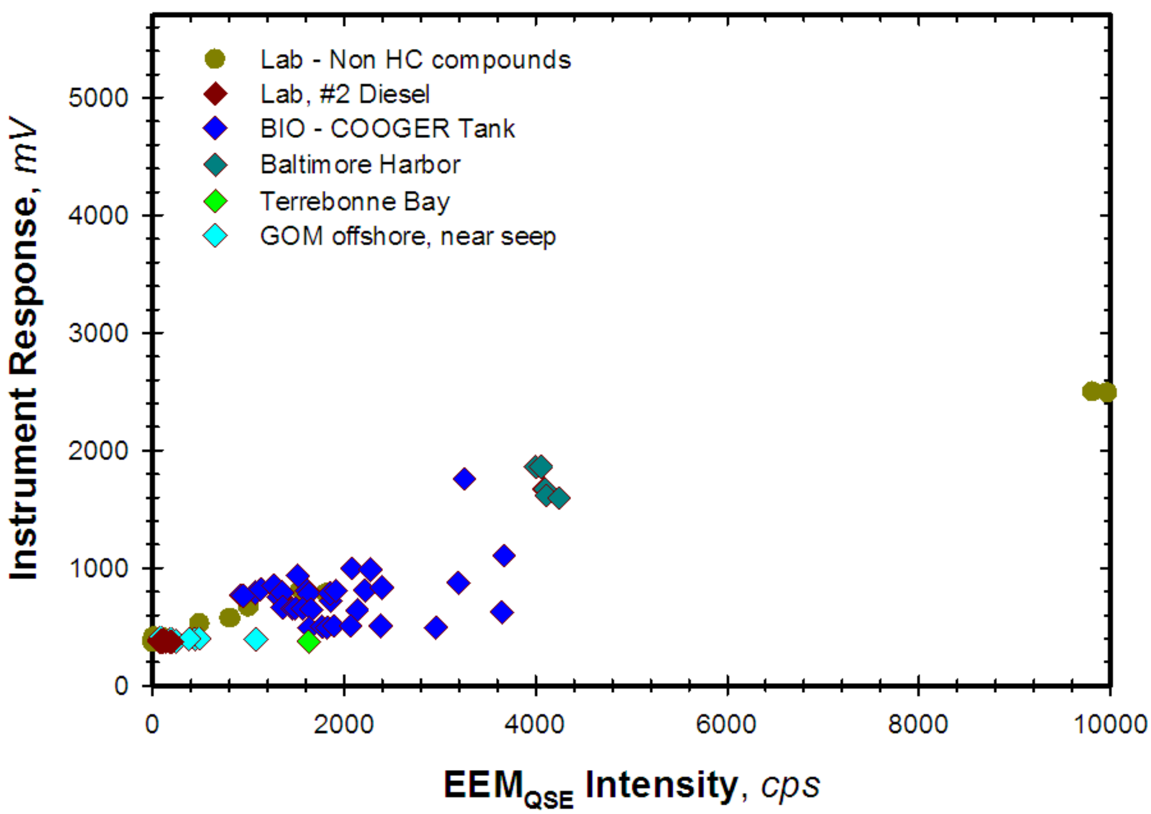


Figure 29. Global response of the CTG UviLux CDOM instrument to water fluorescence properties derived from added challenge compounds, defined crude oils physically dispersed in presence or absence of chemical dispersant (BIO-COOGER Tank) or in natural waters with varying turbidity and CDOM loadings (Baltimore Harbor, Terrebonne Bay, GOM off-shore). EEM_{QSE} axis scaled to range bounding challenge compound additions in these experiments (up to 100 ppm). Field deployment sites varied by over a factor of two in ambient CDOM loads (cf. GOM vs Baltimore Harbor).

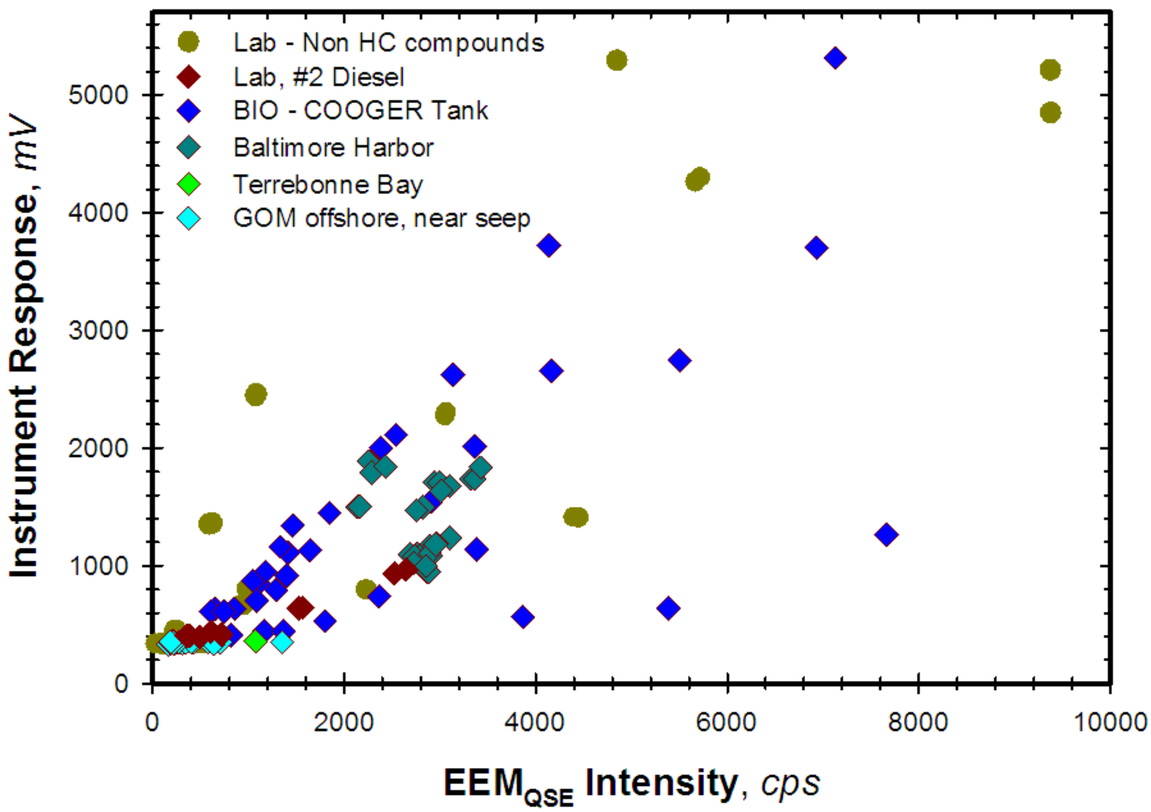


Figure 30. Global response of the CTG UviLux HC instrument to water fluorescence properties derived from added challenge compounds, defined crude oils physically dispersed in presence or absence of chemical dispersant (BIO-COOGER Tank) or in natural waters with varying turbidity and CDOM loadings (Baltimore Harbor, Terrebonne Bay, GOM off-shore). EEM$_{QSE}$ axis scaled to range bounding challenge compound additions in these experiments and saturation of instrument detector (up to 100 ppm). This sensor configuration detected a range of known petroleum based hydrocarbons with sensitivity dependent on dispersion state and chemical composition. The sensor configuration also discriminated differences in ambient CDOM / HC loads among water types (cf. GOM vs Baltimore Harbor).

QUALITY ASSURANCE/QUALITY CONTROL

Quality Assurance (QA)/Quality Control (QC) procedures were performed in accordance with the Test Protocols for this verification test, except where noted specifically within this report. Changes as noted had no impact on the quality of the results. QA/QC procedures and results are described below.

Quality Control Samples

Three types of QA samples were collected as part of our discrete reference sampling protocols: laboratory duplicates, field duplicates, and field trip blanks. Lab duplicates were repeated analysis from the same field collected sample. Field duplicates were two separate field samples collected as close in time and space as possible and processed identically. Field trip blanks were milli-Q DI that was carried into the field in a Van Dorn sampling bottle and then processed identically alongside a normal reference sample. Only one reference sample/field duplicate pair from Baltimore Harbor had a detectable hydrocarbon concentration and the detection was only observed for the field replicate and not the reference sample pair. A summary of the relative percent difference and precision within the QA samples for our ancillary measurements of turbidity, chlorophyll, and CDOM are presented in tables 5-7. QA results for hydrocarbon concentrations in field samples could not be computed, except for the Wave Tank test, because almost all samples were below detection. The average relative precision (95% confidence interval) among triplicate hydrocarbon determinations over all 5 trials was 20 percent, with a range of 1 – 40 percent for this test.

Table 5. Turbidity results for laboratory duplicates and field duplicates of reference samples for the two field test sites in Baltimore Harbor, site 2 in the Gulf of Mexico, and the Wave Tank experiments performed at the Bedford Institute of Oceanography (BIO), Halifax, Nova Scotia. Samples were analyzed on-site with a benchtop Hach 10AN turbidometer.

Site	QA Sample Type	# obs	Mean (s.d.)	95% C.I. Absolute Precision	Average Relative % difference
Baltimore Harbor	Field Blank	3	0.11 (0.05)	0.99	na
	Lab Dup	2	4.1 (0.1)	0.09	6.1
	Field Dup	7	2.6 (0.2)	0.19	13.3
Gulf of Mexico	Field Blank	0	nd[1]	nd[1]	nd[1]
	Lab Dup	0	nd[2]	nd[2]	nd[2]
	Field Dup	5	3.2 (0.02)	0.02	1.6
BIO Wave Tank	Field Blank	1	0.04	na	na
	Lab Dup	7	0.69 (0.04)	0.10	6.9
	Field Dup	5	1.37 (0.08)	0.12	8.6

nd[1]: no data; field trip blank was not collected during profiling
nd[2]: no data; lab duplicates for CDOM were not collected during profiling
na: not applicable

Table 6. Chlorophyll results for laboratory duplicates and field duplicates of reference samples for the two field test sites in Baltimore Harbor, site 2 in the Gulf of Mexico, and the Wave Tank experiments performed at the Bedford Institute of Oceanography (BIO), Halifax, Nova Scotia. Samples were analyzed at the Chesapeake Biological Laboratory, Solomons, MD.

Site	QA Sample Type	# obs	Mean (s.d.)	95% C.I. Absolute Precision	Average Relative % difference
Baltimore Harbor	Field Blank	3	0.03 (0.02)	0.71	na
	Lab Dup	31	16.6 (1.1)	0.15	10.9
	Field Dup	7	13.4 (3.2)	0.45	31.7
Gulf of Mexico	Field Blank	0	nd[1]	nd[1]	nd[1]
	Lab Dup	30	0.83 (0.03)	0.07	5.3
	Field Dup	5	0.84 (0.80)	0.11	7.9
BIO Wave Tank	Field Blank	1	0.00 (0.00)	na	na
	Lab Dup	34	0.51 (0.02)	0.07	5.3
	Field Dup	5	0.50 (0.01)	0.08	5.5

nd[1]: no data; field trip blank was not collected during profiling
na: not applicable

Table 7. CDOM (a[400]) results for laboratory duplicates and field duplicates of reference samples for the two field test sites in Baltimore Harbor, for site 2 in the Gulf of Mexico, and the Wave Tank experiments performed at the Bedford Institute of Oceanography (BIO), Halifax, Nova Scotia. Samples were analyzed at Moss Landing Marine Lab, Moss Landing, CA.

Site	QA Sample Type	# obs	Mean (s.d.)	95% C.I. Absolute Precision	Average Relative % difference
Baltimore Harbor	Field Blank	3	0.05 (0.05)	1.8	na
	Lab Dup	2	1.44 (0.04)	0.053	3.8
	Field Dup	6	1.50 (0.05)	0.065	4.6
Gulf of Mexico	Field Blank	0	nd[1]	nd[1]	nd[1]
	Lab Dup	0	nd[2]	nd[2]	nd[2]
	Field Dup	5	0.18 (0.04)	0.49	34.4
BIO Wave Tank	Field Blank	1	0.08 (0.01)	na	na
	Lab Dup	5	0.10 (0.07)	0.16	11.5
	Field Dup	5	0.88 (0.04)	0.08	5.7

nd[1]: no data; field trip blank was not collected during profiling
nd[2]: no data; lab duplicates for CDOM were not collected during profiling
na: not applicable

Audits

Three types of audits were performed during the verification test: a PE audit of the reference method measurements (GC-MS analyses), a technical systems audit (TSA) of the verification test performance, and a data quality audit.

Performance Evaluation Audit

A PE audit was conducted to assess the quality of the reference method measurements (GC-FID analyses) made in this verification test. The reference method PE audit was performed by supplying “blind” PE samples to TestAmerica, in Sacramento, CA as part of the laboratory tests.

A quantitative hydrocarbon standard for TPH analysis was formulated from freshly opened vial of #2 Diesel Fuel Oil (5000 ppm in methanol; Spex Certiprep S-WDF-25; Lot#T1101213004) using a 1:10000 dilution in MilliQ water for a final concentration of 500 ppb directly in the sampling jars provided by Test America. These spiked samples were shipped to and analyzed by Test America along with lab test samples for the same challenge compound. One of the spiked replicate samples was lost during transport. For the remaining blind sample the Test America analysis report indicated a TPH content of 410 ppb compared to the calculated original concentration of 500 ppb, or an underestimate of TPH of 18% by Test America. Comparison of paired samples from the laboratory tests with the same lot of #2 Diesel Fuel CRM indicated an average underestimate of predicted TPH of 9.5%.

Technical Systems Audit

Two TSAs were performed during this verification. The ACT Quality Manager performed a TSA on May 24-25, 2011 at Moss Landing Marine Laboratory during the initial laboratory tests; and the ACT Chief Scientist performed a TSA on October 31-November 2, 2011 during the tank tests at the Bedford Institute of Oceanography. The purpose of the TSAs was to assess and document the conformance of on- site testing procedures with the requirements of the Test Protocols and associated SOPs. The TSAs consisted of observations of instrument deployments, reference sample collections and analysis, and data acquisition and handling procedures. The TSAs also included an inspection of test records and documents, e.g., chain of custody (COC) documentation, record books, and instrument calibration logs. The audits confirmed that:

- Test instrument set-up and deployment was performed according to the Test Protocols and vendor instructions.
- Reference sample preparation procedures were performed according to the Test Protocols requirement.
- Test documentation provided a complete and traceable record of reference sample collection and analysis.
- Equipment used in the test was calibrated and monitored according to Test Protocols requirements and standard laboratory procedures.

There were no adverse findings. However, there were a number of deviations in the test procedures specified in the Test Protocols. These deviations are documented in this report and had no negative effects on the test data quality and objectives.

A TSA of the field tests in Baltimore Harbor was scheduled for August 15-16, 2011. The breakage of the mooring structure on August 14, 2011 resulted in a 4-day suspension of the field tests. The ACT QA Manager observed the recovery and inspection of the test instruments and repair of cables prior to redeployment on August 18, 2011. The mooring and instruments were restored to their initial condition, with the exception of the redeployment of the UViLux due to the absence of a replacement cable.

Data Quality Audit

The objective of the DQA is to determine if the test data were collected according to the requirements of the Test Protocols and associated SOPs. At least 10% of the data acquired during the verification test was required to be audited for completeness, accuracy and traceability. The ACT QA Manager traced data from the laboratory tests at Moss Landing Marine Labs and the Nova Scotia field tests from the initial acquisition, through reduction and statistical analysis, to final reporting to ensure the integrity of the reported results. Any calculations performed on the data undergoing the audit were checked. The DQA confirmed that no systematic errors were introduced during data handling and processing.

REFERENCES

Coble, P., Schultz, C., Mopper, K. 1993. Fluorescence contouring analysis of DOC intercalibration experiment samples: a comparison of techniques. Marine Chemistry: 41(1-3), 173–178.

Murphy, K., Butler, K., Spencer, R., Stedmon, C., Boehme, J., Aiken, G. 2010. Measurement of dissolved organic matter fluorescence in aquatic environments: an interlaboratory comparison. Environ. Sci.Technol.: 44 (24), 9405–9412.

Murphy, K.R. 2011. A note on determining the extent of the water Raman peak in fluorescence spectroscopy. Appl. Spectrosc: 65, 233-236.

Parsons, T. R., Maita, Y., and Lalli, C.M. 1984. A manual of chemical and biological methods for seawater analysis. Pergamon Press, New York. 174 p.

Zepp, R.G., Sheldon, W.M., Moran, M.A., 2004. Dissolved organic fluorophores in southeastern U.S. coastal waters: correction method for eliminating Rayleigh and Raman scattering peaks in excitation–emission matrices. Marine Chemistry: 89, 15– 36.

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February 10, 2013	
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February 10, 2013	
Date	**Approved By: Dr. Tom Johengen** **ACT Chief Scientist**
February 10, 2013	
Date	**Approved By: Dr. Earle Buckley** **Quality Assurance Supervisor**

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Dear Dr Tamburri,

COMMENTS ON ACT ASSESSMENT OF CTG'S UVILUX SENSORS

Many thanks for sending us the ACT report on our UviLux CDOM and Hydrocarbon fluorometers. We strongly support the aims of ACT and have been happy to participate in this specific programme, as we value any independent assessment of our sensor technology.

For this study we supplied two versions of our UviLux sensors: one targeting Polycyclic Aromatic Hydrocarbons (Hydrocarbon) the other Coloured Dissolved Organic Matter (CDOM). Both use a UV LED source and a photomultiplier detector. High quality optical filtration is used to define specific excitation and emission wavelengths with minimal crosstalk, thus providing excellent turbidity rejection. Both instruments provide high measurement sensitivities.

Our specific comments on the report are outlined below:

Laboratory testing

As for the programme of work undertaken on our UV AquaTracka sensors, we were generally impressed with the thoroughness undertaken in all the laboratory and field analyses. The EEMs studies in particular provided a clear indication of how well the UviLux sensors' excitation and emission wavelengths are matched to the various challenge compounds.

We were pleased to see that excellent regressions were achieved for all the test compounds that would be expected to generate a response with each of the sensors, based on the EEMs spectra. From this EEMs data it is clear that, while the challenge compounds used to test the CDOM UviLux were not well matched to this instrument's optical response, good correlations were still achieved for Quinine Sulphate and Carbazole, albeit at a significantly lower sensitivity than for the Hydrocarbon UviLux, as would be expected.

ISO 9001:2008 SGS UKAS MANAGEMENT SYSTEMS 005 ISO 13485:2003 SGS UKAS QUALITY MANAGEMENT 005

Registration No: 00832429
Registered at the above address

It would have been good to see the sensors challenged below 1ppb to demonstrate their lower limits of detection, this is particularly true for the Hydrocarbon UviLux's response to Carbazole in Figure 4, which extends well beyond the sensor's dynamic range of 10ppb. I would add the comment that since the study the lower detection limits have been further improved from the values quoted in the report to 0.005μg/l (Carbazole) and 0.002μg/l (Perylene) for the Hydrocarbon and CDOM UviLux sensors respectively.

Wave tank tests
As expected the EEMs for the crude oil samples used in the wave tank tests are not well matched to the CDOM UviLux's response, hence the weaker response. The Hydrocarbon UviLux on the other hand is well matched to the EEMs spectra and shows a strong response to all samples. The enhanced response seen with the dispersant treated samples might be explained by better dissolution or dispersion of the oil. For example, small oil droplets in untreated samples are likely to generate fluorescence only from their surface, due to inner filter effects within each droplet, hence a lower signal will be generated than for a better dispersed sample at the same concentration.

Moored field tests
Based on the original Hydrocarbon UviLux factory calibration the deployment in Baltimore is registering relatively low levels of hydrocarbon, equivalent to 2 - 4ppb Carbazole. At these levels it would be useful to know if the reference GC-FID analysis is working below its detection limits, hence the lack of any correlation.

The deployment trace presented in Figure 14 for the Hydrocarbon UviLux appears to be providing significant data over time, particularly for the peaks observed between the 23rd and 26th of August. It's a pity that the CDOM UviLux was non-operational due to cable damage for the latter part of the deployment, as this sensor is well matched to the sample fluorescence peaks observed in the EEMs data and would have helped to determine whether the Hydrocarbon UviLux was picking-up genuine hydrocarbon peaks or just variations in the background. For this reason, we agree with the statement made in the report's summary that it may be beneficial to use the CDOM sensor to interpret the results from the Hydrocarbon sensor in deployments where the background fluorescence it likely to vary significantly.

It was good to see that the sensors are not correlating with any of the other parameters measured during this deployment.

Vertical profiling field tests
No further comments to add to those already made in the report.

Yours sincerely,

John Attridge.

Dr John Attridge
Technical Director, Chelsea Technologies Group