PERFORMANCE VERIFICATION STATEMENT
for the Turner C3 Fluorometer

TECHNOLOGY TYPE: Hydrocarbon (Refined and Crude Oil) and CDOM sensors

APPLICATION: In situ estimates of hydrocarbons (oil-in-water) for coastal moored and profiled deployments

PARAMETERS EVALUATED: Response linearity, 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 a Turner Designs C3 in situ fluorometer configured for concurrent measurement of refined fuel, crude oil, and CDOM levels. The C3 package tested in this verification integrated the Cyclops Refined Fuel Fluorometer (Ex 254 nm, Em 350 nm), the Cyclops Crude Oil Fluorometer (Ex 320, Em 510) and the Cyclops CDOM fluorometer (Ex 320, Em 470). Response specificity of each individual sensor on the C3 to a range of organic compounds was evaluated in a series of lab tests. Instrument response with respect to challenge compound concentration varied with respect to the inherent fluorescence properties of the challenge compound, as well as, sensor optical configuration. The CDOM sensor detected quinine sulfate (QS) over a large dynamic range (0-5000 ppb tested) and was insensitive to other organic compounds tested. The Crude Oil sensor exhibited a similar but dampened response to QS challenges, as expected based on the optical overlap with the CDOM sensor, but additionally displayed a weak response to carbazole and #2 Diesel Fuel challenges. The Refined Fuel sensor exhibited a lower linear dynamic range (0-100 ppb) and distinct sensitivity to challenge compounds, with sensitivity ranked as Carbazole > #2 Diesel Fuel> NDSA > QS. Instrument responses to various challenge compounds linearly scaled with standardized EEMs fluorescence intensity for each compound as estimated to correspond with the instruments emission optics.

The C3 package provided discrimination between crude oil source type and dispersion state during wave tank trials at the COOGER facility at the Bedford Institute of Oceanography. Response sensitivity among the three sensors ranked as Refined Fuel > Crude Oil > CDOM. The Crude Oil and CDOM sensors response baselines were sensitive to daily changes in ambient water CDOM while the Refined Fuel sensor response was dampened to this ambient background.

Field deployments in Baltimore Harbor and northern Gulf of Mexico were equivocal as all but three field reference samples were below the limit of detection for total petroleum hydrocarbons (≤ 25 ppb), yet the output of each of the three sensors was significantly 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. 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. 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 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 Turner Designs' C3 Submersible Fluorometer was configured for this ACT evaluation with Refined Fuels, Crude Oil, and CDOM sensors used for measuring *in situ* fluorescence of these compounds in water. Each sensor uses a specific LED and filter set

(excitation/emission) for detecting fluorophores of interest. The excitation and emission filters are oriented at 45 degree angles to one another to allow fluorescence detection at a 90 degree angle. The Refined Fuels sensor uses a deep UV LED (260 nm) for excitation and detects compounds that fluoresce between 300-400 nm. Both the Crude Oil and CDOM sensors use a UV LED, centered at 365 nm, as an excitation source. The Crude Oil sensor has a wide band pass filter (400-600 nm), which allows detection of a broad range of oils in water, whereas the CDOM sensor has a narrower band pass filter (470 nm with a band pass of 60 nm) optimized for the fluorescence of CDOM. These three sensors are mounted equidistant from each other on the C3 Fluorometer's optical head which comes standard with a temperature probe.

The C3 Submersible Fluorometer was configured with a mechanical wiper located on the optical head used to reduce biofouling of the sensors during long term deployments. Opposite the optical head is the end cap which has a pressure sensor for measuring depth and an 8-pin stainless steel bulkhead connector that is used for communication and data transfer. The optical head and the end cap are fastened to a Delrin plastic housing using non-metal fasteners. The C3 Fluorometer is 9.1 inches long and has a diameter of 3.9 inches over the length of the instrument. The C3 Fluorometer is factory sealed and pressure tested to ensure its operation up to a depth of 600 meters and between -2 and 50 degrees Celsius. C-Soft is Turner Designs' proprietary software used to configure, calibrate, communicate with and download data from the C3 Submersible Fluorometer. Each sensor can be custom calibrated using a two point calibration method. Up to 64,000 data lines may be stored onto the C3's internal flash memory. The C3 Fluorometer may also be configured to stream real-time digital (ASCII) or analog (voltage) data to be used for integrating with CTD or third party dataloggers.

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 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 (as provided by manufacturers) were mapped onto corrected EEM space and fluorescence intensity similarly regressed and compared to regression of instrument reads versus challenge concentration in order to identify sources of response variation observed.

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.

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

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 oil challenge compounds 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 (BIO-COOGER). 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 BIO-COOGER 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 BIO-COOGER 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 18th and operated for 9 days prior to retrieval.

Instrument Setup – The test instruments was 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 site 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, wind speed and 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.
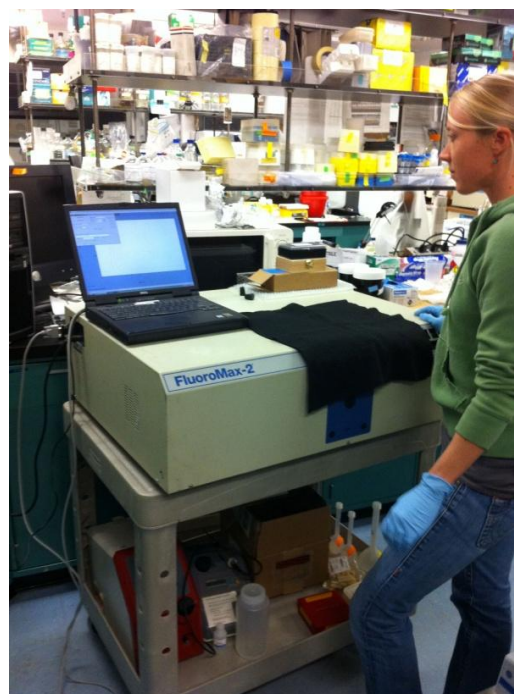
Photo 2. EEM's Generation

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 Turner C3-CDOM (Fig. 1), -Crude Oil (Fig. 2) and -Refined Fuel (Fig. 3) sensors. Excitation and Emission maximums of the challenge compounds varied by over 100 nm, with Quinine Sulfate mapping most closely with the optical windows of the CDOM and Crude Oil sensor filter sets, and Carbazole mapping most closely with the optical window of the Refined Fuel sensor filter set. Response curves for all three sensors were generated against each of the challenge compounds over concentrations ranging from 1 – 1000 ppb (see Fig. 4 for CDOM, Fig. 5 for Crude Oil, and Fig. 6 for Refined Fuel). Results show instrument response (in RFU) presented against both concentration and estimated EEM_{QSE} (Quinine Sulfate equivalent) fluorescence intensity for each challenge compound. Baseline signal in deionized water was less than 0.5 RFU for the CDOM and Crude Oil Sensors while the Refined Fuel sensor exhibited a higher baseline offset of approximately 6 RFU. As anticipated from their optical design, the CDOM and

Crude Oil sensors exhibited their greatest response sensitivity to QS, with near linear response through 5000 ppb QS (Figs. 4 & 5). The Crude Oil sensor exhibited a lower detection limit for QS (1 ppb) than the CDOM sensor (10 ppb). The CDOM and Crude Oil sensor response to increasing challenge concentration was approximately 20 times lower for those compounds with fluorescence maxima outside the optical window wavelength centers of the instruments filter set (e.g. Carbazole) and hence lower predicted EMM_{QSE} intensities (Figs. 4-6, panel B). In contrast the Refined Fuel sensor exhibited highest sensitivity to carbazole followed by NDSA and #2 Diesel Fuel and approximate 4.5 x lower sensitivity to QS through 1000 ppb challenges.

Ancillary water quality conditions for turbidity and CDOM are presented for each challenge compound at each of the concentrations tested (Fig. 7). 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 (Fig. 7, panel A). BB3 additions increased measured turbidity, but since none of individual sensors 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. 7, panel B) and reached asymptotes above 10 ppb additions, well below the instruments' response capacity (>100 ppb for QS and #2 diesel fuel oil).
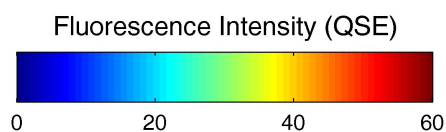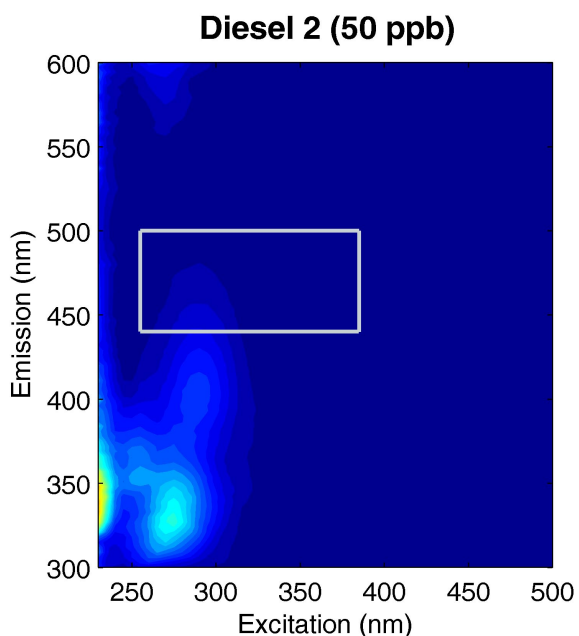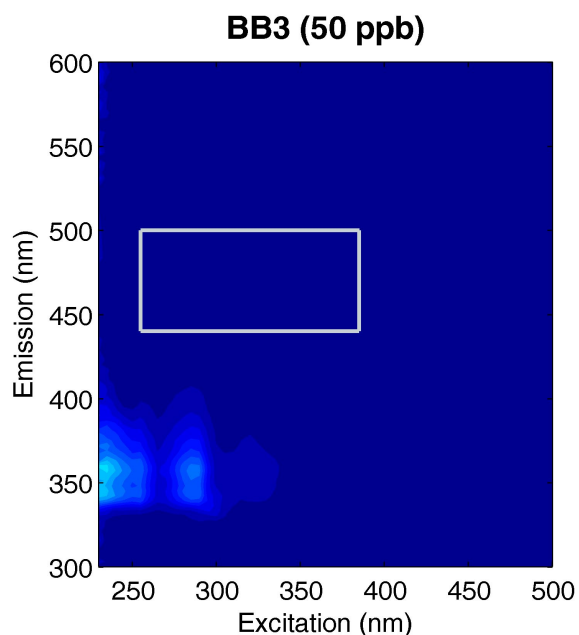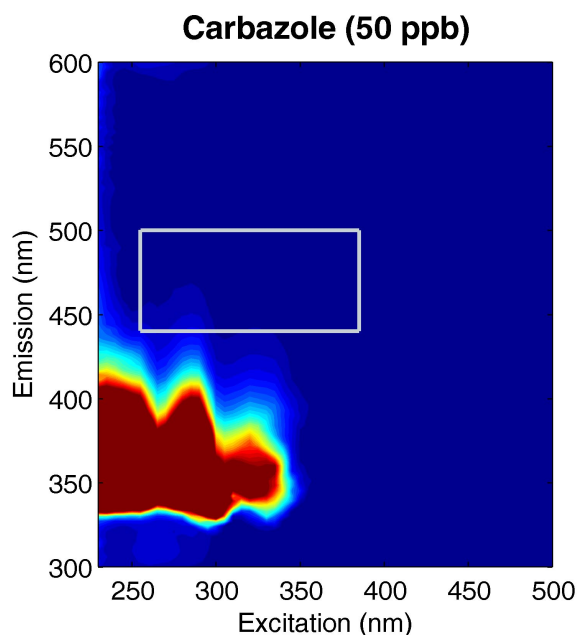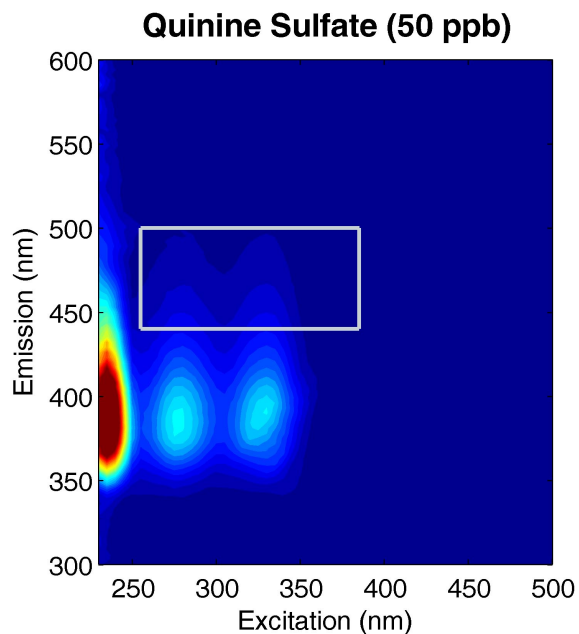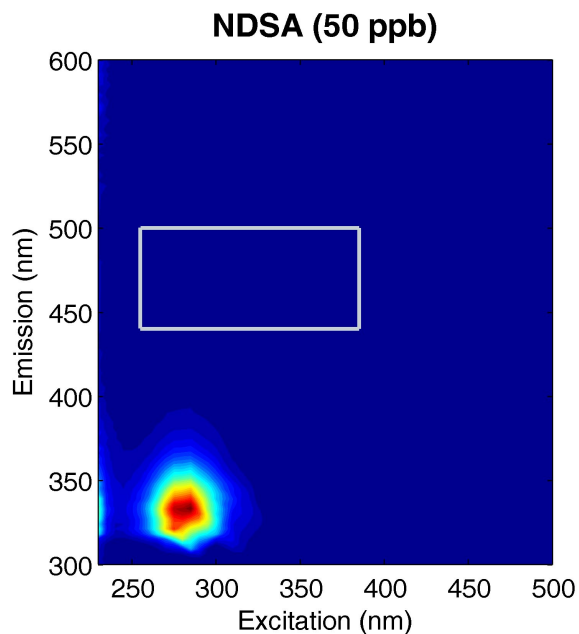
Figure 1. Excitation-Emission Matrix (EEM) fluorescence maps of challenge compounds used in the laboratory trials at Moss Landing Marine Laboratories. Reported fluorescence intensities (cps) are normalized to Quinine Sulfate Equivalents (QSE). Boxes denote optical window for the Turner C3 CDOM sensor 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.

NDSA (50 ppb)

Quinine Sulfate (50 ppb)

Carbazole (50 ppb)

BB3 (50 ppb)

Diesel 2 (50 ppb)

Fluorescence Intensity (QSE)
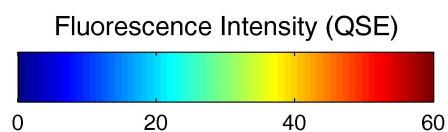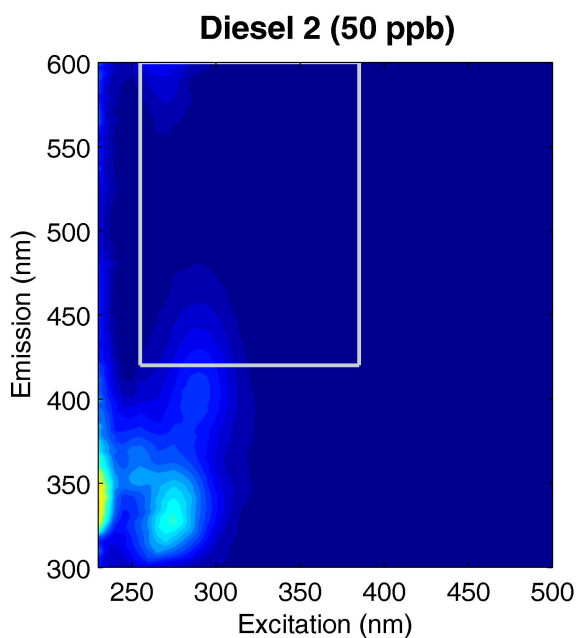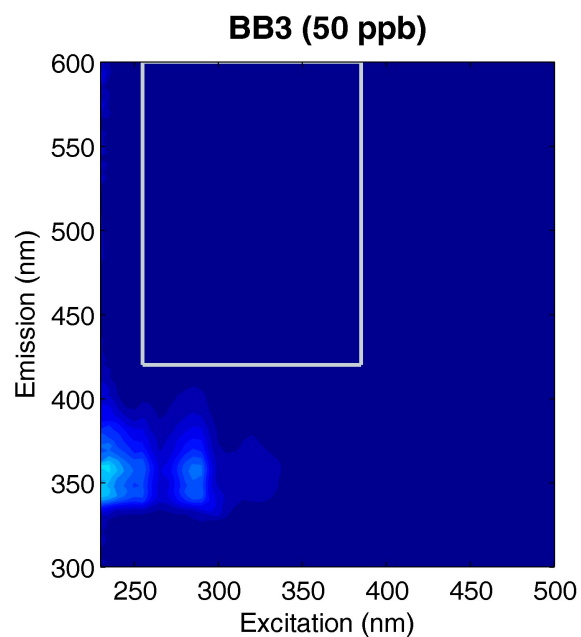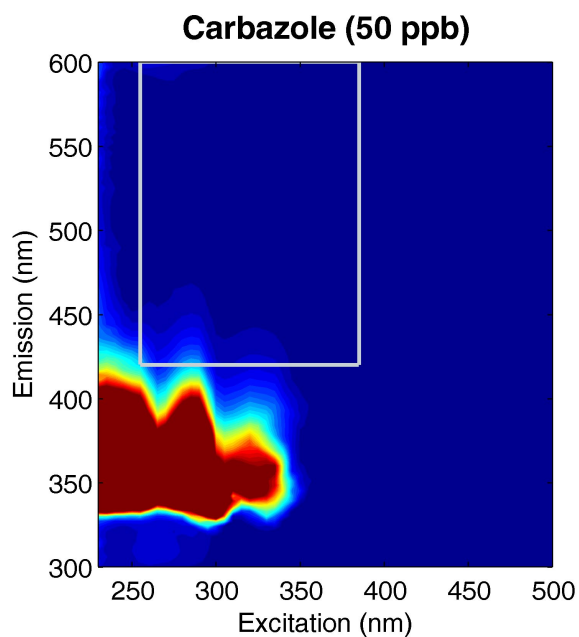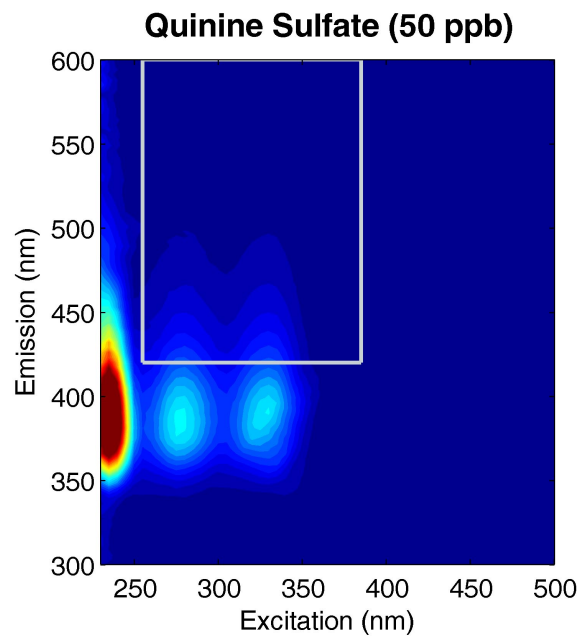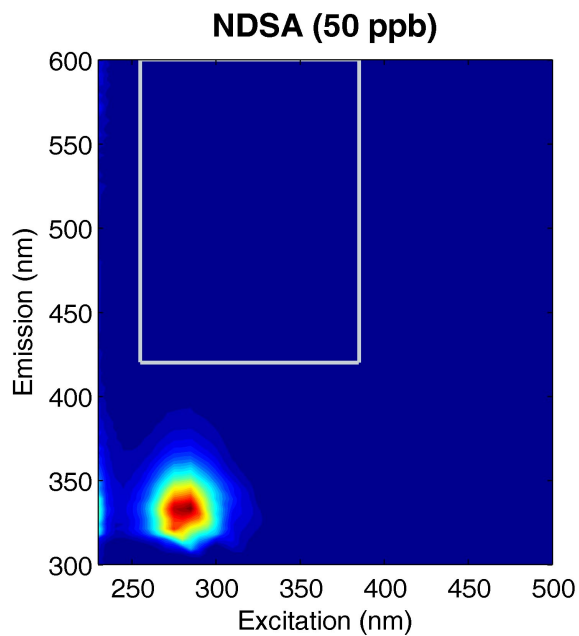
Figure 2. The Turner C3 Crude Oil Sensor optical window specification mapped onto EEM_{QSE} of lab challenge compounds as in Fig. 1.

NDSA (50 ppb)

Quinine Sulfate (50 ppb)

Carbazole (50 ppb)

BB3 (50 ppb)

Diesel 2 (50 ppb)

Fluorescence Intensity (QSE)
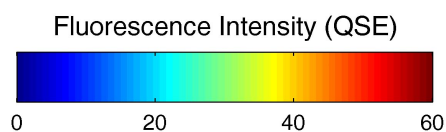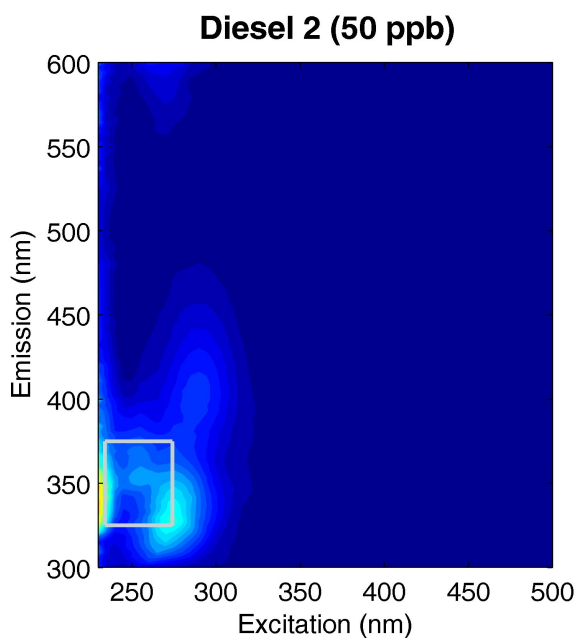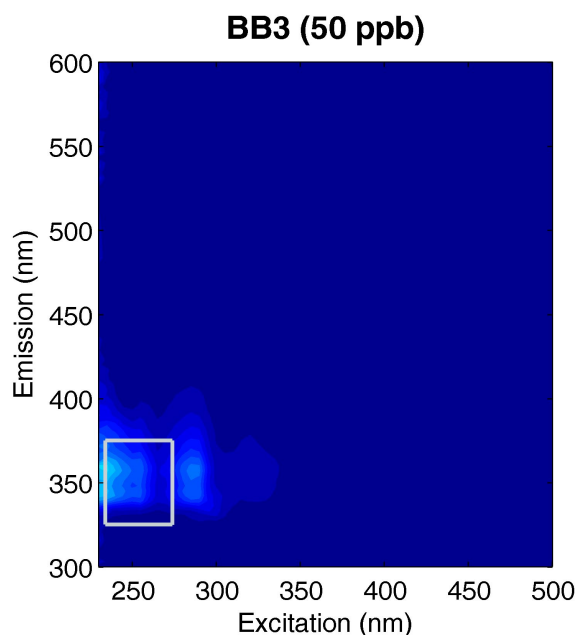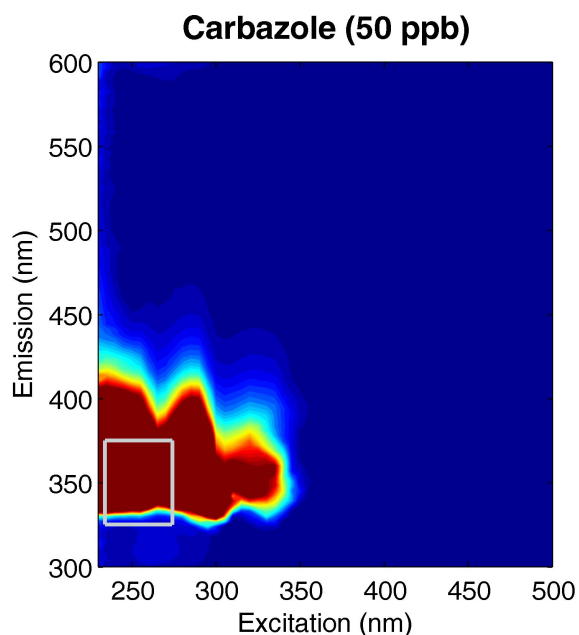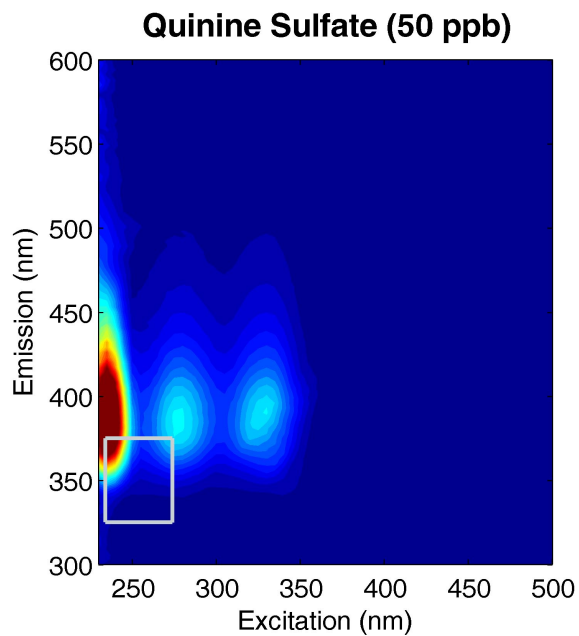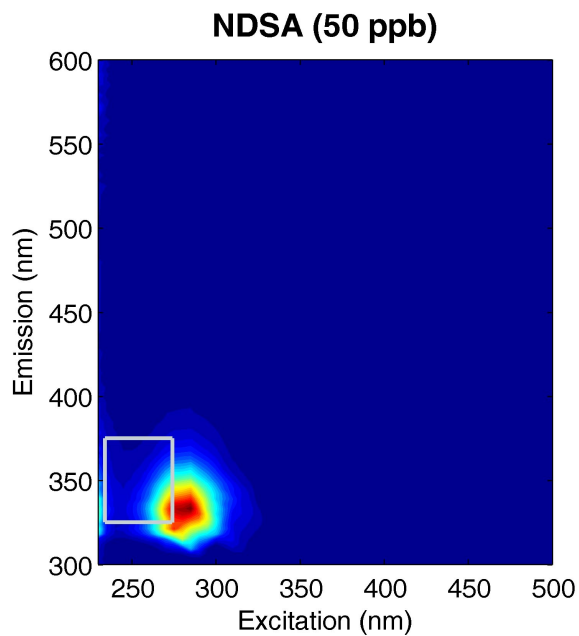
Figure 3. The Turner C3 Refined Fuel sensor optical window specification mapped onto EEM_{QSE} of lab challenge compounds as in Fig. 1.
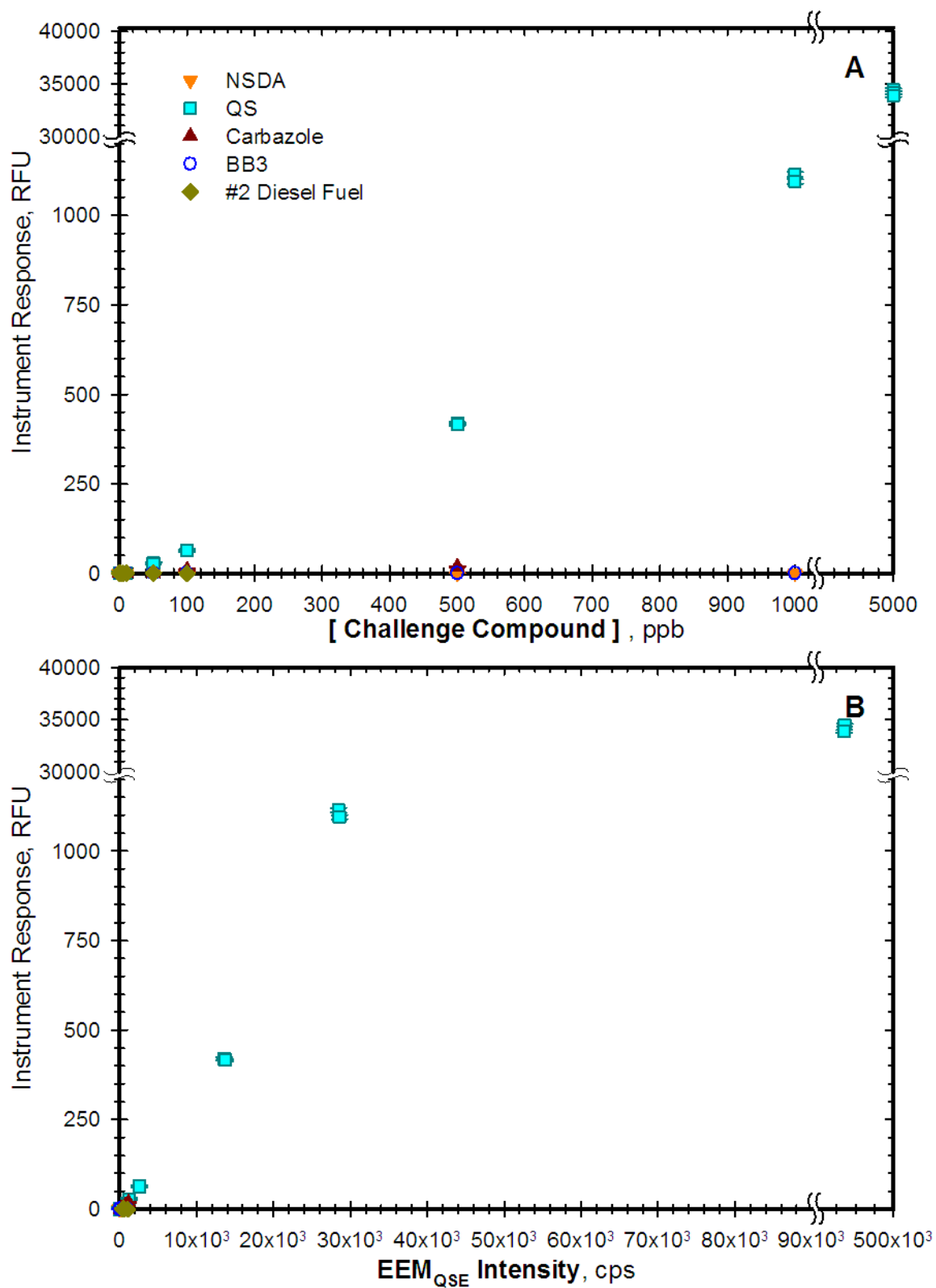
Figure 4. Response of Turner C3 CDOM sensor to challenge compound additions. Reported measurements represent the average (+/- S.D.) instrument response over 10 minute windows starting 30 minutes after addition of the challenge compound. (A) Instrument response to concentration of challenge compound in 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. After axis break, EEM$_{QSE}$ scaling changes to 100,000 cps per tick, RFU scaling changes to 1000 per tick.
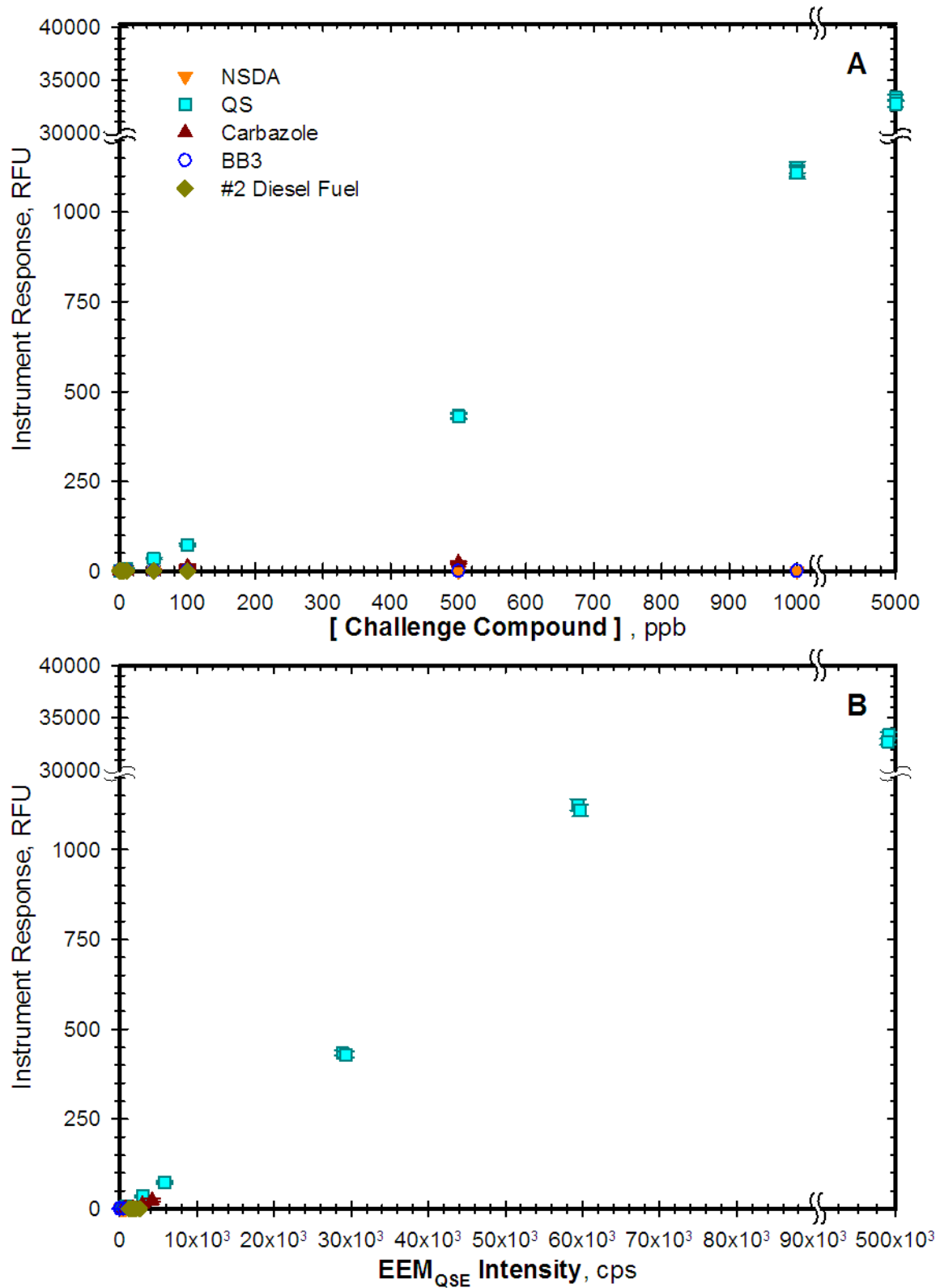
Figure 5. Response of Turner C3 Crude Oil sensor to challenge compound additions. Reported measurements represent the average (+/- S.D.) instrument response over 10 minute windows starting 30 minutes after addition of the challenge compound. (A) Instrument response to concentration of challenge compound 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. After axis break, EEM_{QSE} scaling changes to 100,000 cps per tick, RFU scaling changes to 1000 per tick.
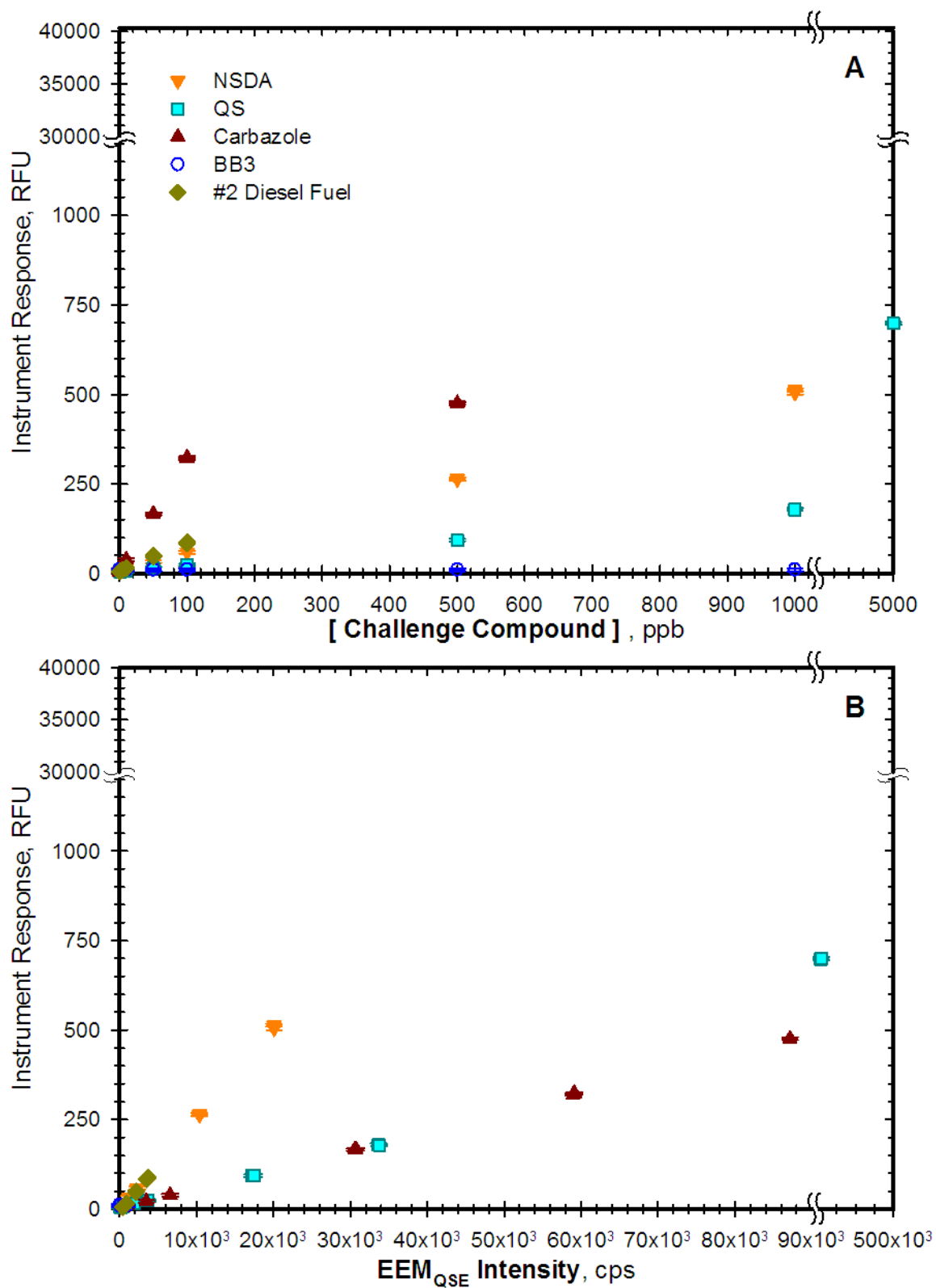
Figure 6. Response of Turner C3 Refined Fuel sensor to challenge compound additions. Reported measurements represent the average (+/- S.D.) instrument response over 10 minute windows starting 30 minutes after addition of the challenge compound. (A) Instrument response to concentration of challenge compound 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. After axis break, EEM_{QSE} scaling changes to 100,000 cps per tick, RFU scaling changes to 1000 per tick.
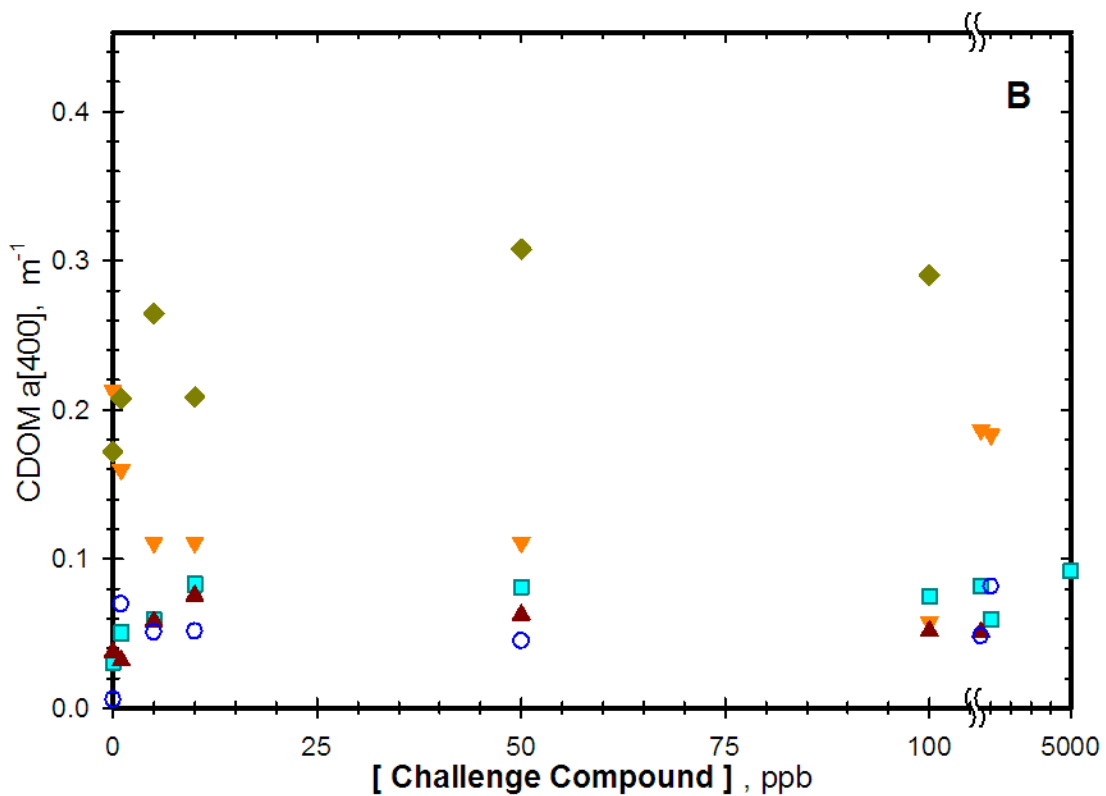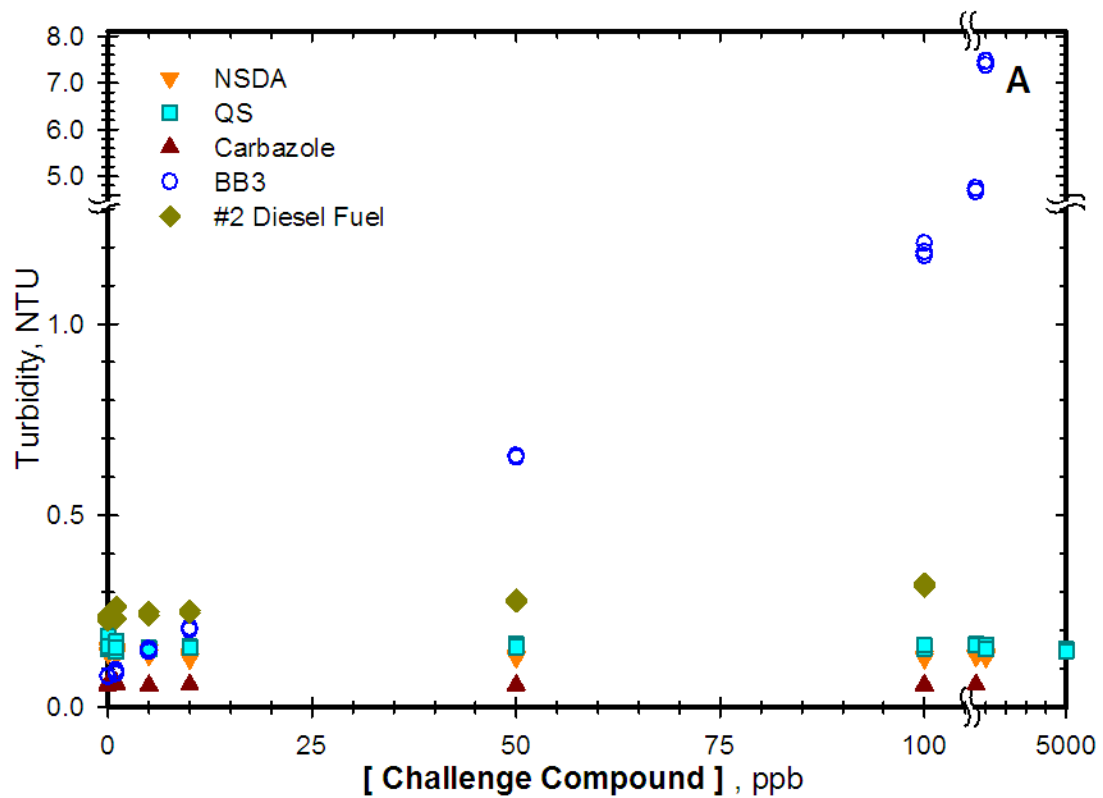
Figure 7. Variation in Turbidity and CDOM measurements with respect to challenge compound and challenge concentration during laboratory trials. (A) Turbidity (NTU) measured with a calibrated Hach 2100 AN. (B) Relative CDOM (chromophoric dissolved organic matter) concentration estimated as the absorbance 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 (^{o}C)	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 CDOM, Crude Oil and Refined Fuel sensors to the stepwise addition of crude oil and dispersant are plotted in figures 8, 9, and 10, respectively. Each lettered panel represents a day-long test of specific source oil and dispersant ratio at seven different concentrations, ranging from background to approximately 12 ppm oil (see figure legends). The highest concentration was not tested on day 1, but this challenge experiment was repeated on day 3 during which the highest concentration level was included. The background fluorescence of the incoming seawater varied with each daily trial averaging between $100 - 300$ RFU for the CDOM sensor, $100 - 450$ RFU for the Crude Oil sensor, and $10 - 20$ RFU for the Refined Fuel sensor. The elevated ambient background fluorescence is also evident in the non-zero EEM_{QSE} values at the start of each trial series (Figs. 14, 15, & 16, 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 11, 12, and 13, with the optical windows used for estimating the integrated fluorescent intensities of the CDOM, Crude Oil, and Refined Fuel sensors overlaid on the maps, respectively. The degree of overlap of the optical windows to the region of maximum fluorescence of the oil mixtures varies for each of the sensors on the C3. Overall, the Crude Oil sensor had the largest potential EMM fluorescence intensity based on both the bandwidth size and location and produced the greatest instrument response.

Cross plots of instrument response versus oil concentration and estimated EEM_{QSE} intensity clearly reveal differences in the detection capabilities of the three individual sensors within the C3 unit (Figs. 14-16). In all cases, the background reading in seawater was not subtracted from the instrument response during oil additions in order to reveal response characteristics above ambient background. The response of the CDOM sensor to the oil additions was minimal and positively linear only in trials conducted with the chemical dispersant Corexit 9500 (Fig. 14, panel A). The response of the Crude Oil sensor was significantly greater than that of the CDOM sensor but again only responded positively linear when chemical dispersant was added in addition to physical wave dispersion (Fig. 15). The Refined Fuel sensor had an overall lower response signal, reflective of the smaller range magnitude and range in EMM Intensity within the optical window (Fig. 16). In addition, this sensor showed a positive response to all of the oil addition treatments, even without chemical dispersion. However, when dispersant was not added, the instrument response plateaued for concentrations greater than approximately 2-3 ppm oil. It is not clear why the response of all three sensors differed between day 1 and day 3 experiments using the same source oil with dispersant, beyond the small differences that occurred from the initial ambient background signal.

Figure 17 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.
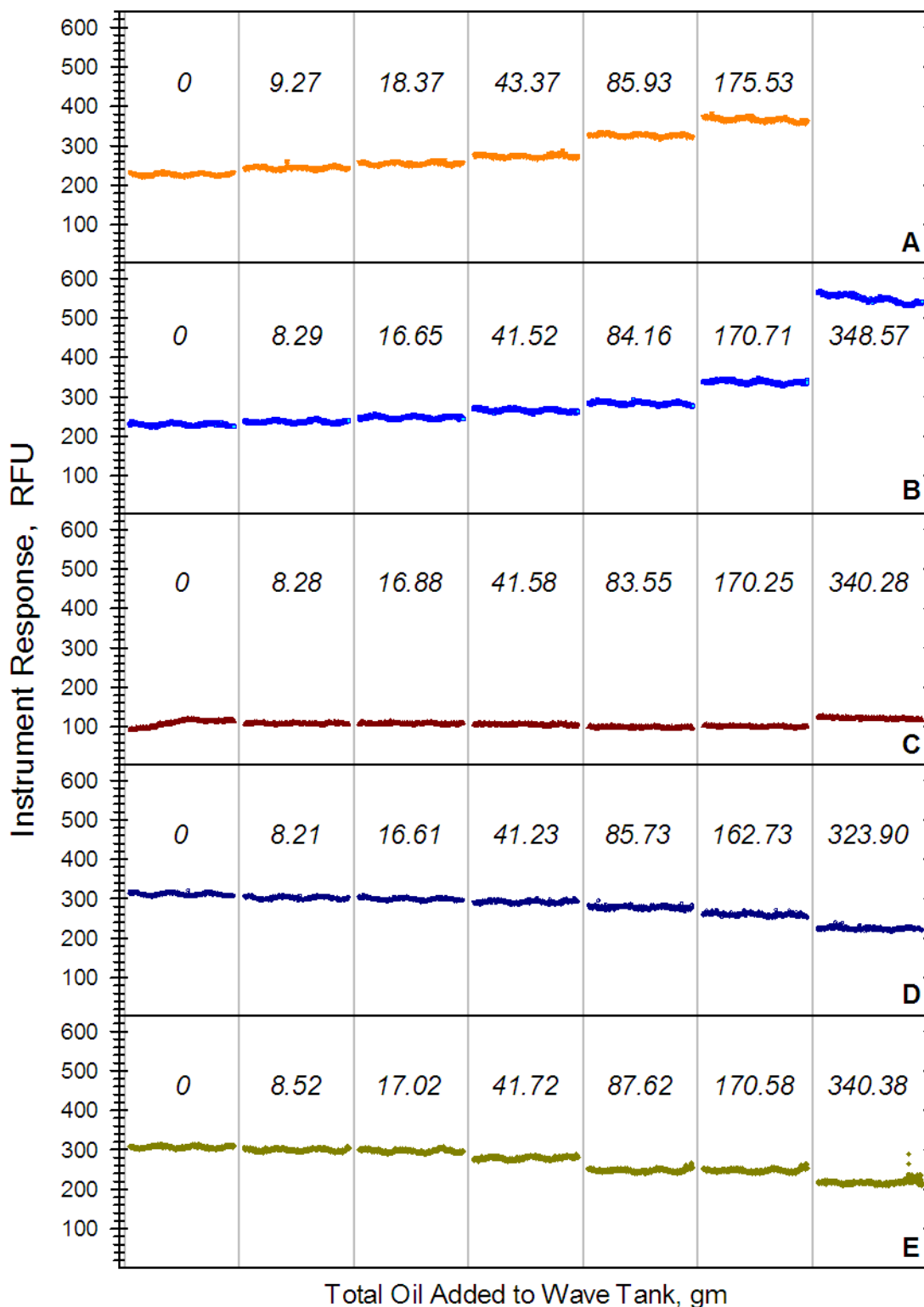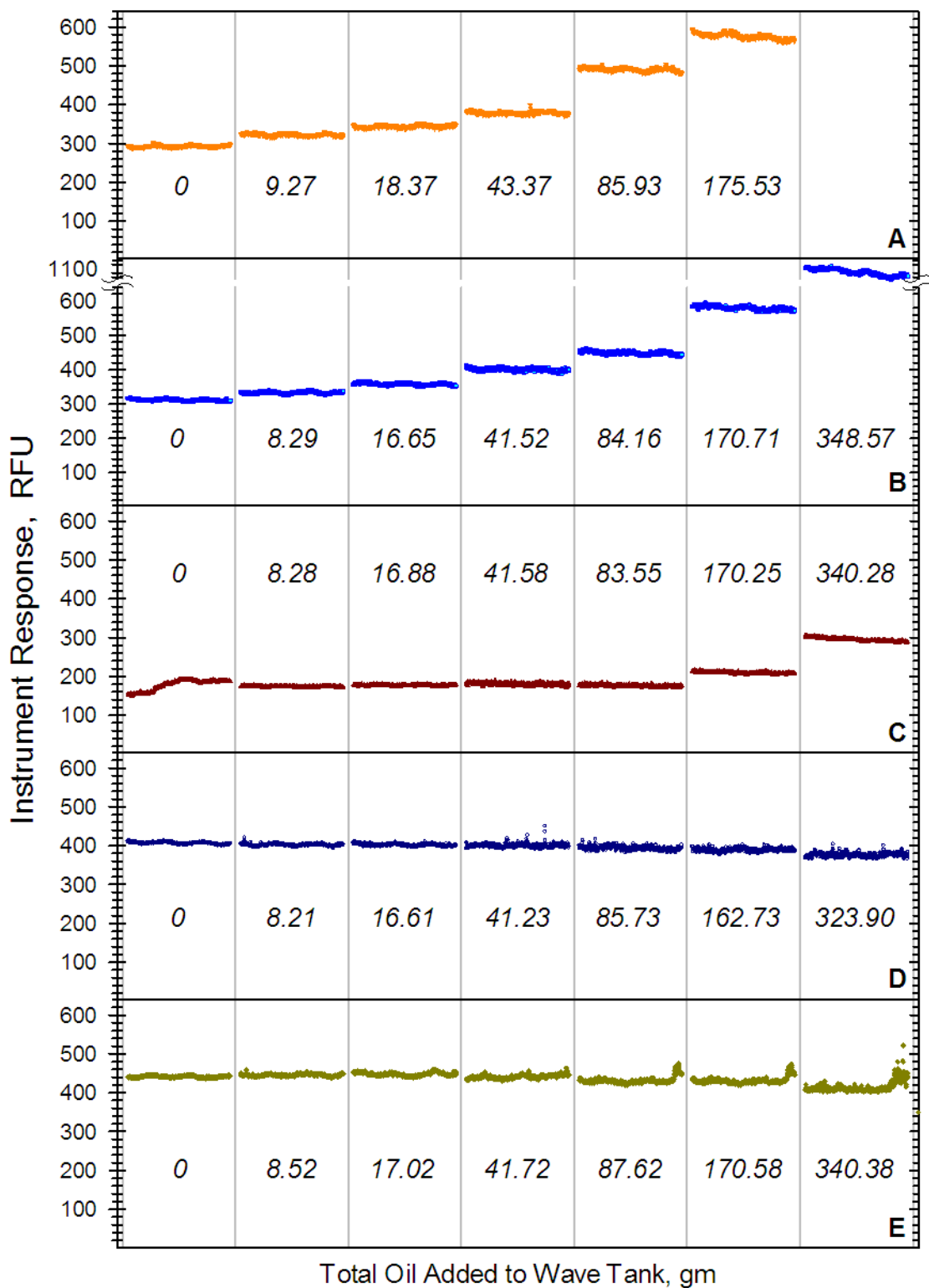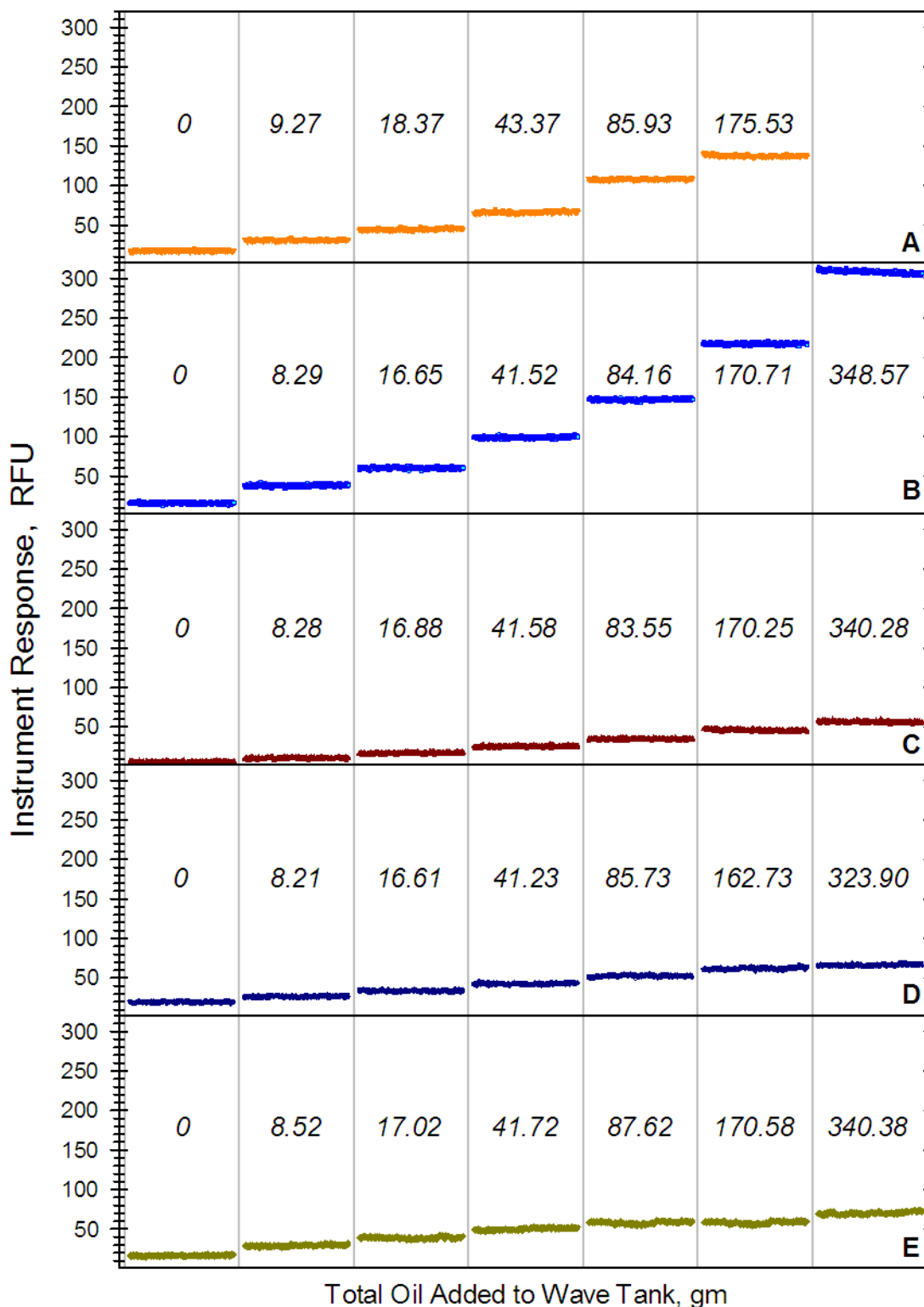
Figure 8. Time-series response of the Turner C3 CDOM sensor to crude oil additions in the COOGER wave tank at the Bedford Institute of Oceanography, Bedford, NS. Instrument response reported over the 10 minute interval starting 40 minutes after addition of the indicated challenge compound level. Each 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 9. Time-series response of the Turner C3 Crude Oil sensor 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 10. Time-series response of the Turner C3 Refined Fuel sensor 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. Note reduce RFU scale.
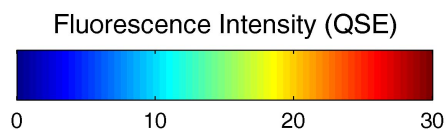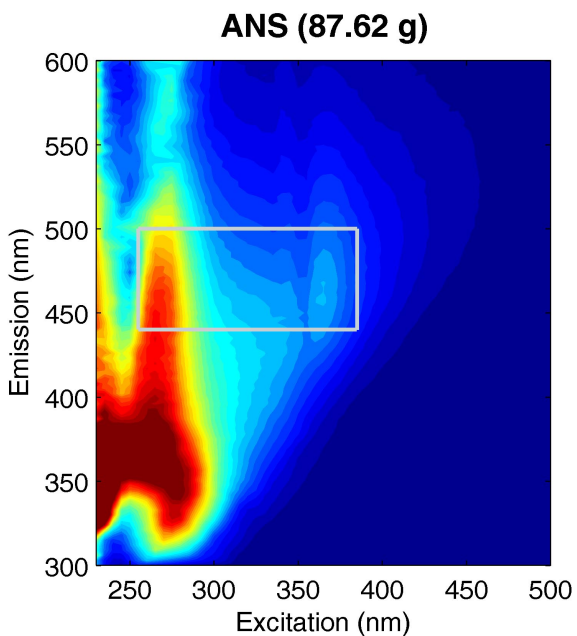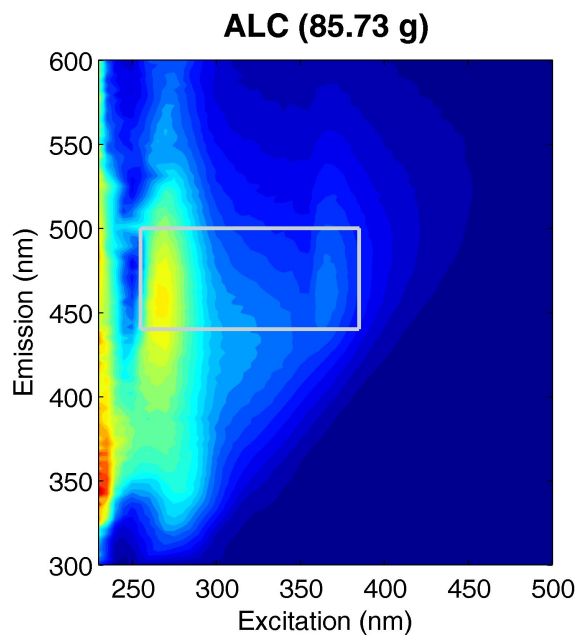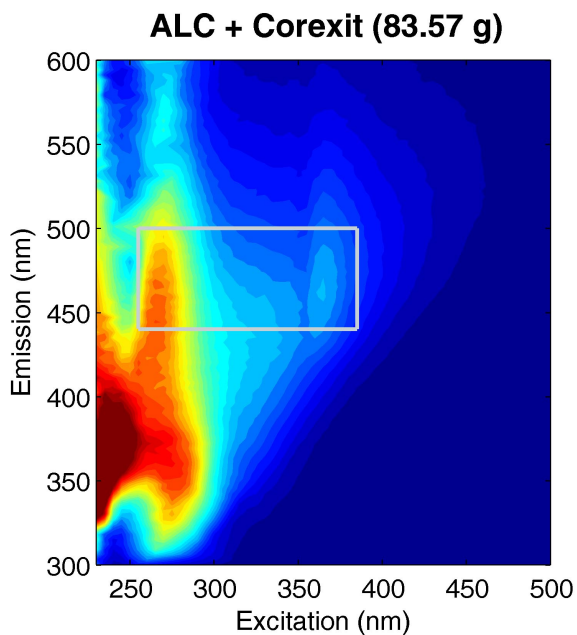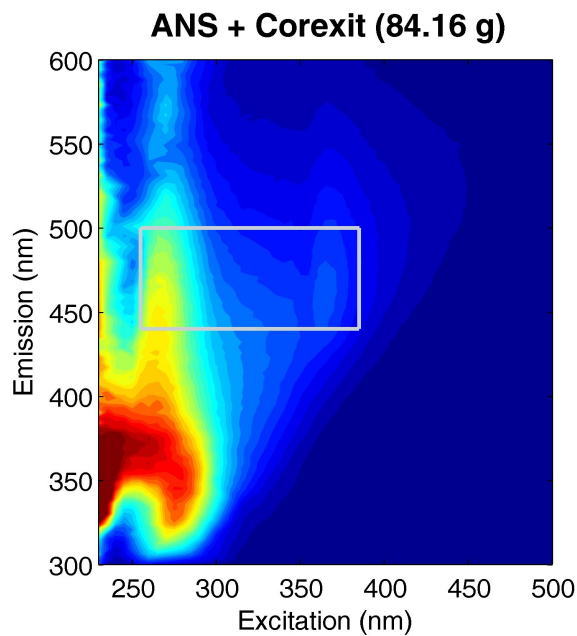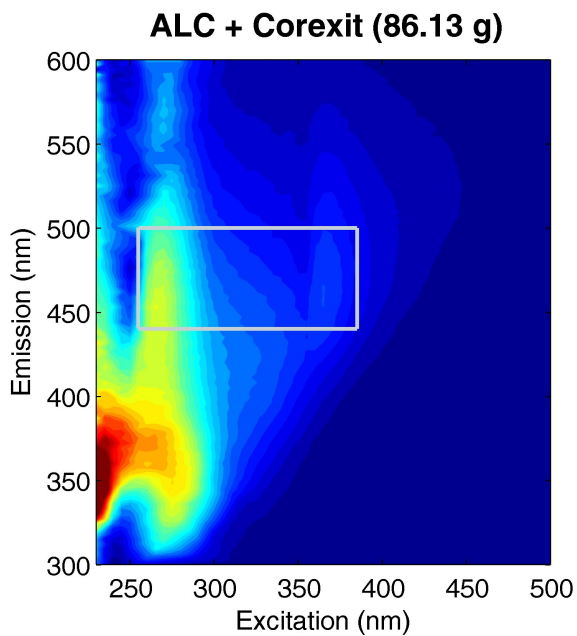
Figure 11. Representative EEM$_{QSE}$ for crude oil treatments during the COOGER trials. Grey boxes represent the Turner C3 CDOM sensor optical windows used for estimation of integrated fluorescent intensities.
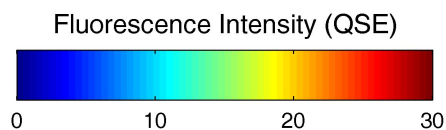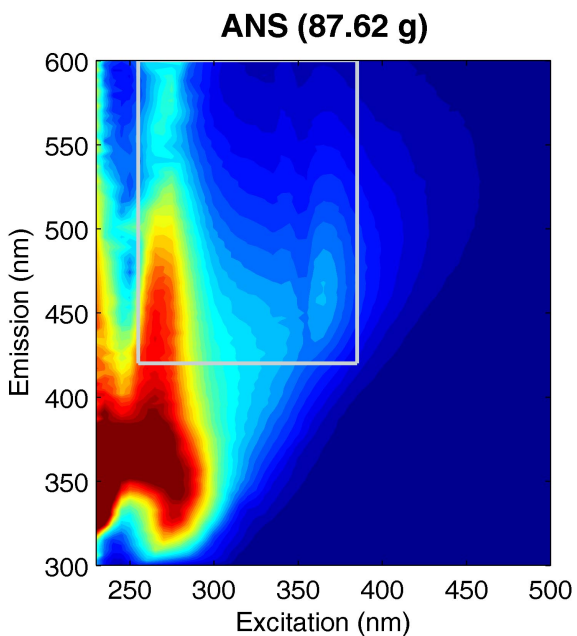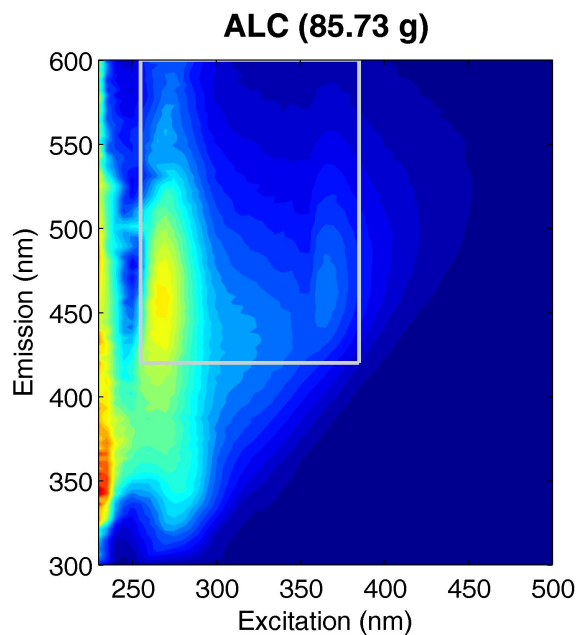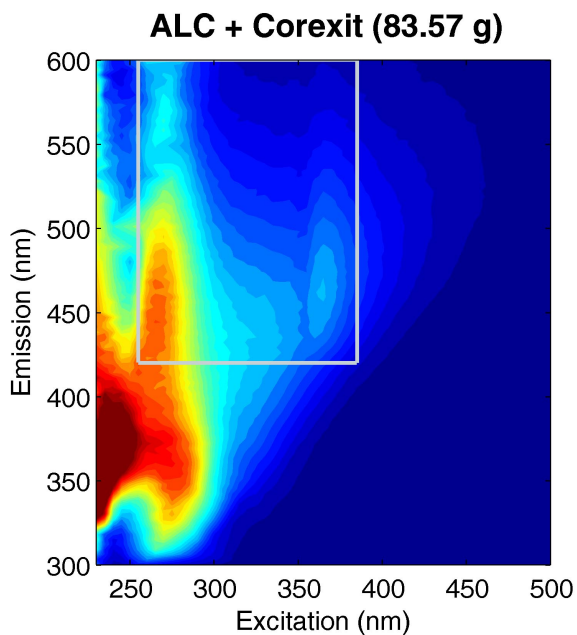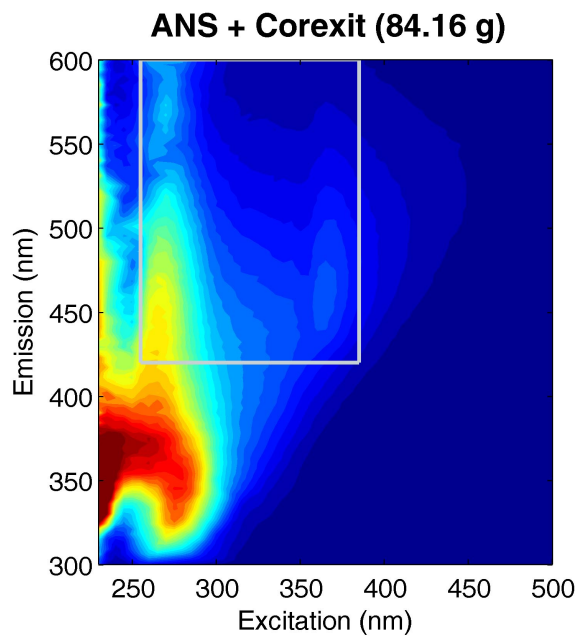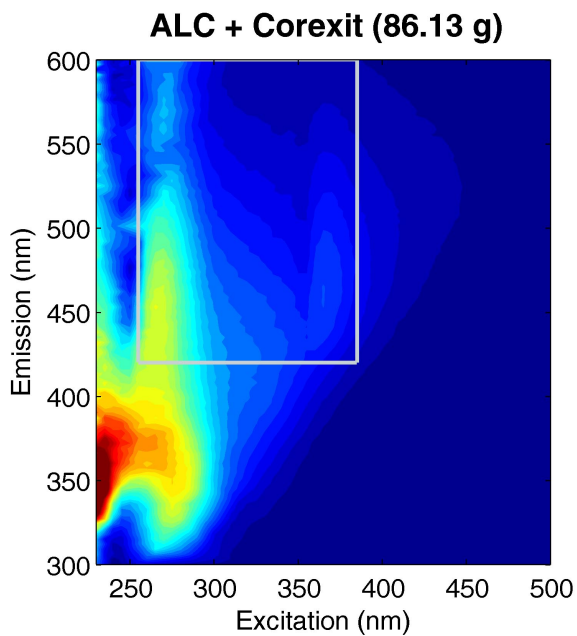
Figure 12. Representative EEM$_{QSE}$ for crude oil treatments during the COOGER trials. Grey boxes represent the Turner C3 Crude Oil sensor optical windows used for estimation of integrated fluorescent intensities.
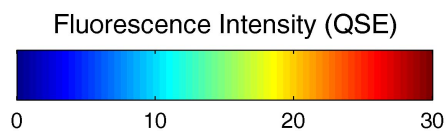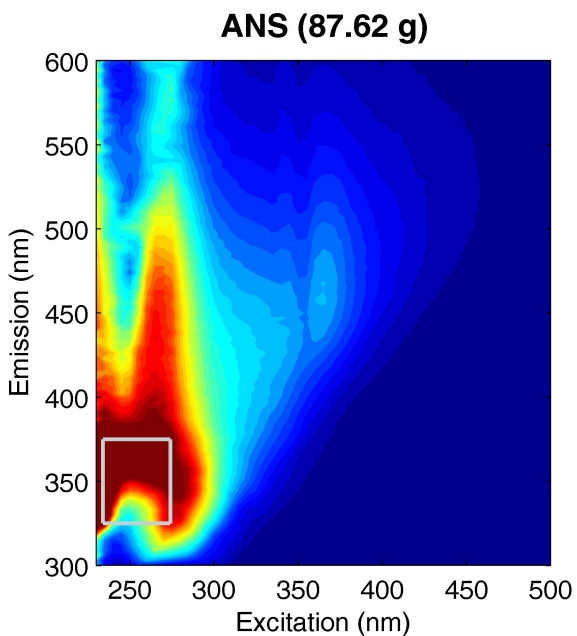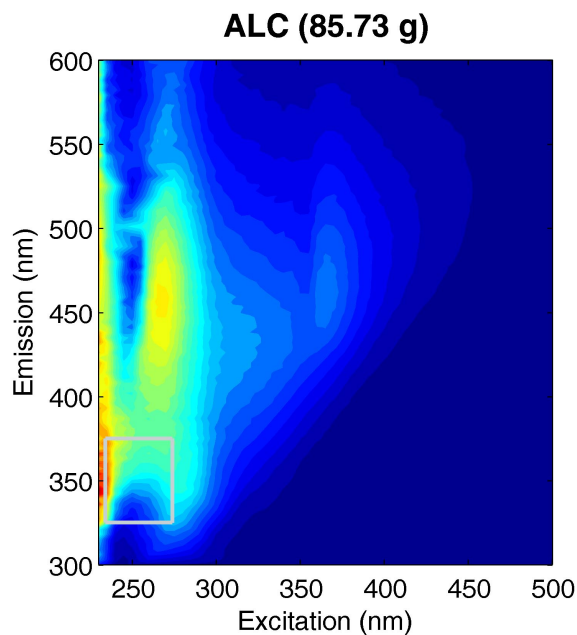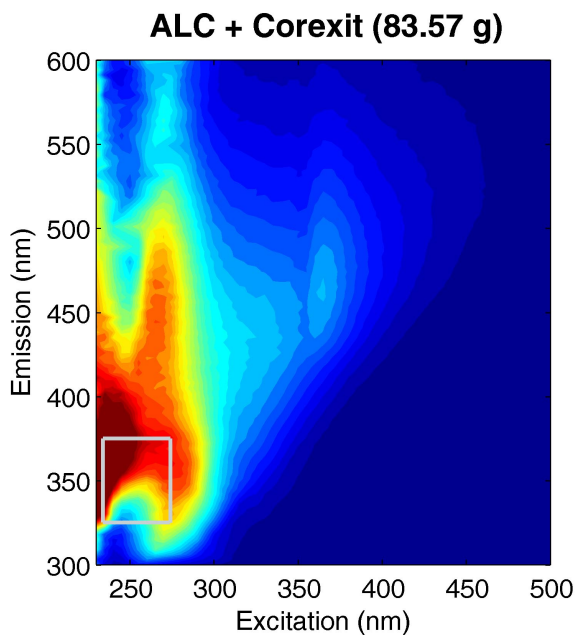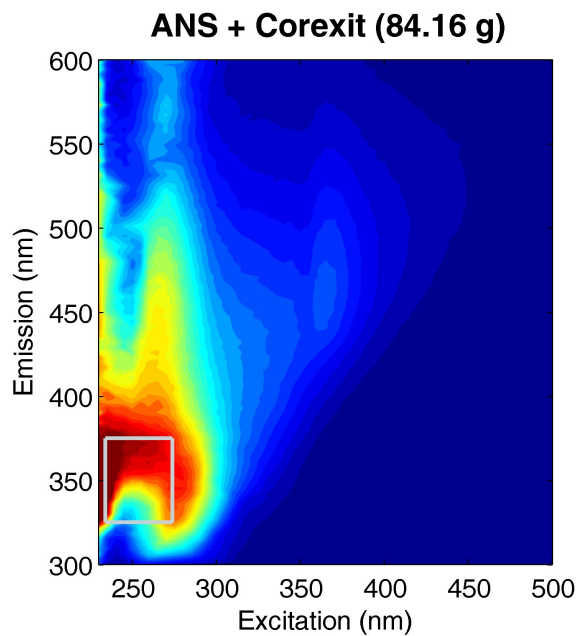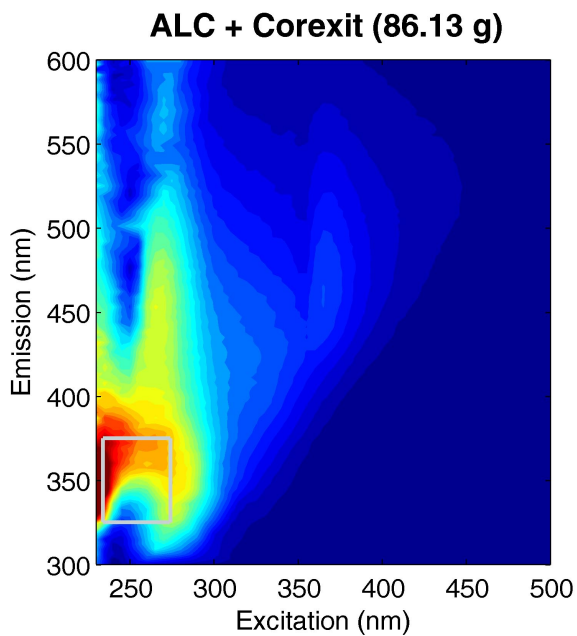
Figure 13. Representative EEM$_{QSE}$ for crude oil treatments during the COOGER trials. Grey boxes represent the Turner C3 Refined Fuel sensor optical windows used for estimation of integrated fluorescent intensities.
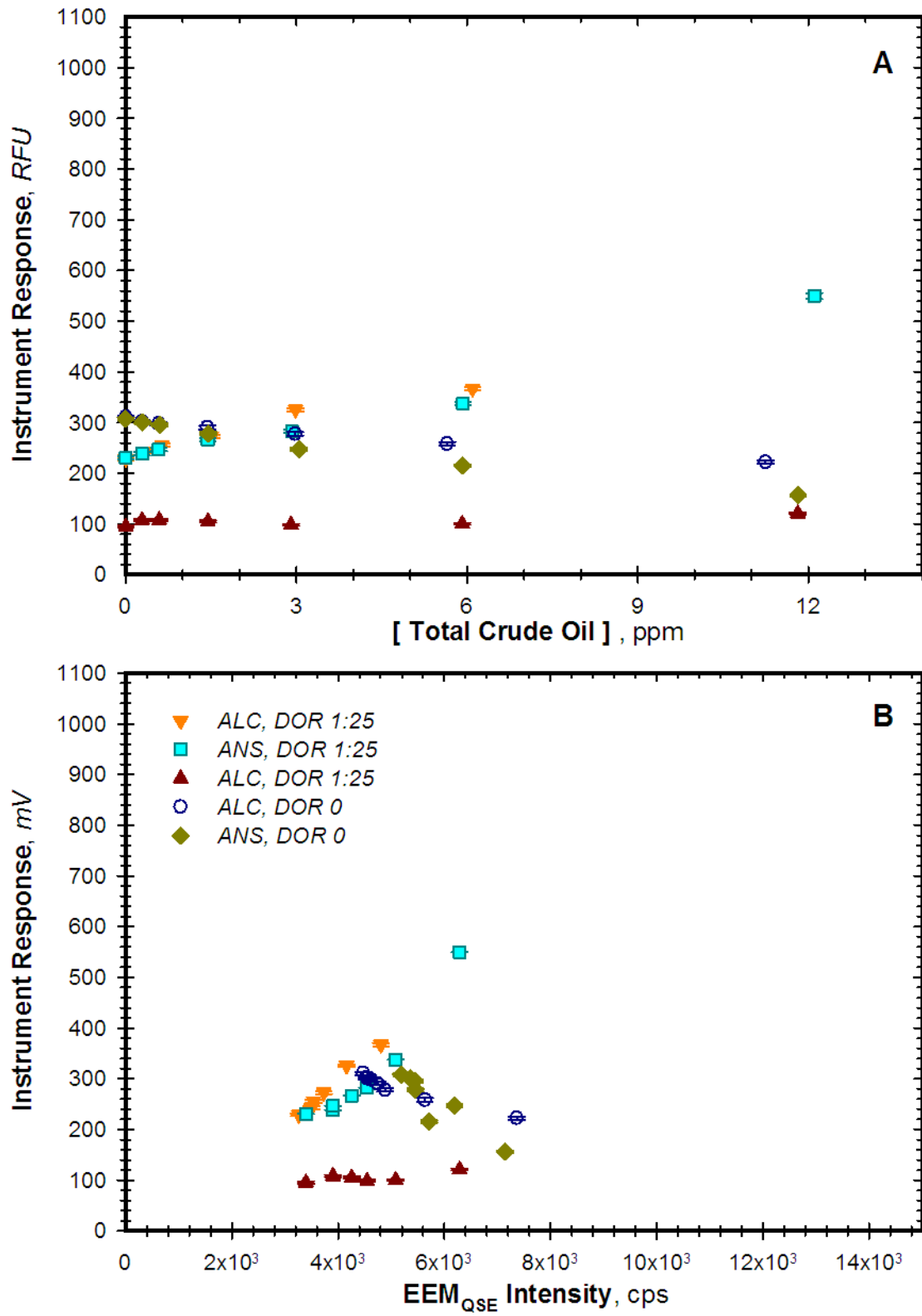
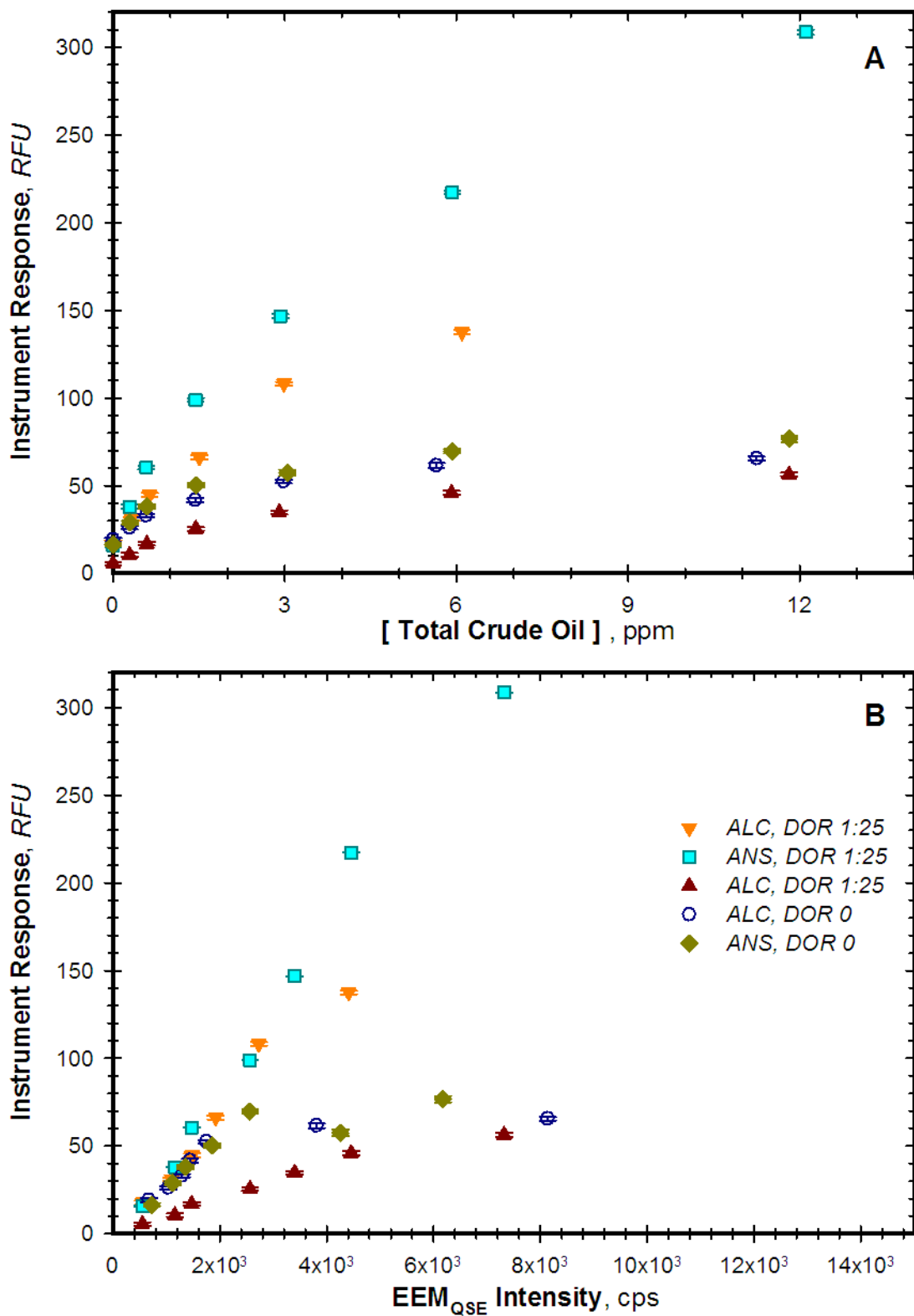**Figure 14.** Response of Turner C3 CDOM sensor to total crude oil concentration in presence/absence of the chemical dispersant Corexit 9500. All exposures experienced breaking waves. Reported measurements represent the average (+/- S.D.) instrument response over 10 minute windows starting 40 minutes 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.
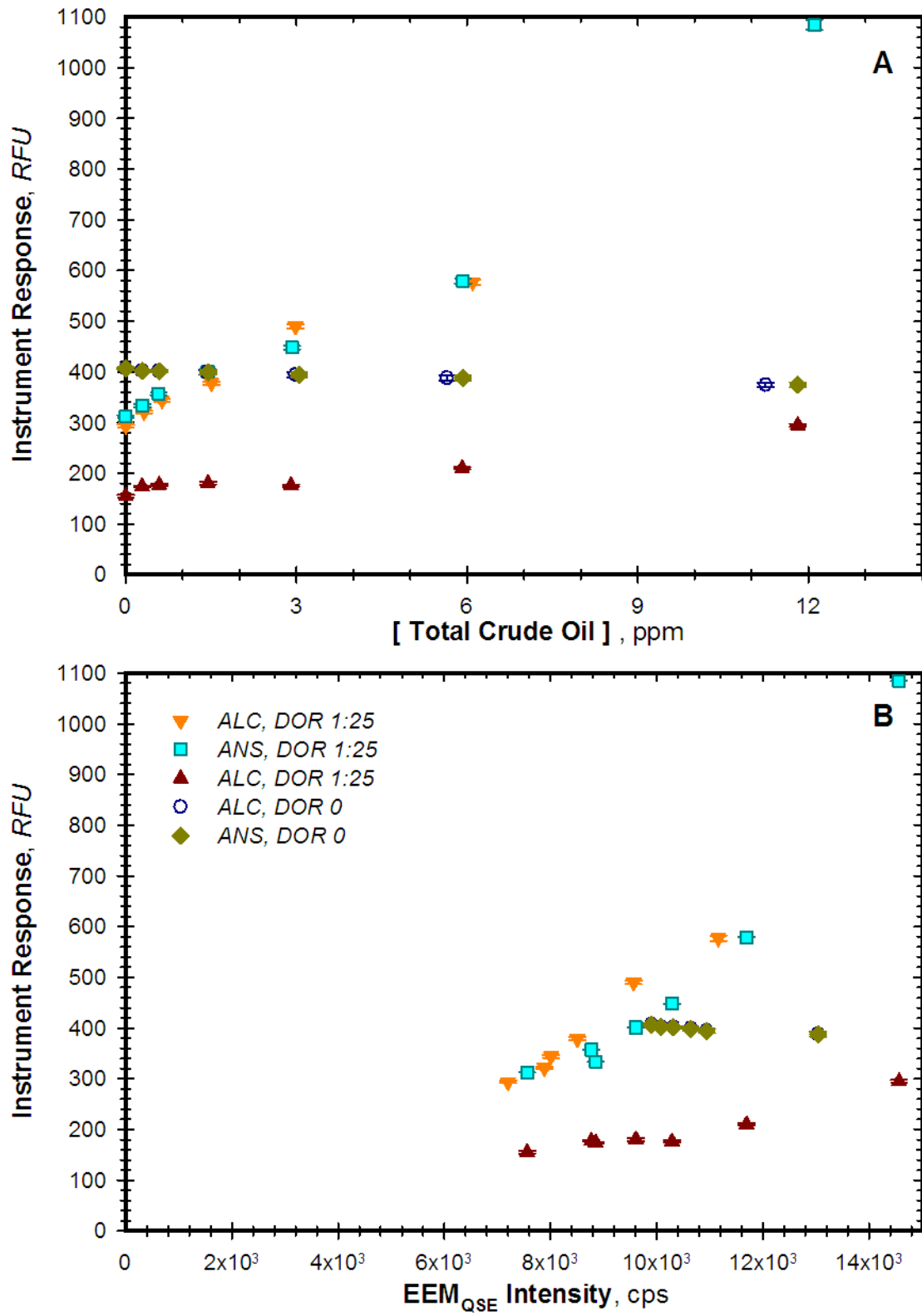
Figure 15. Response of Turner C3 Crude Oil sensor to total crude oil concentration in presence/absence of the chemical dispersant Corexit 9500. All exposures experienced breaking waves. Reported measurements represent the average (+/- S.D.) instrument response over 10 minute windows starting 40 minutes 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_{OSE} indicative of daily variation in ambient CDOM loads in tank source waters.

Figure 16. Response of Turner C3 Refined Fuel sensor 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_{OSE} indicative of daily variation in ambient CDOM loads in tank source waters. Note reduction in RFU scale.
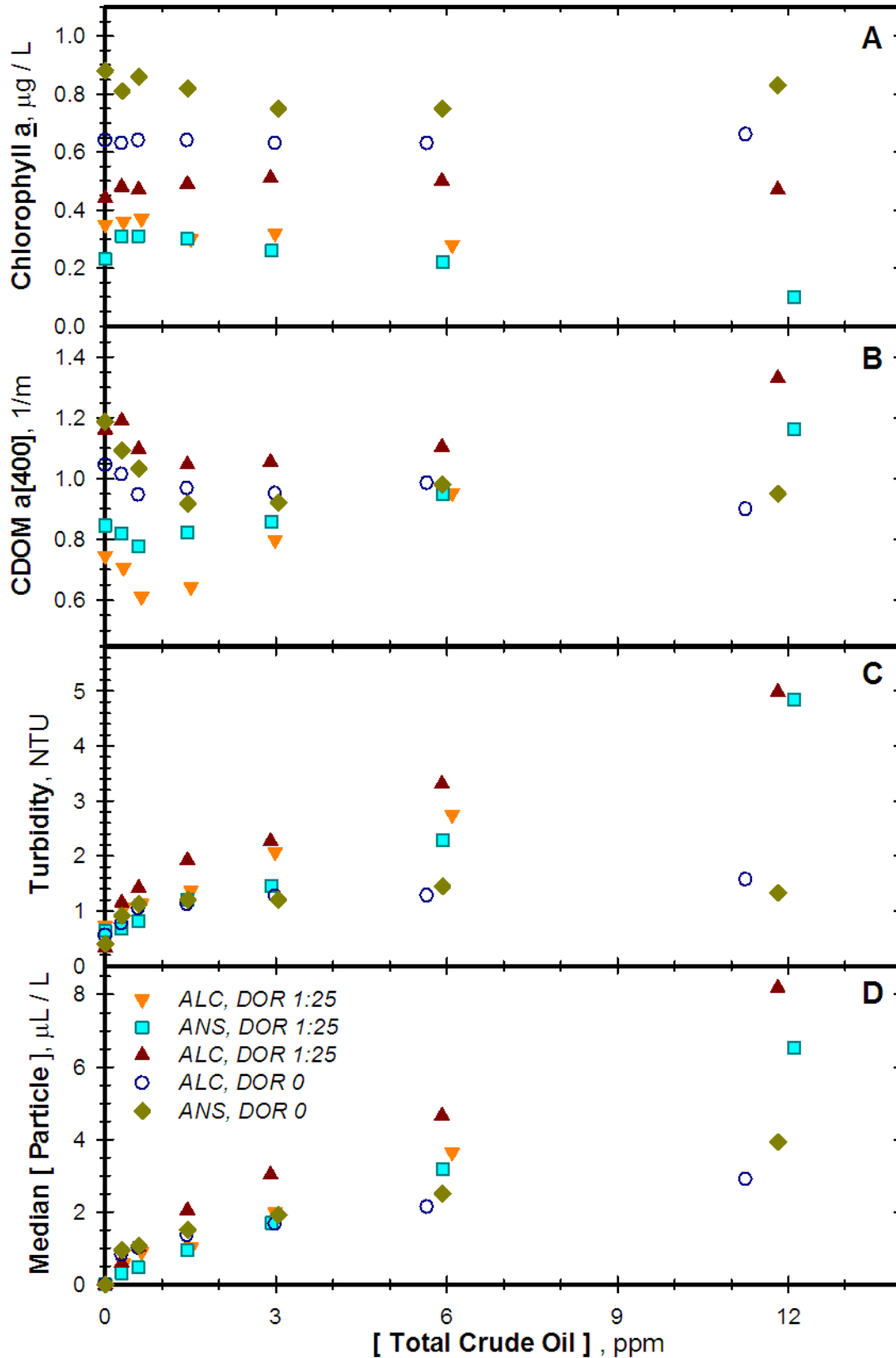
Figure 17. Variation of ancillary water quality parameters with crude oil additions during the COOGER trials. (A) Extracted chlorophyll a . (B) Absorbance at 400 nm as a proxy for CDOM. (C) Turbidity measured with a Hach 2100 AN. (D) Median particle concentration measured in situ using a LISST (Sequoia 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.
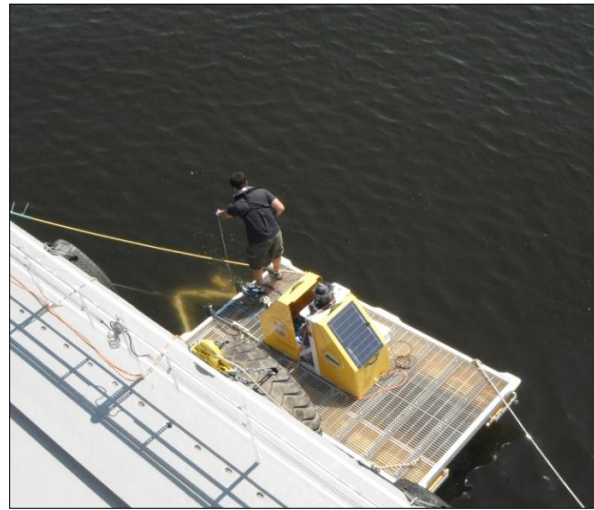
Photo 7. Site map and photo of the field test site located in Winans Cove, Baltimore Harbor MD.

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.

A summary of the physical and water quality conditions experienced over the duration of the moored deployment are presented in Table 3. Water temperature ranged from 25.3 to 29.4 °C and salinity varied from 3.9 to 9.9. Chlorophyll and CDOM are quite high at this location and can contribute significantly to the fluorescent properties of the ambient seawater.

Table 3. Ancillary physical and water quality conditions for the moored field deployment test conducted in Winans Cove, Baltimore Harbor, Baltimore, MD.

Site		Temperature (^{o}C)	Salinity	Chlorophyll (µg/L)	CDOM A_{400}, m^{-1}	Turbidity (NTU)
Baltimore Harbor	Min	25.3	3.9	2.6	1.17	1.3
	Max	29.4	9.9	44.8	2.48	6.0
	Mean	27.0	8.2	16.6	1.52	3.0

The time series response of the C3-CDOM, -Crude Oil, and -Refined Fuel sensors during the moored deployment in Baltimore Harbor are shown in figures 18, 19, and 20, respectively. During the deployment 33 discrete reference samples were collected and analyzed for total petroleum hydrocarbons (TPH). Only three samples, one on 8/22 and two on 8/24, had any detectable level of hydrocarbons as analyzed by TestAmerica using GC-FID. The instrument response of the CDOM unit averaged 739 (± 55) RFU during the field deployment but no clear response was observed corresponding to the three positive TPH detections in corresponding reference samples (Fig. 18). The Crude Oil sensor response averaged around 920 (± 82) RFU over this same period and again there was no observable increased instrument response for the three positive TPH reference sampling timepoints (Fig. 19). The Refined Fuel sensor averaged only 22 (± 7) RFU (Fig. 20), but did show an 8 RFU change at the highest reference TPH detect of 35 ppb consistent with the responses observed during the COOGER trials (see Fig 26). All three sensors exhibited a small but repeated diurnal pattern that appeared to track with temperature. The pattern is the most obvious in Fig. 20, where the scale has been significantly reduced and reveals a maximum level of variation. There was no apparent tracking of instrument response by any of the sensors to concentrations of chlorophyll, CDOM, or turbidity despite substantial variation in these parameters (Table 3 and Figs. 18-20, panel C).

Representative EEM fluorescent maps for reference samples collected on five different dates are shown in figure 21-23, with the CDOM, Crude Oil, and Refined Fuel sensor optical window depicted, respectively. EEM characteristics were fairly consistent over time and fluorescence intensity maxima were much more closely matched for the CDOM and Crude Oil configuration relative to the Refined Fuel configuration.

Cross plots of instrument response to concentrations of TPH detected by GC-FID and predicted EEM$_{QSE}$ intensities are shown in figures 24-26. For the CDOM sensor, the average EEM$_{QSE}$ for the reference samples yielding non-detects was 12221 (± 283) cps, compared to 12284 (±210) cps for the three positive TPH samples. For the Crude Oil sensor the average EEM$_{QSE}$ was 27013 (± 678) for the TPH non-detect reference samples and 27194 (± 450) cps for reported TPH detects, which although higher, was within the environmental range observed during this deployment. Corresponding instrument responses for the CDOM (745 (± 18) vs 744 (± 17) RFU) and Crude Oil sensors (924 (± 20) vs 921 (± 16) RFU) were little changed from

their time-series averages. The EEM$_{QSE}$ for the Refined Fuel sensor was 2670 ($\pm$ 158) cps for non-detect reference samples and 2779 ($\pm$ 102) cps for the reported TPH detects respectively representing a similar absolute increase but larger relative change in EEM$_{QSE}$ for this sensor window. Corresponding instrument response from the Refined Fuel sensor was 19 ($\pm$ 3). And 24 ($\pm$ 4) RFU for the non-detect and detect reference samples respectively. Only the referenced 35 ppb detect sample clearly corresponded to an instrument signal (29 RFU) above the deployment average. These patterns point to the difficulty in the quantitative interpretation of instrument signal responses in environments with variable ambient fluorescence properties.

Instrument Photographs

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

Turner C3 Prior to Deployment

Turner C3 Prior to Deployment

Turner C3 After Deployment

Turner C3 After Deployment

Photo 8. Turner C3 photos taken at Baltimore Harbor test site before and after deployment.
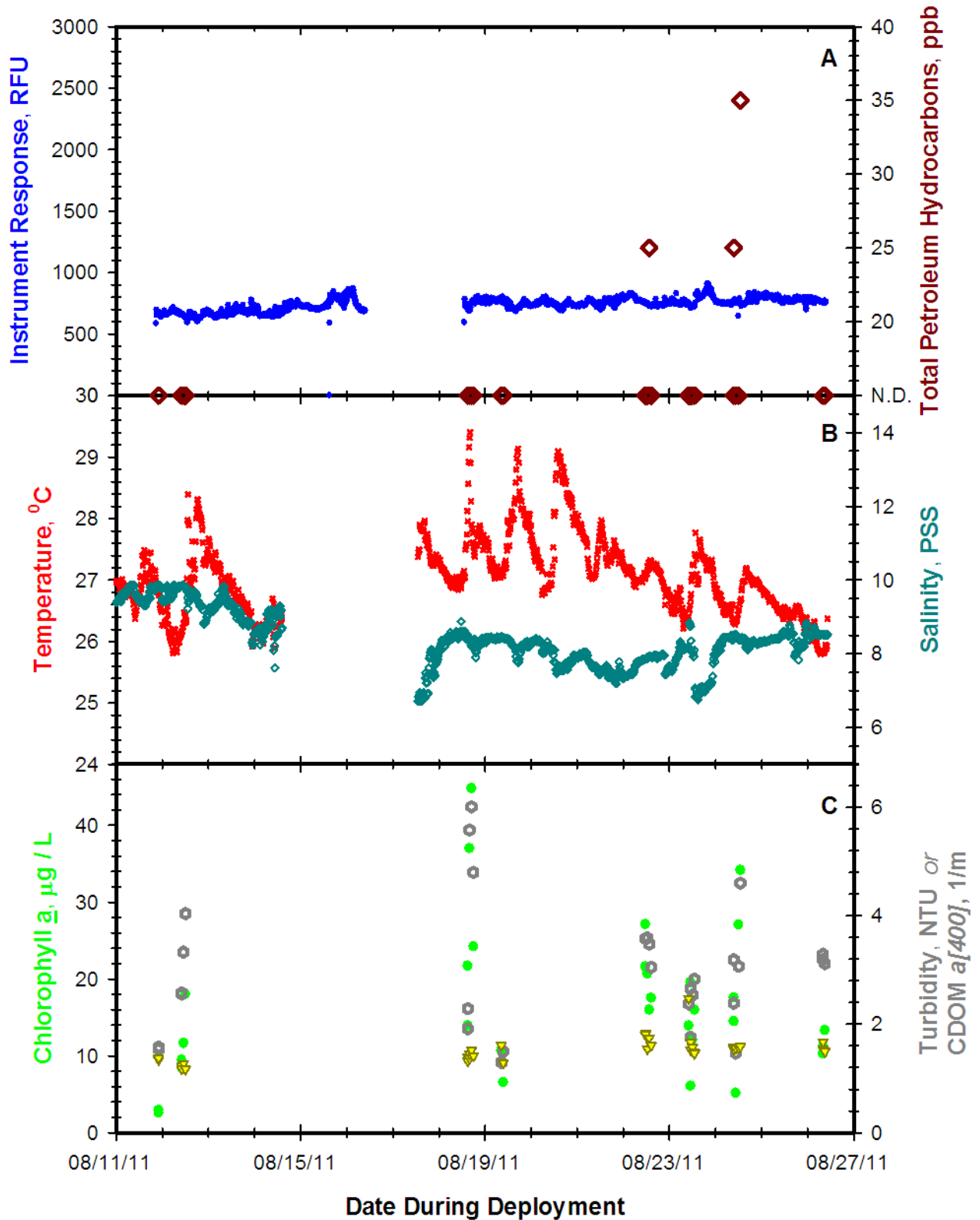
Figure 18. Summary of field deployment results for the Turner C3 CDOM sensor at the MERC facility in Baltimore, MD. (A) Instrument time series response during deployment along with GC-FID measurements of total petroleum hydrocarbons (TPH, Test America- Sacramento) in grab samples taken at the time of 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.
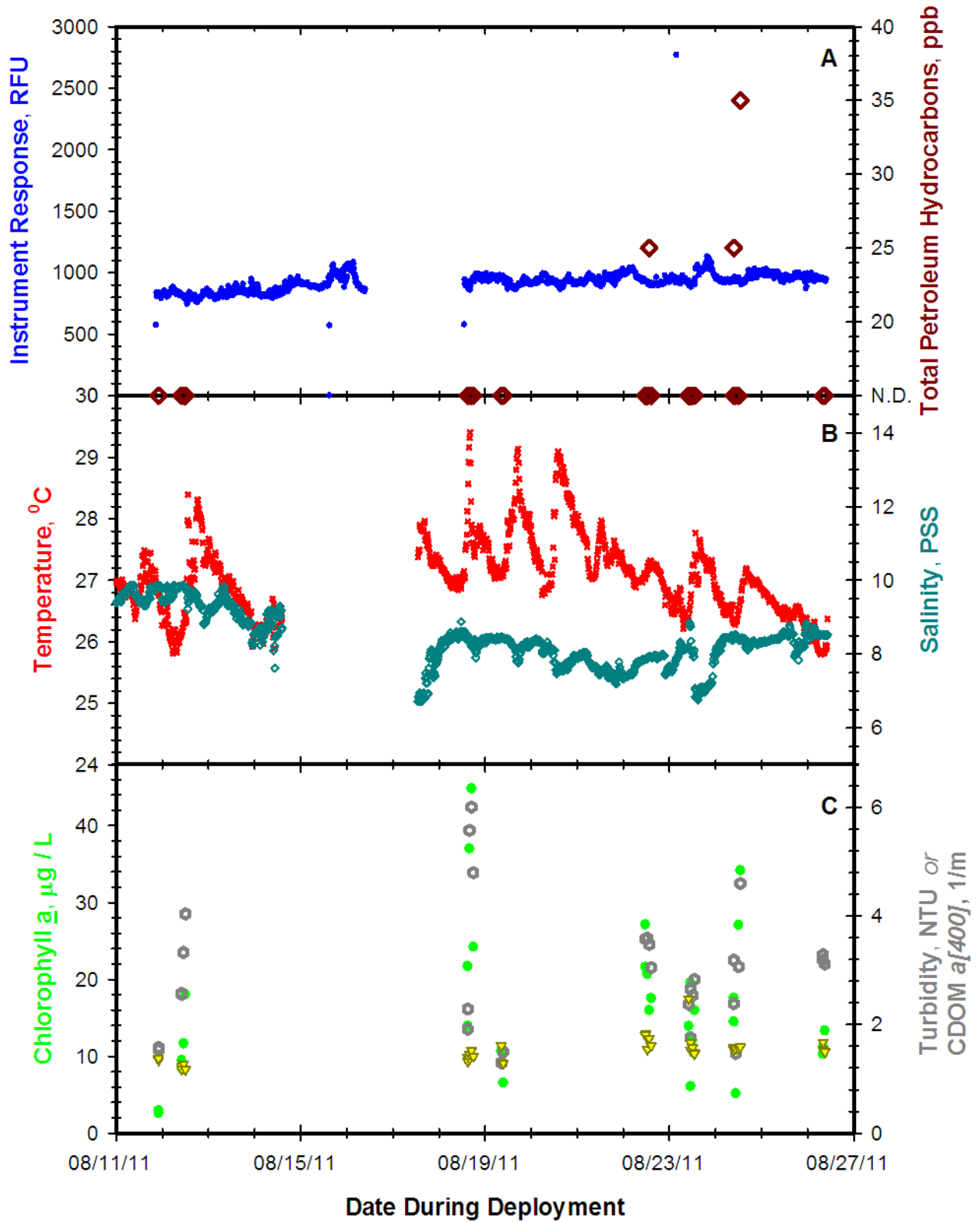
Figure 19. Summary of field deployment results for the Turner C3 Crude Oil sensor at the MERC facility in Baltimore, MD. (A) Instrument time series response during deployment along with GC-FID measurements of total petroleum hydrocarbons (TPH, Test America- Sacramento) in grab samples taken at the time 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.
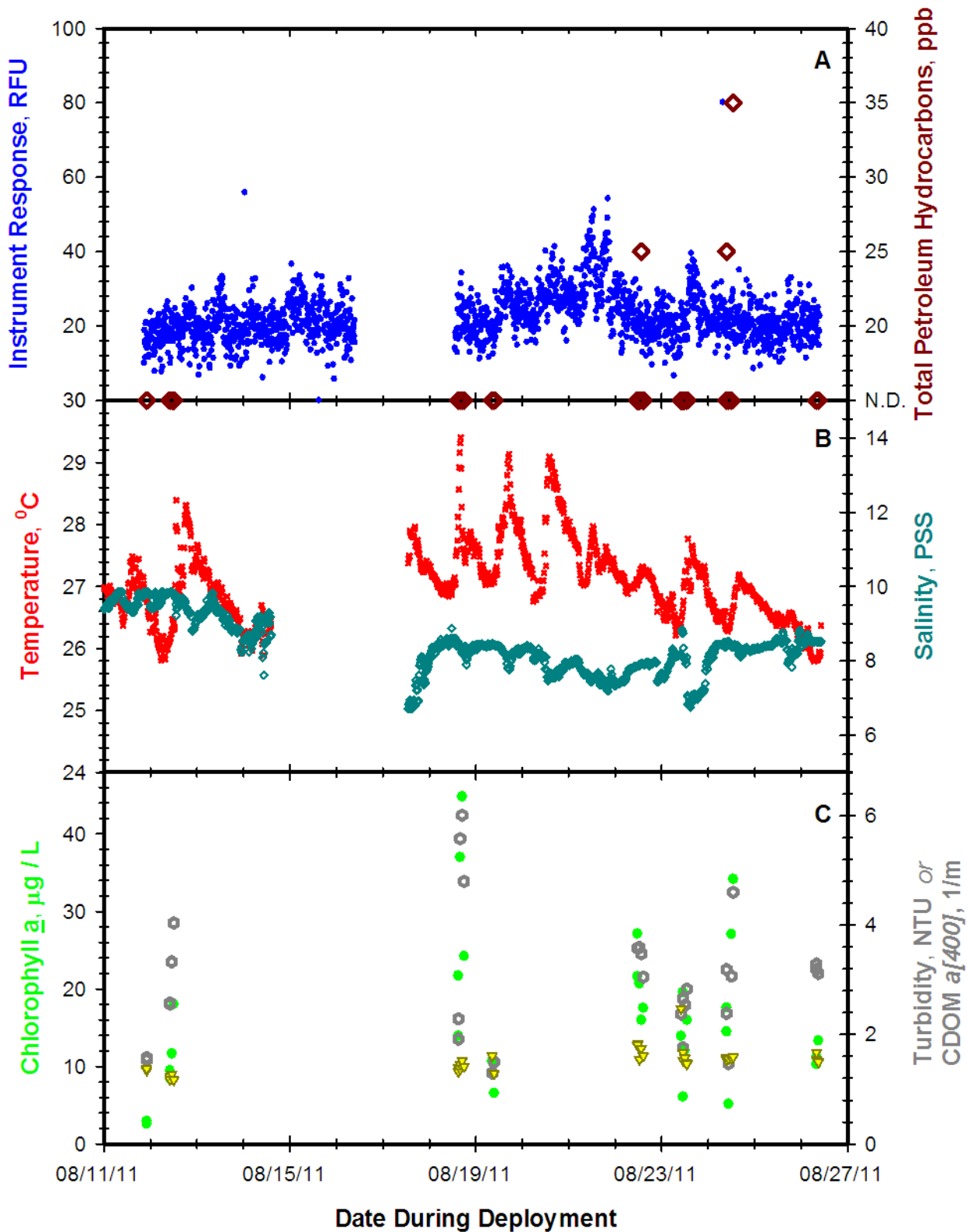
Figure 20. Summary of field deployment results for the Turner C3 Refined Fuel sensor at the MERC facility in Baltimore, MD. (A) Instrument time series response during deployment along with GC-FID measurements of total petroleum hydrocarbons (TPH, Test America- Sacramento) in grab samples taken at the 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 reduced RFU scale.
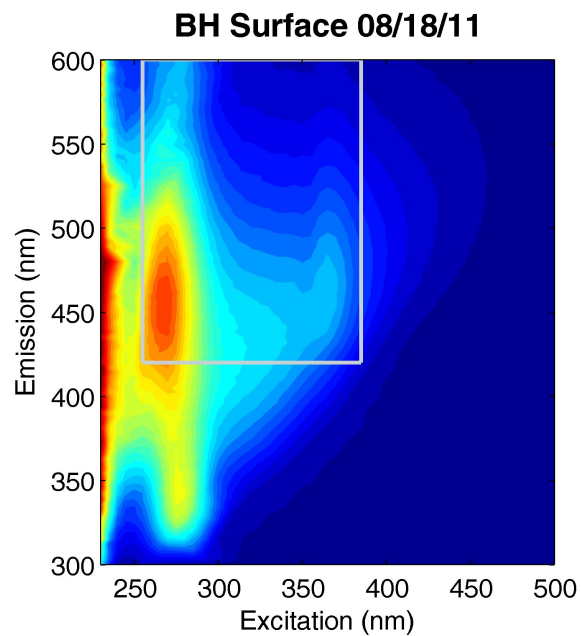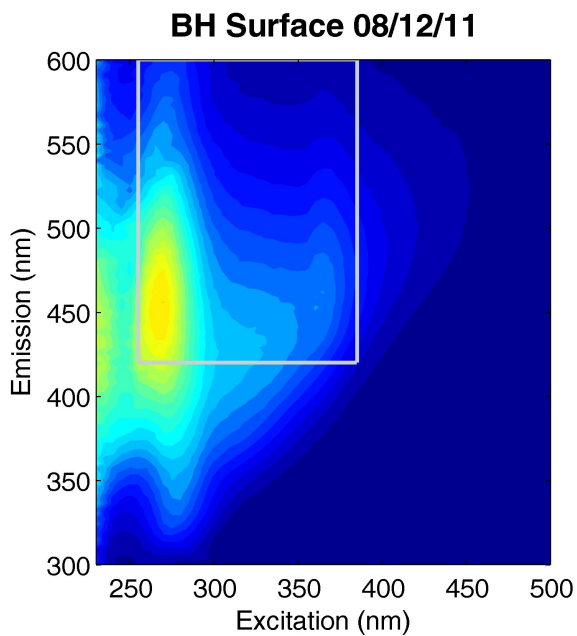
39
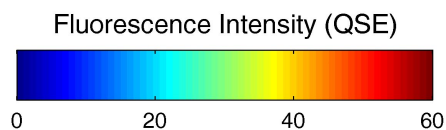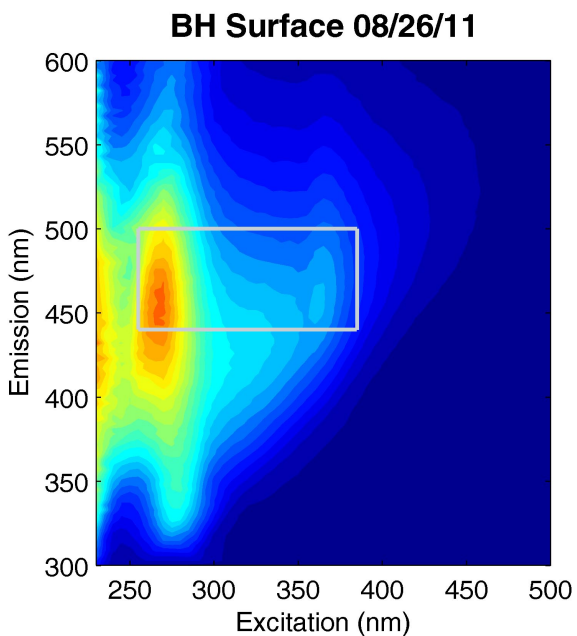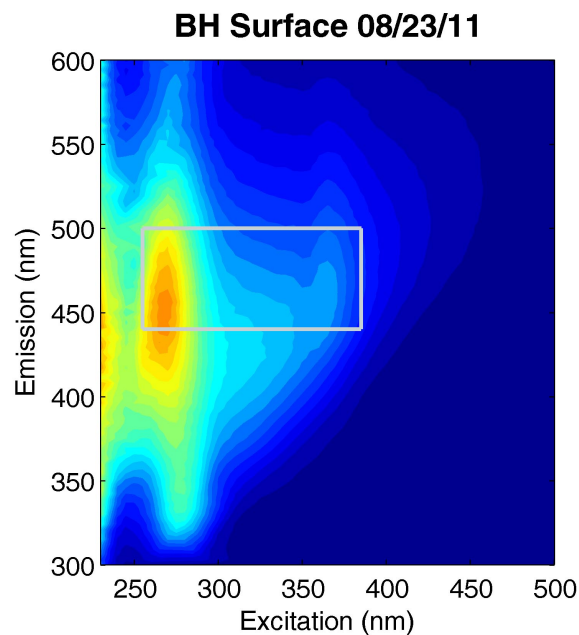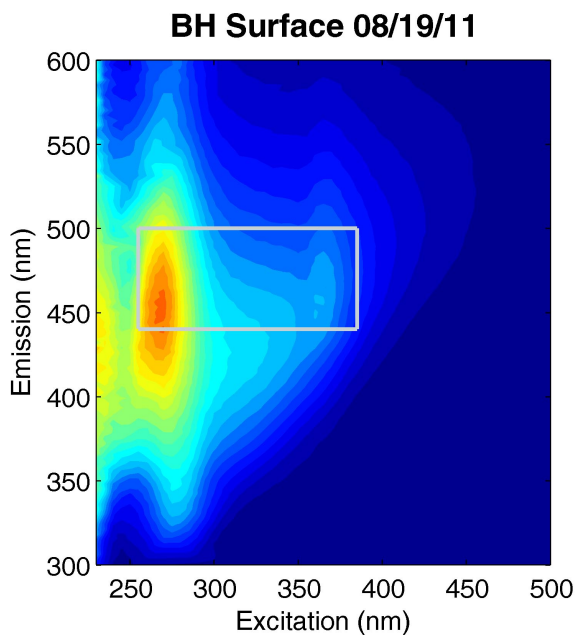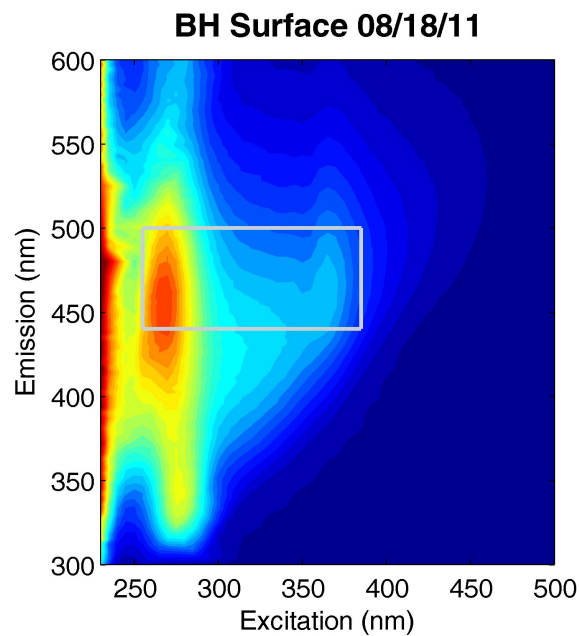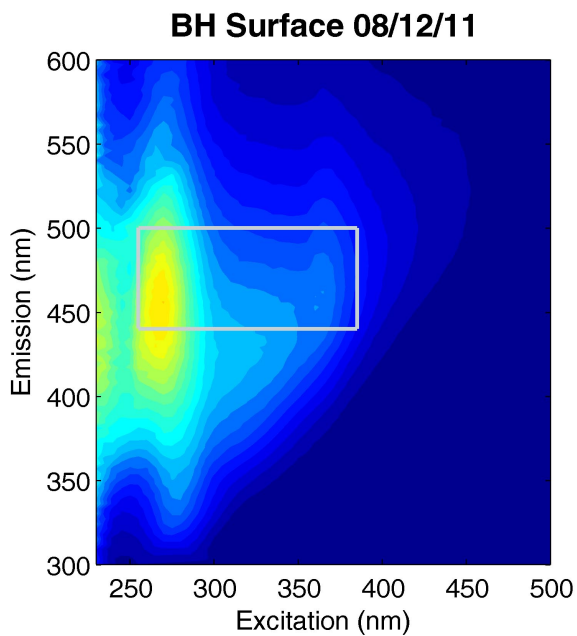
Figure 21. Representative EEM$_{QSE}$ of Baltimore Harbor water from grab samples taken during the deployment. Grey box represents the optical window of the Turner C3 CDOM sensor used for estimation of integrated fluorescence intensity available to the instrument sensor. The 8/22 and 8/24 samples had significant TPH detections by independent analytical methods.
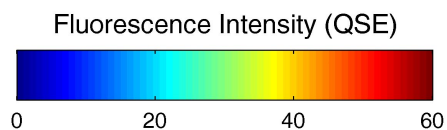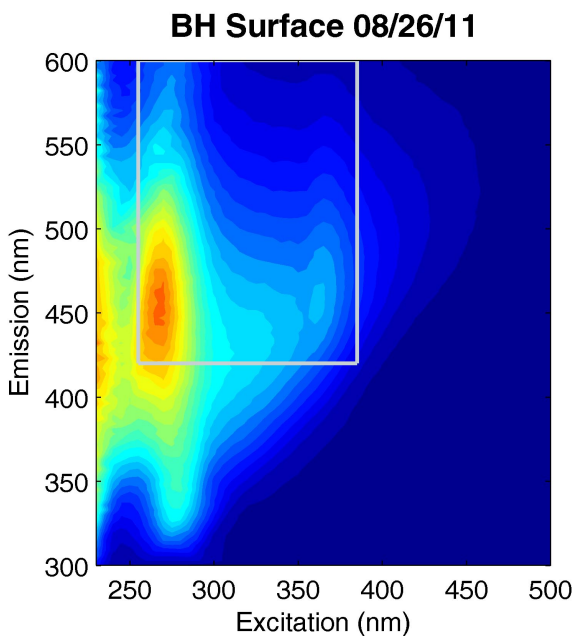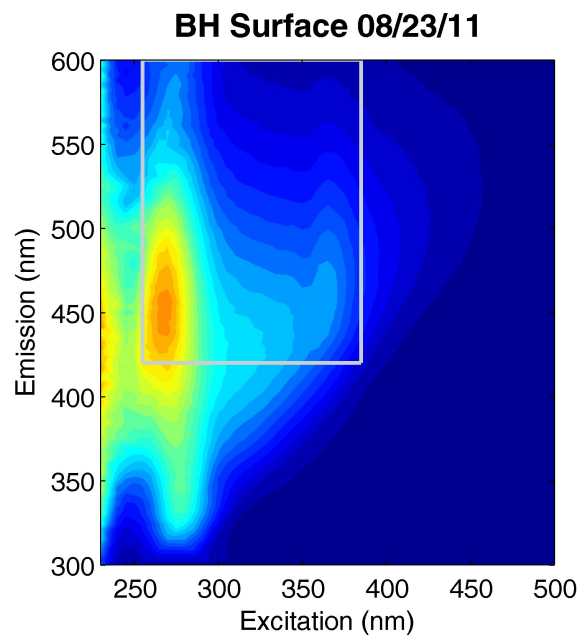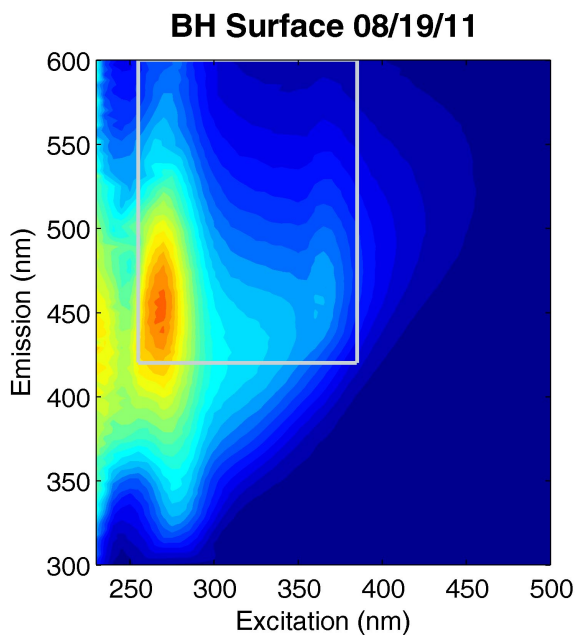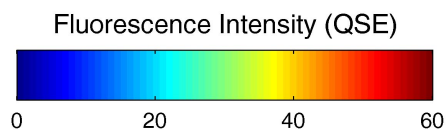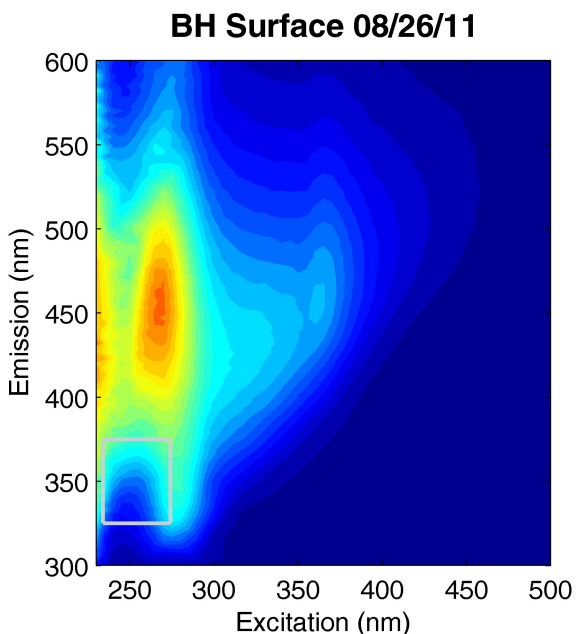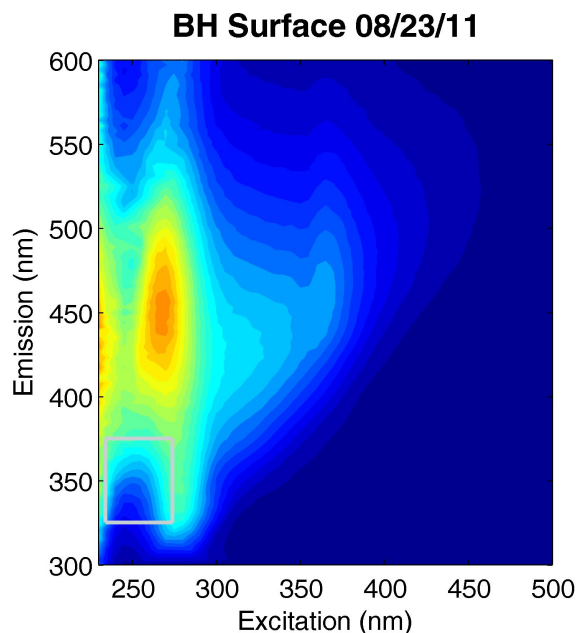
Figure 22. Representative EEM_{QSE} of Baltimore Harbor water from grab samples taken during the deployment. Grey box represents the optical window of the Turner C3 Crude Oil sensor used for estimation of integrated fluorescence intensity available to the instrument sensor. The 8/22 and 8/24 samples had significant TPH detections by independent analytical methods.
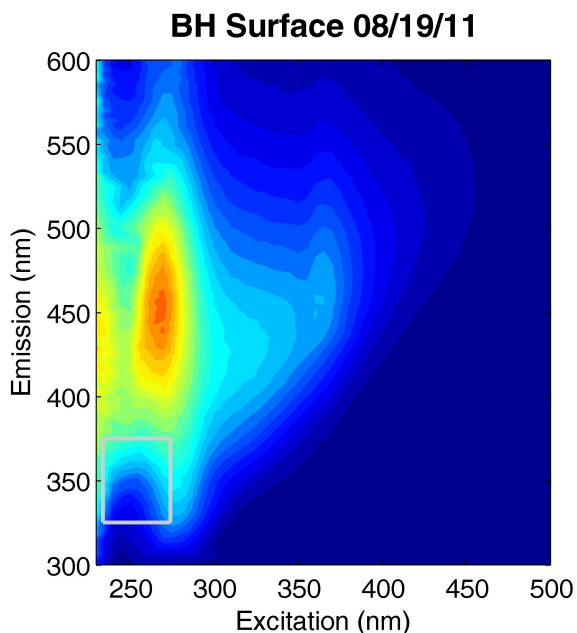
BH Surface 08/12/11

BH Surface 08/18/11

BH Surface 08/19/11

BH Surface 08/23/11

BH Surface 08/26/11

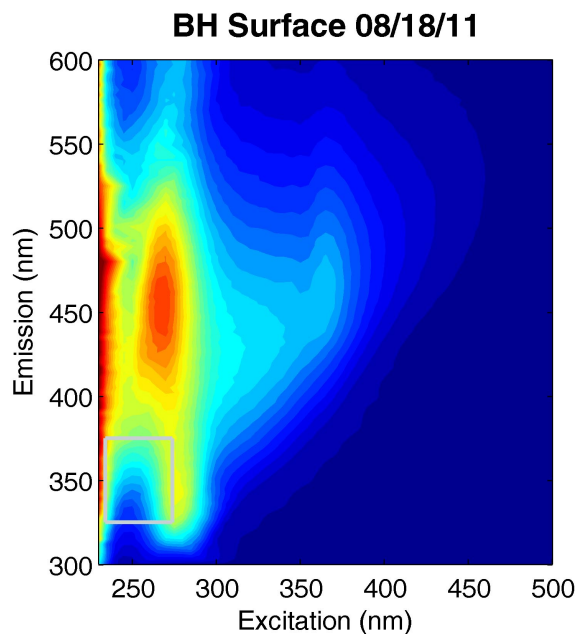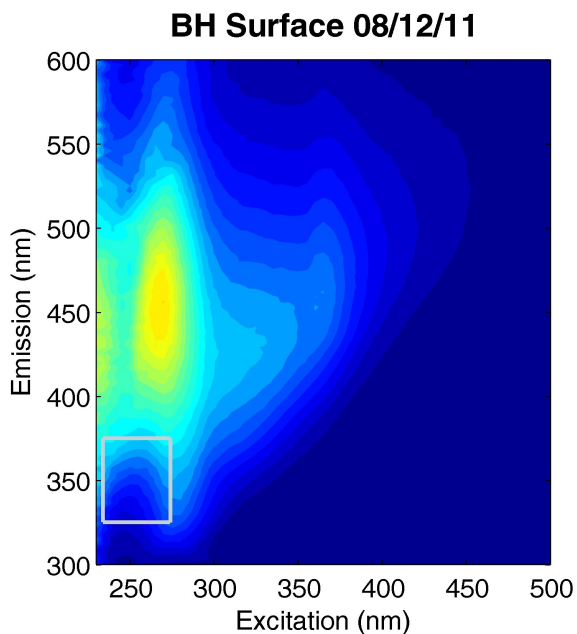Fluorescence Intensity (QSE)

Figure 23. Representative EEM$_{QSE}$ of Baltimore Harbor water from grab samples taken during the deployment. Grey box represents the optical window of the Turner C3 Refined Fuel sensor 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.
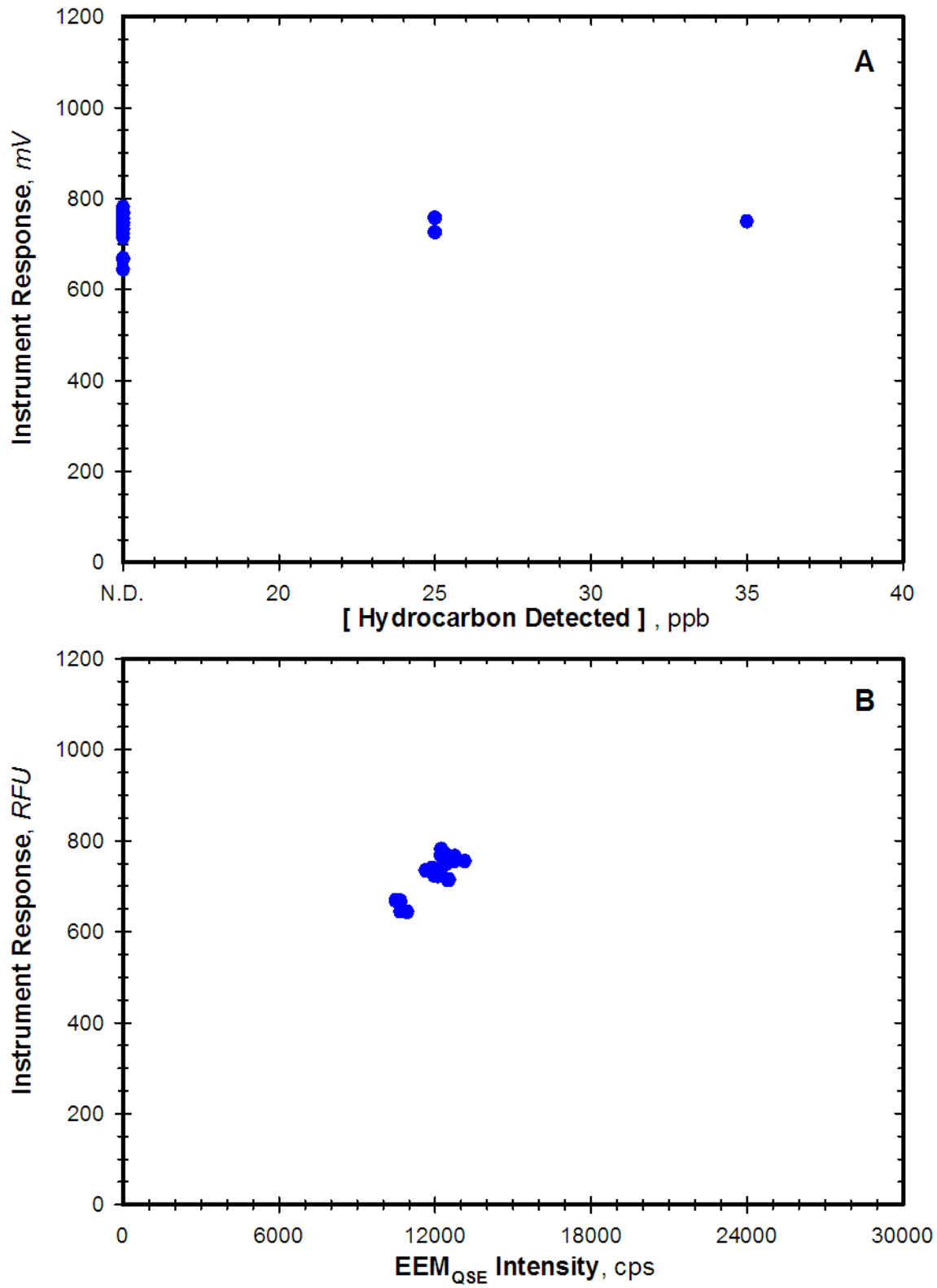
42

Figure 24. Response of Turner C3 CDOM sensor to ambient hydrocarbons and water fluorescence properties during the Baltimore Harbor deployment. (A) Instrument response to TPH detected by GC-FID. (B) Instrument response relative to predicted EEM_{QSE} fluorescent intensity.
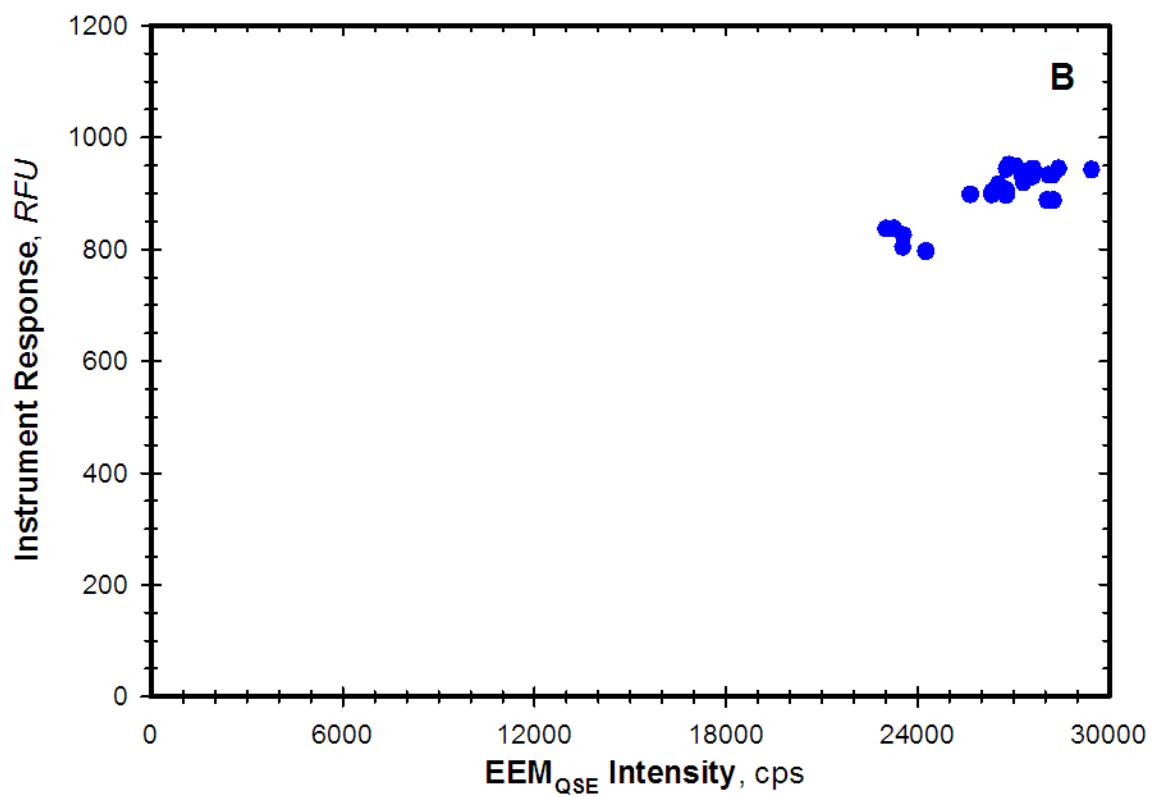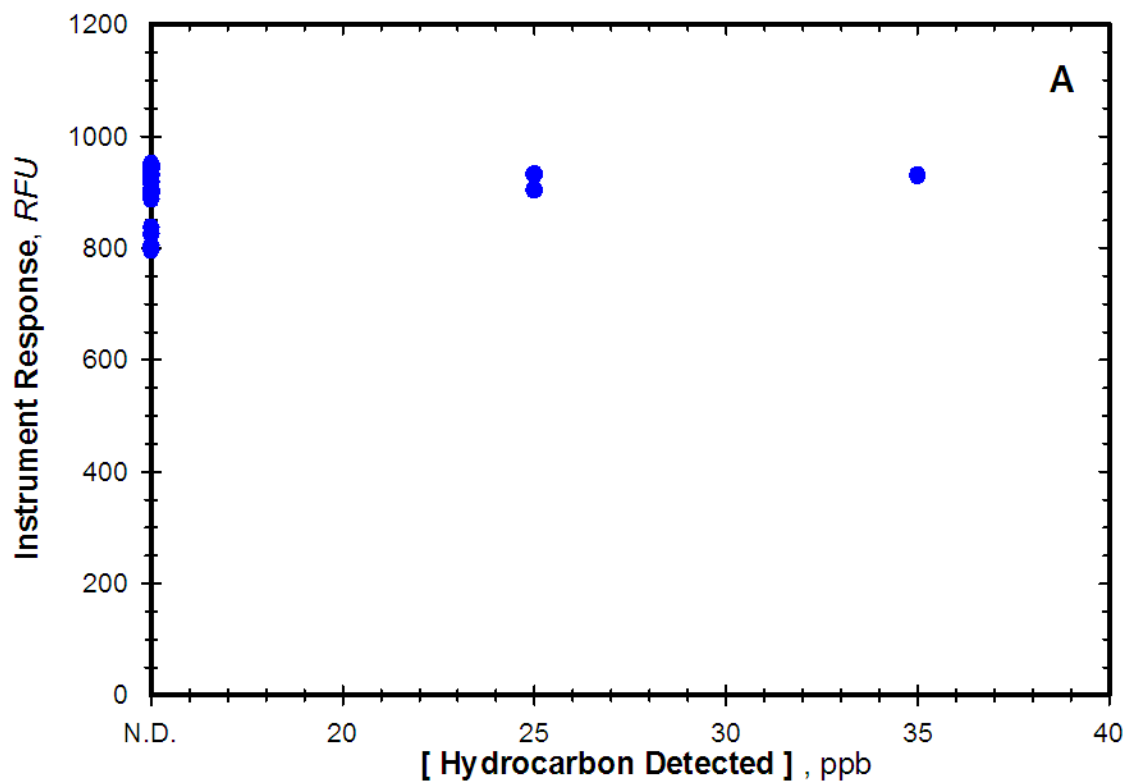
Figure 25. Response of Turner C3 Crude Oil sensor to ambient hydrocarbons and water fluorescence properties during the Baltimore Harbor deployment. (A) Instrument response to TPH detected by GC-FID. (B) Instrument response relative to predicted EEM_{QSE} fluorescent intensity.
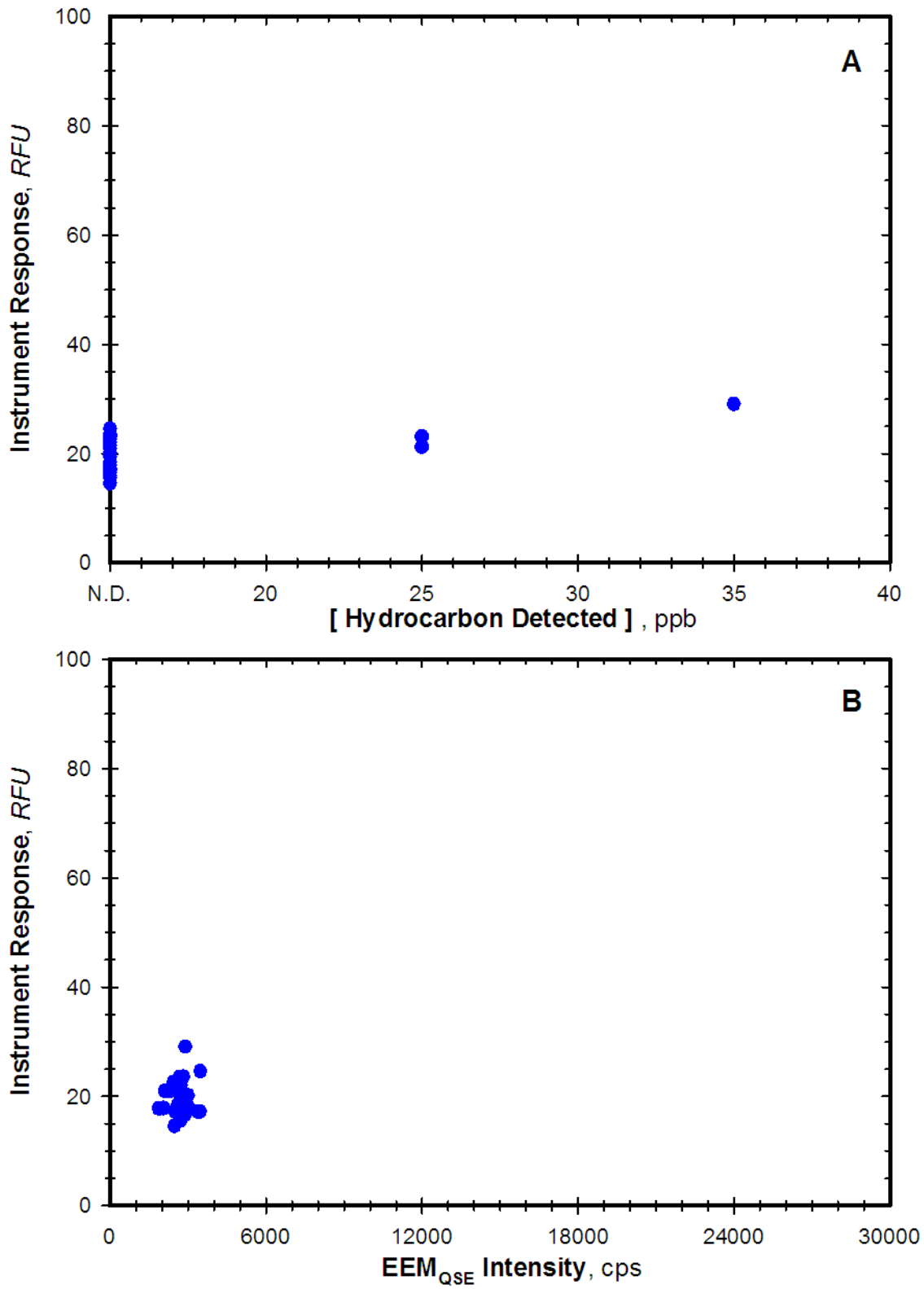
Figure 26. Response of Turner C3 Refined Fuel sensor to ambient hydrocarbons and water fluorescence properties during the Baltimore Harbor deployment. (A) Instrument response to TPH detected by GC-FID. (B) Instrument response relative to predicted EEM_{QSE} fluorescent intensity. Note reduced RFU scale.

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).
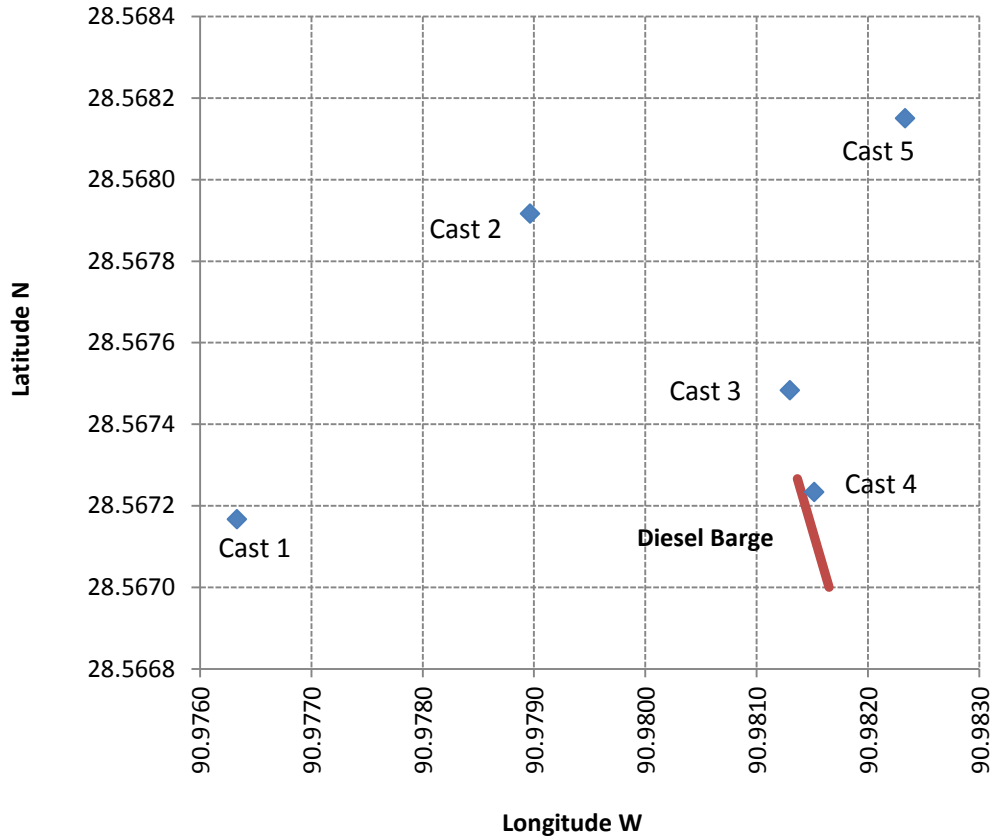
Photo 9. Site 2 of Gulf of Mexico vertical profiling over diesel barge.

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 Rig and Oil on Surface over 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 (^{o}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 27-35. Results are presented sequentially for the CDOM, Crude Oil and Refined Fuel sensors. Each panel displays the sensor response in RFU 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). The CDOM sensor showed an elevated response in Terrebonne Bay, with values of approximately 105 (± 24) RFU (Fig. 27). At site 2, the CDOM sensor response was significantly lower (4.2 RFU, 0-18 RFU range) and were quite variable with depth (Figs. 28-29). The sensor response matched elevated CDOM levels measured in the bottom water reference samples of casts 4 and 5 (Fig. 29). The Crude Oil sensor also showed an elevated response in Terrebonne Bay (233 ± 16 RFU) compared to offshore in the Gulf (5.5 RFU, 0-14 RFU range) (Figs. 30-32). The Crude Oil sensor showed similar variability with depth and with elevated bottom water levels at site 2, casts 4 and 5 (Fig. 32). The response of the Refined Fuel sensor was significantly lower than the other two sensors at all sites. The response in Terrebonne Bay was 14.4 (± 3) RFU (Fig. 33) and for all casts at site 2 the response only ranged from 0-4 RFU (average 2 RFU) (Figs. 34-35), similar to the baseline response observed in deionize water challenges (see Fig 6).

EEM characterizations of the reference samples revealed some possible evidence of hydrocarbon-like compounds in the surface sample of site 2, cast 5 and mid-depth sample for site 2, cast 3 (Figs. 36-38) but these all yielded non-detect results by our analytical methods. The observed fluorescence peaks mostly fell outside the optical windows of the CDOM sensor, but did show overlap with the Crude Oil and Refined Fuel optical windows. Only minimum levels of fluorescent signal was observed throughout the EEM maps of the other samples.

Cross plots of instrument response versus TPH concentration and estimated EEM_{QSE} intensity are presented in figures 39-41 for the CDOM, Crude Oil, and Refined Fuel sensors, respectively. As previously noted there were no detectable hydrocarbons within the collected reference water samples, so little or no signal for the sensors to respond to. The CDOM, Crude Oil and Refined Fuel sensors did show an elevated response to the waters within Terrebonne Bay, 98, 229 and 13 RFU respectively, well above baseline responses. The corresponding EEM_{QSE} values were 5406 cps, 12635 cps and 944 cps for the CDOM, Crude Oil and Refined fuel sensors respectively, values lower than observed during the Baltimore Harbor deployment. Offshore in the Gulf of Mexico predicted EEM_{QSE} values were lower ranging 331-579 cps, 1105-2977 cps and 298-609 cps for the CDOM, Crude Oil and Refined Fuel sensors, respectively. Corresponding instrument responses were also dampened, ranging from 2.9-4.5 RFU, 4.7-6.3 RFU and 1.6-2.9 RFU for the CDOM, Crude Oil and Refined Fuel sensors, respectively. Only the CDOM and Crude Oil sensors were noticeably above baseline response levels.
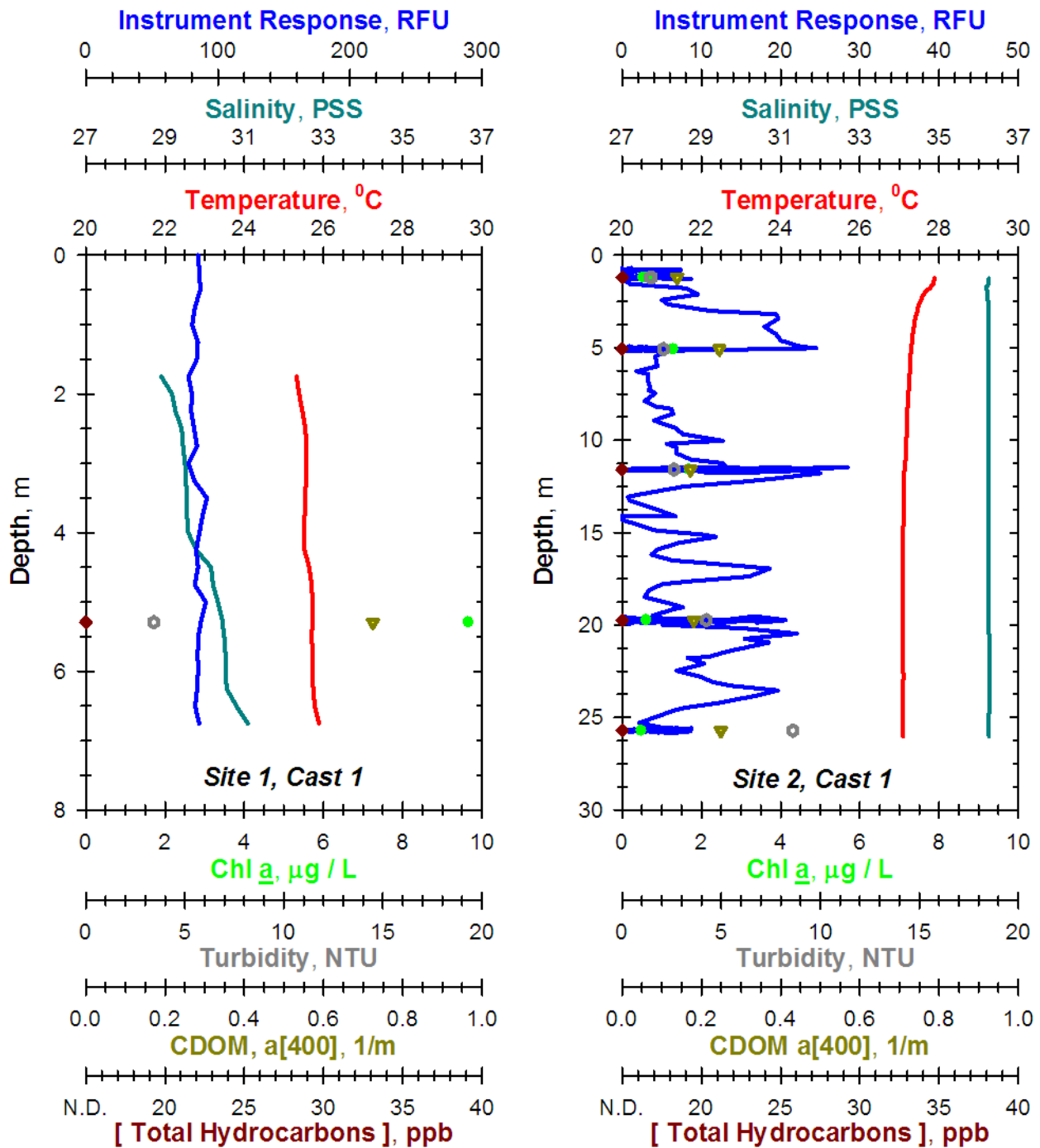
Figure 27. Gulf of Mexico field trials with the Turner C3 CDOM sensor, Site 1 and Site 2, Cast 1. Hydrocast profiles in a nearshore and offshore environment. Left panel: Site 1; Terrebonne Bay (29.02.791N. 90.33.410W). Right Panel: Site 2, Cast1; 1000' due East of a sunken fuel oil barge (28.34.03N, 90.58.58W). Note that scale is expanded relative to previous figures to reveal small responses in this environment.
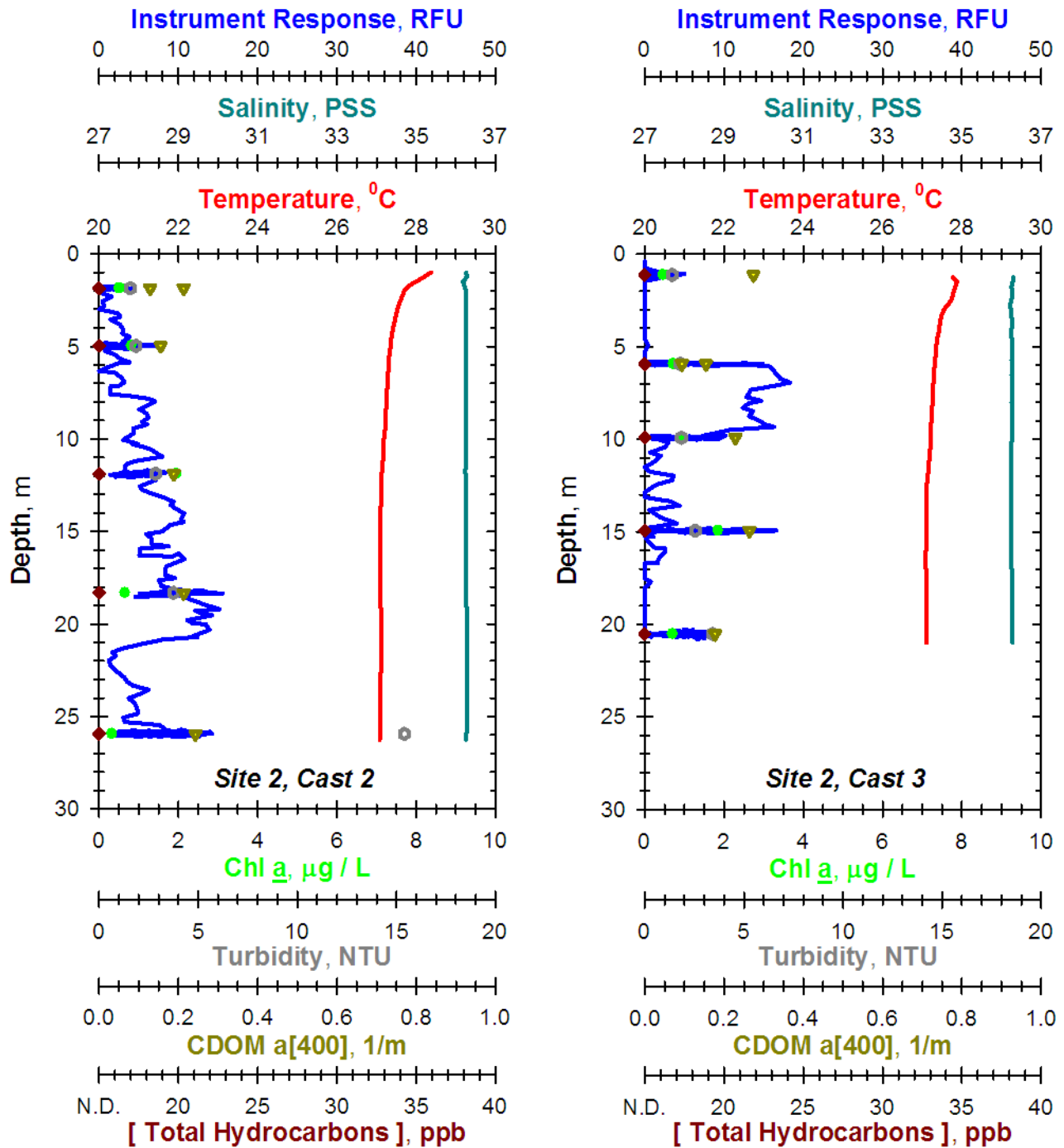
Figure 28. Gulf of Mexico hydrocast profiles from the Turner C3 CDOM sensor, Site 2, Cast 2 and Cast 3. Left panel: Cast 2; Inside slick from oil barge (28.34.075N, 90.58.738W). Right panel: Cast 3; Inside plume near submerged barge (28.34.049N, 90.58.878W). Refer to Fig. 27 for details.
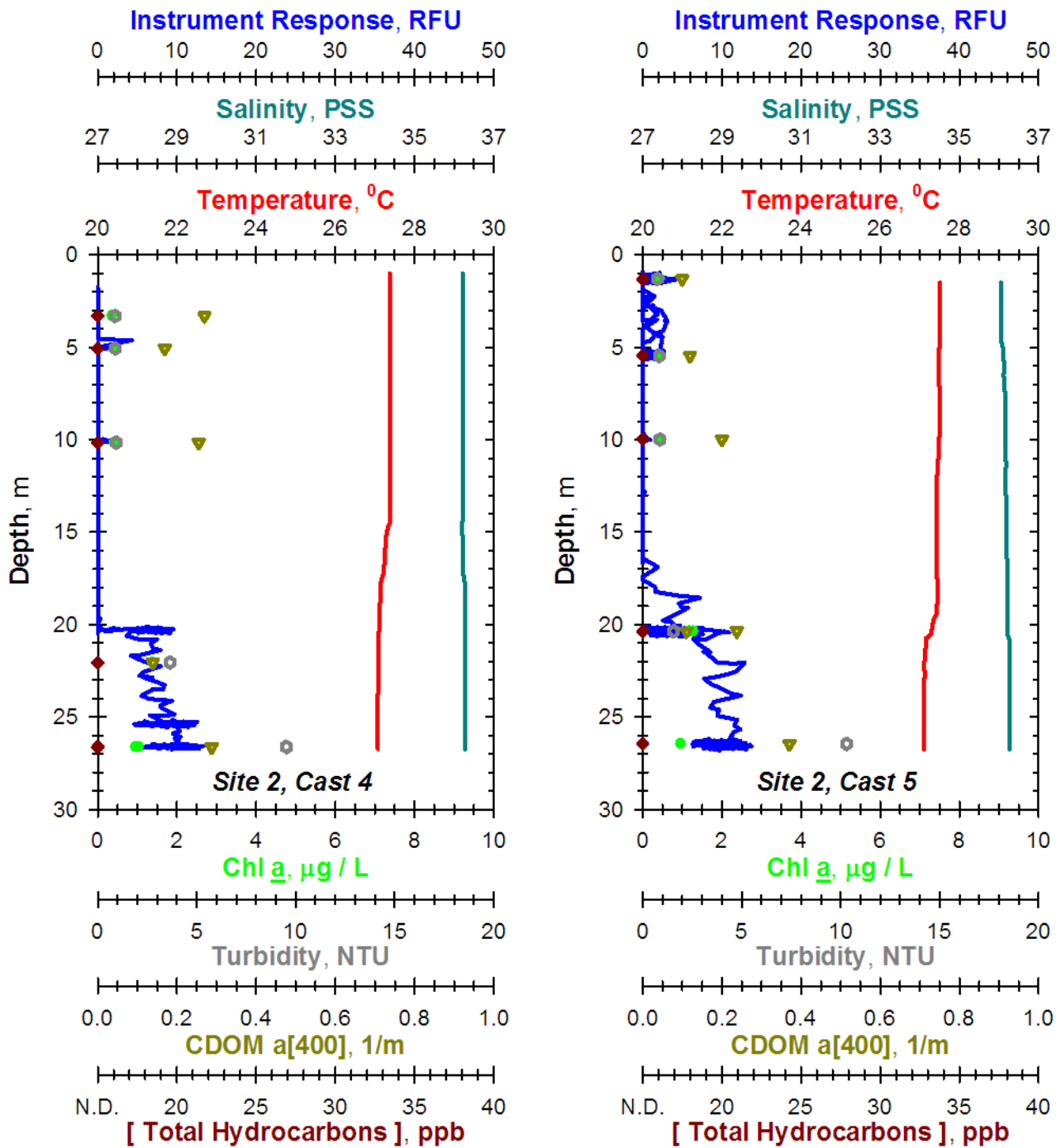
Figure 29. Gulf of Mexico hydrocast profiles from the Turner C3 CDOM sensor, Site 2, Cast 4 and Cast 5. Left panel: Cast 4; Over sunken barge, drifting with surface slick (28.34.034N, 90.58.891W). Right panel: Cast 5; Down stream of sunken barge (28.34.089N 90.58.940W). Refer to Fig. 27 for details.
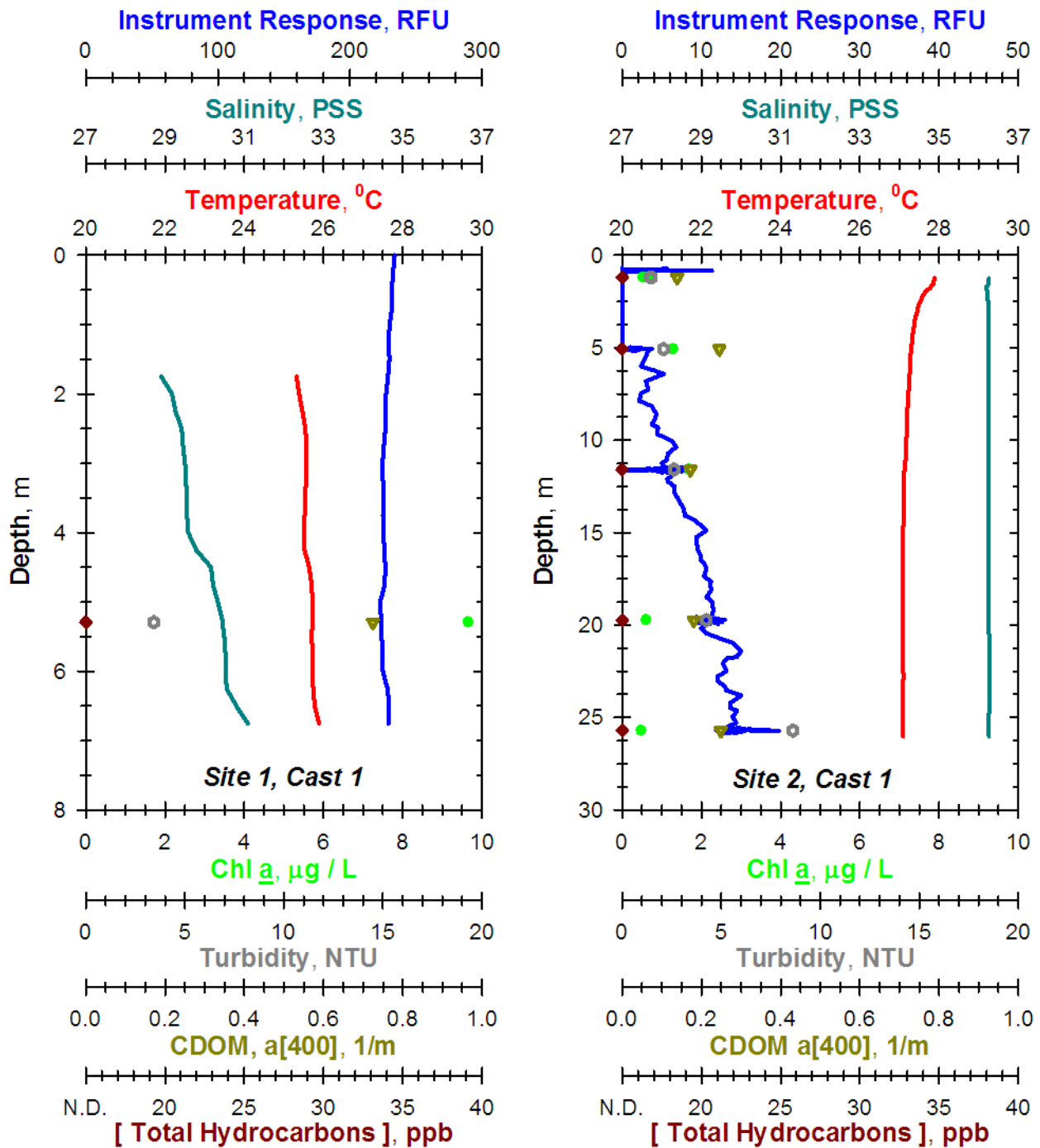
Figure 30. Gulf of Mexico field trials with the Turner C3 Crude Oil sensor, Site 1 and Site 2, Cast 1. Hydrocast profiles in a nearshore and offshore environment. Left panel: Site 1; Terrebonne Bay (29.02.791N. 90.33.410W). Right Panel: Site 2, Cast1; 1000' due East of a sunken fuel oil barge (28.34.03N, 90.58.58W). Note that scale is expanded relative to previous figures to reveal small responses in this environment.
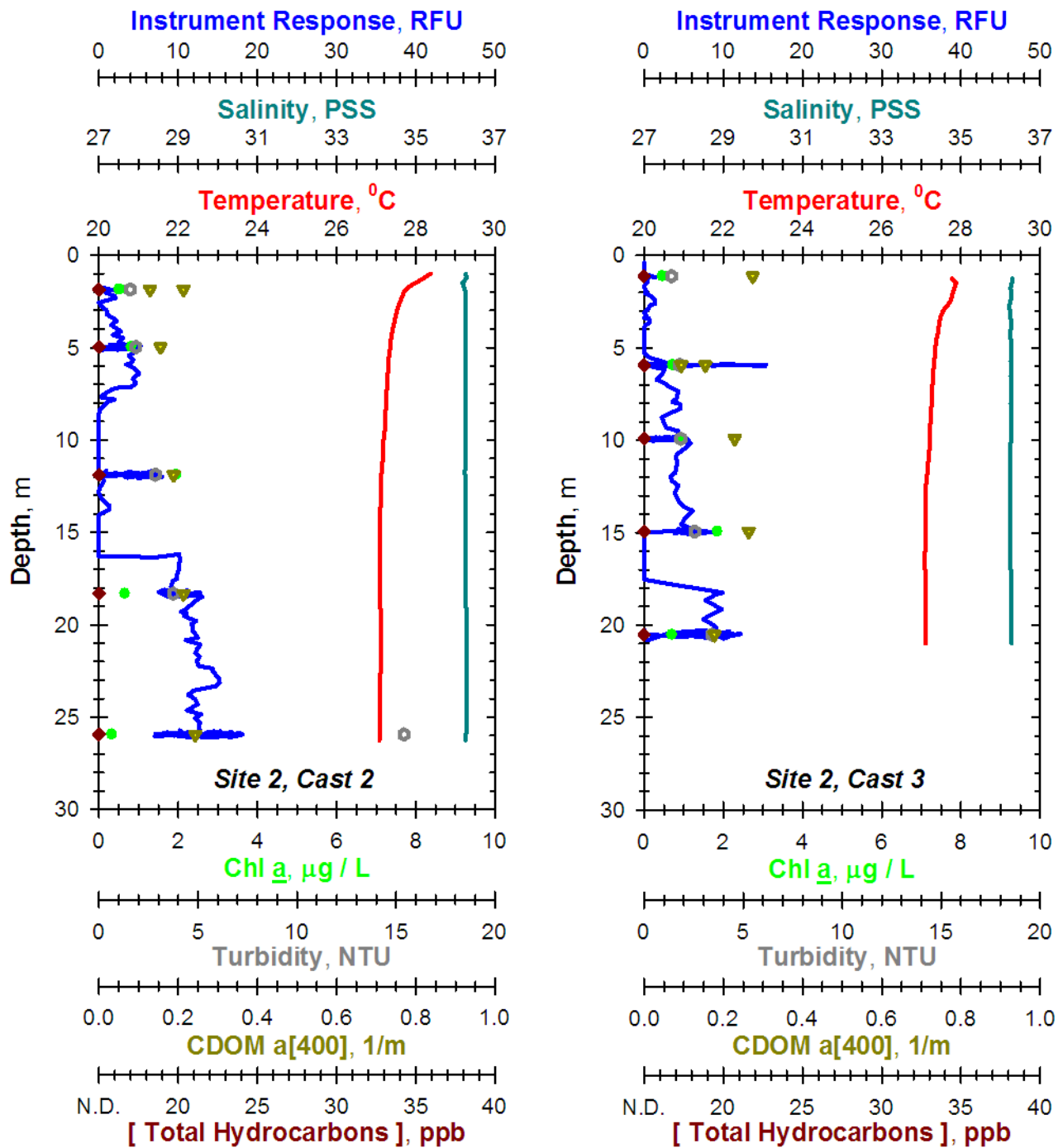
Figure 31. Gulf of Mexico hydrocast profiles from the Turner C3 Crude Oil sensor, Site 2, Cast 2 and Cast 3. Left panel: Cast 2; Inside slick from oil barge (28.34.075N, 90.58.738W). Right panel: Cast 3; Inside plume near submerged barge (28.34.049N, 90.58.878W). Refer to Fig. 30 for details.
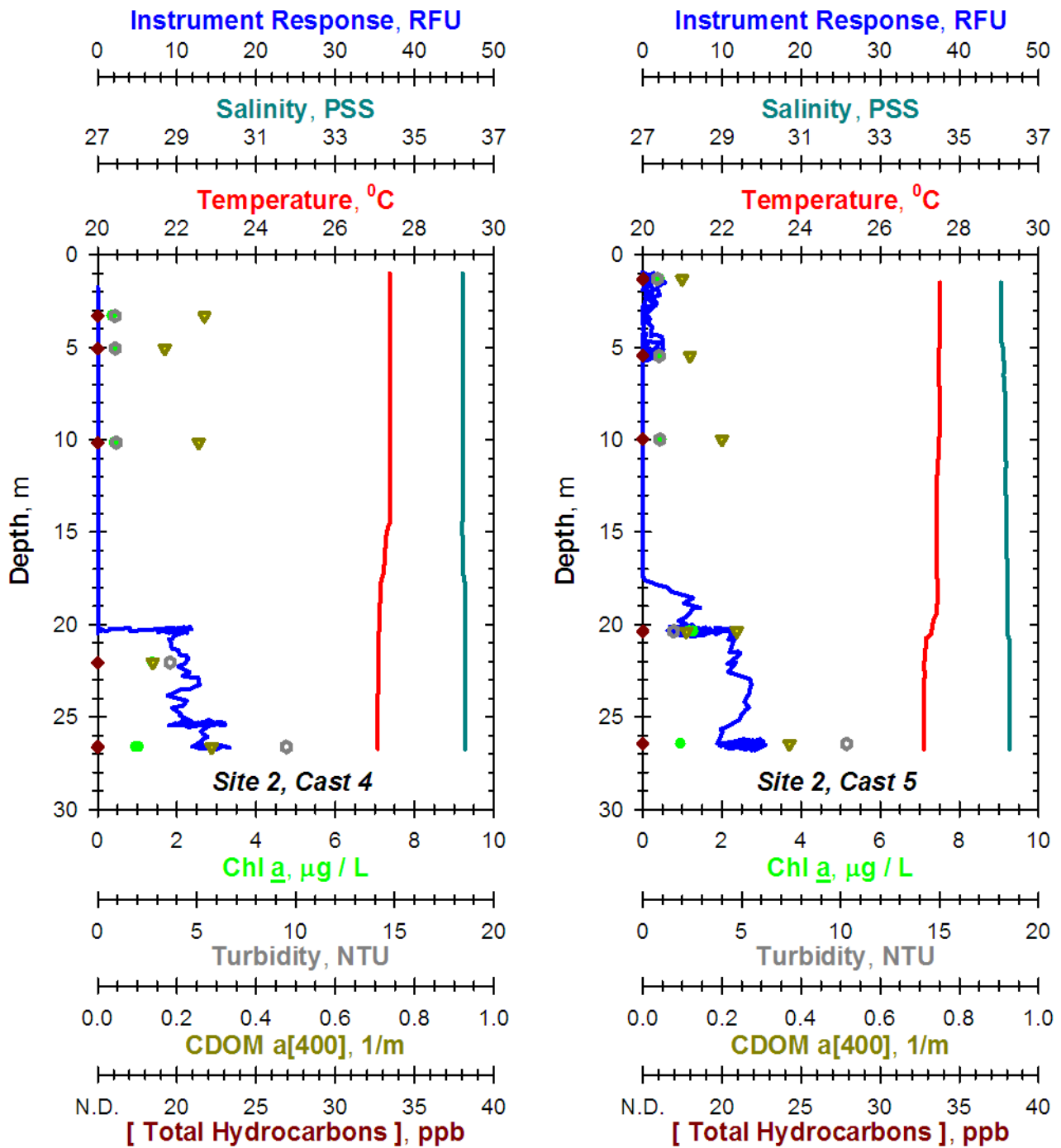
Figure 32. Gulf of Mexico hydrocast profiles from the Turner C3 Crude Oil sensor, Site 2, Cast 4 and Cast 5. Left panel: Cast 4; Over sunken barge, drifting with surface slick (28.34.034N, 90.58.891W). Right panel: Cast 5; Down stream of sunken barge (28.34.089N 90.58.940W). Refer to Fig. 30 for details.
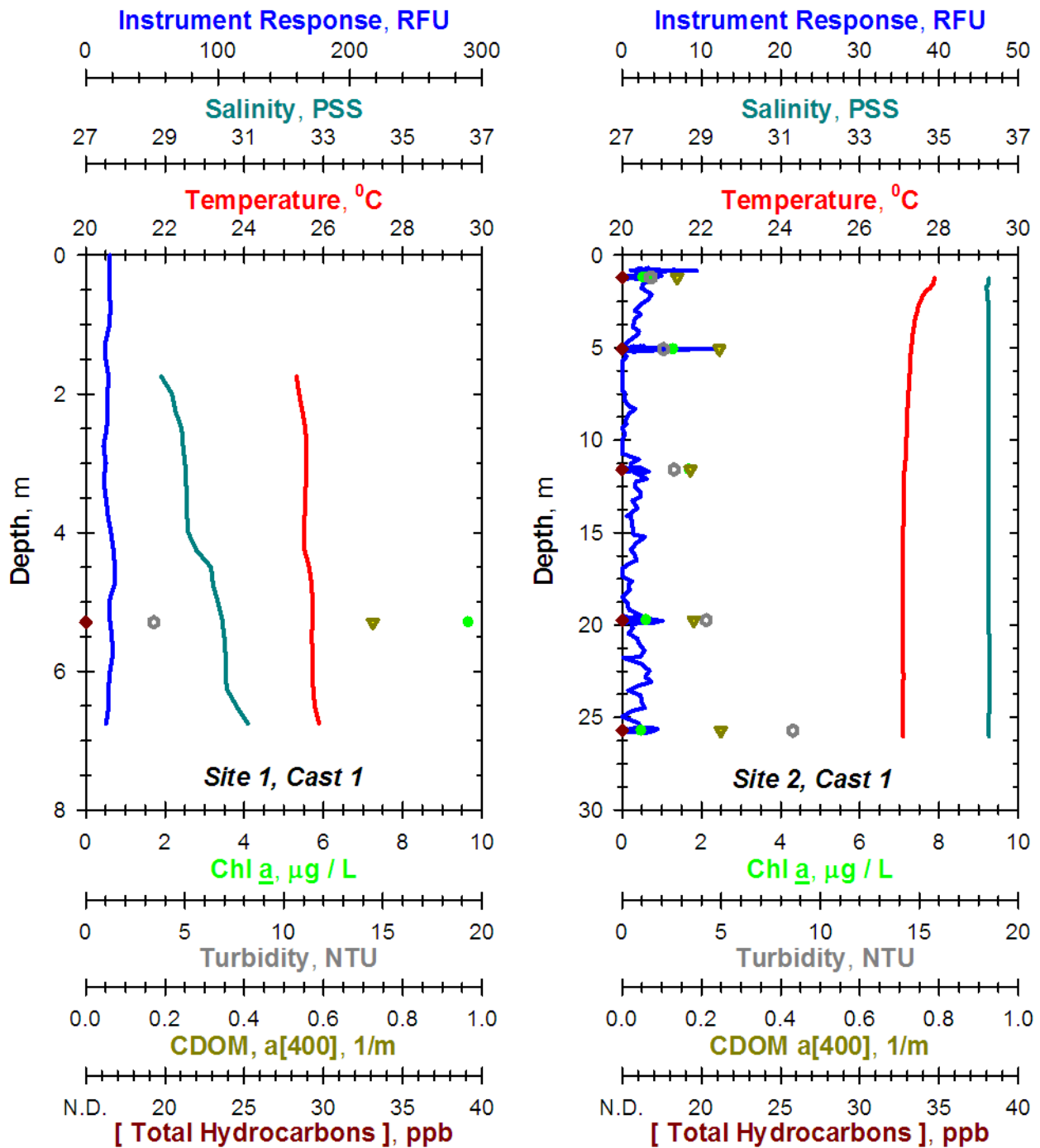
Figure 33. Gulf of Mexico field trials with the Turner C3 Refined Fuel sensor, Site 1 and Site 2, Cast 1. Hydrocast profiles in a nearshore and offshore environment. Left panel: Site 1; Terrebonne Bay (29.02.791N. 90.33.410W). Right Panel: Site 2, Cast1; 1000' due East of a sunken fuel oil barge (28.34.03N, 90.58.58W). Note that scale is expanded relative to previous figures to reveal small responses in this environment.
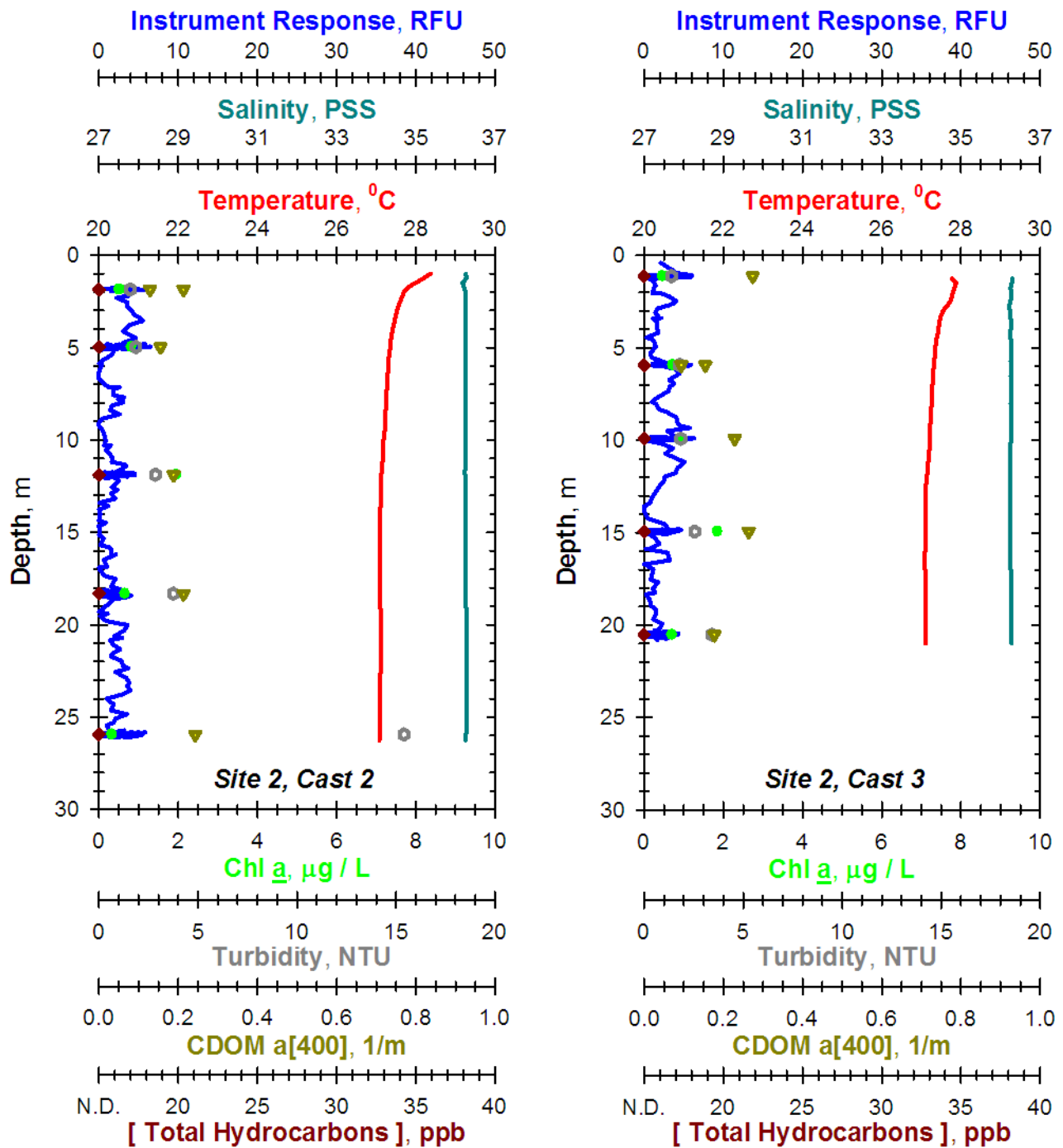
Figure 34. Gulf of Mexico hydrocast profiles from the Turner C3 Refined Fuel sensor, Site 2, Cast 2 and Cast 3. Left panel: Cast 2; Inside slick from oil barge (28.34.075N, 90.58.738W). Right panel: Cast 3; Inside plume near submerged barge (28.34.049N, 90.58.878W). Refer to Fig. 33 for details.
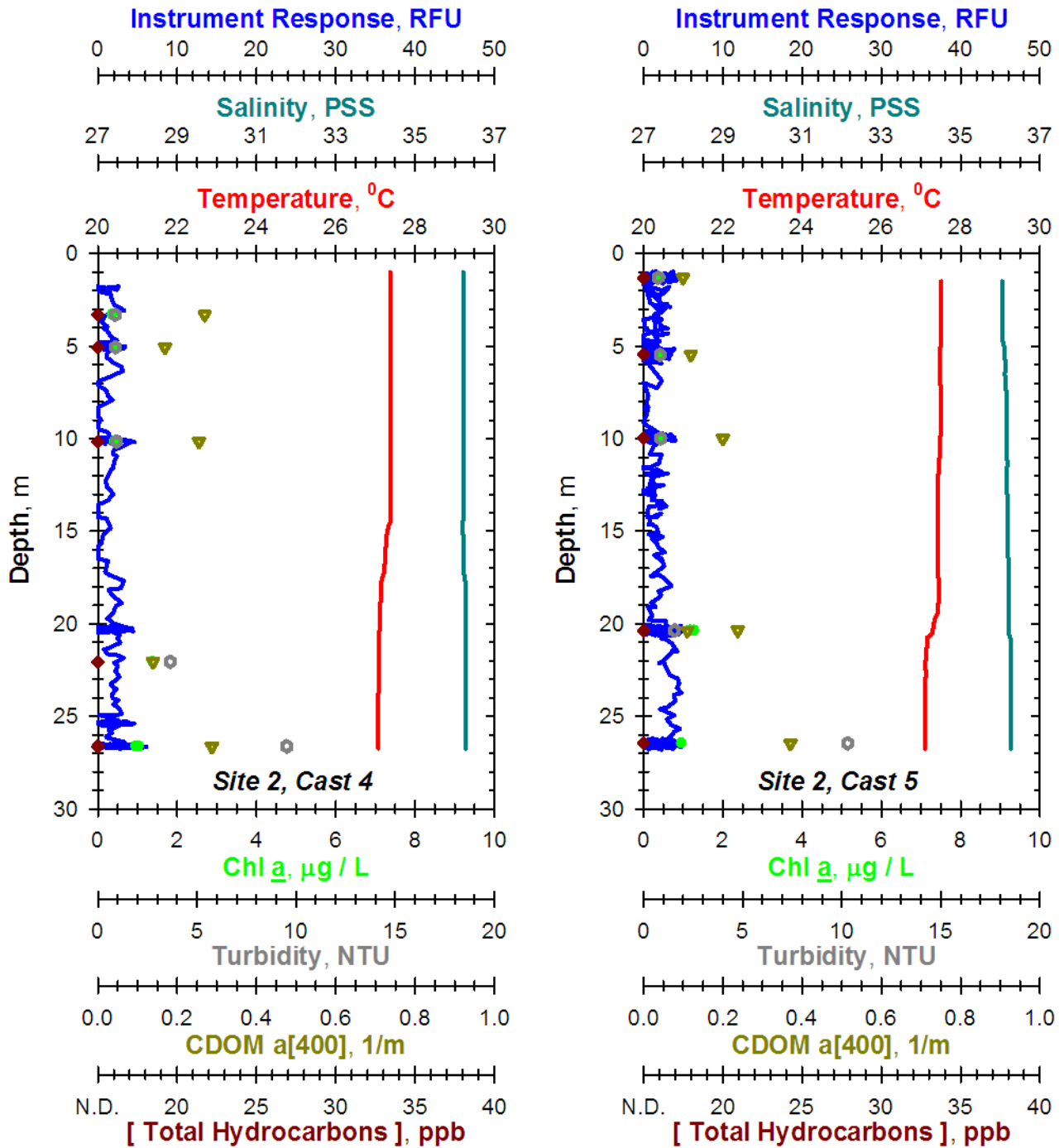
Figure 35. Gulf of Mexico hydrocast profiles from the Turner C3 Refined Fuel sensor, Site 2, Cast 4 and Cast 5. Left panel: Cast 4; Over sunken barge, drifting with surface slick (28.34.034N, 90.58.891W). Right panel: Cast 5; Down stream of sunken barge (28.34.089N 90.58.940W). Refer to Fig. 33 for details.
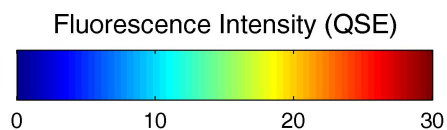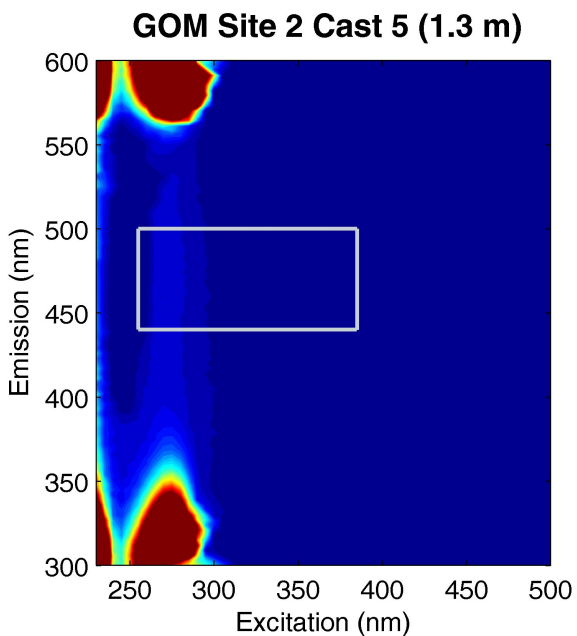
GOM Site 2 Cast 1 (19.7 m)

GOM Site 2 Cast 1 (1.2 m)

GOM Site 2 Cast 3 (14.9 m)

GOM Site 2 Cast 5 (20.35 m)

GOM Site 2 Cast 5 (1.3 m)

Fluorescence Intensity (QSE)

0   10   20   30
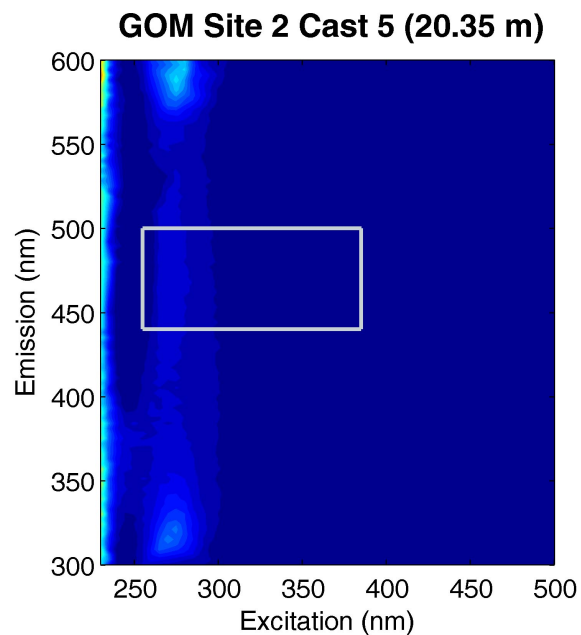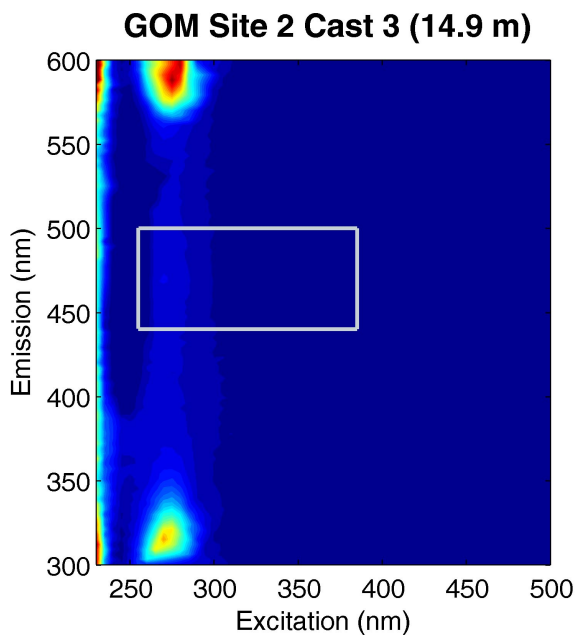
Figure 36. Representative EEM$_{QSE}$ for Gulf of Mexico hydrocast samples with the optical window for the Turner C3 CDOM sensor represented by the grey outline box. No petroleum hydrocarbons were detected by GC-FID.

GOM Site 2 Cast 1 (19.7 m)

GOM Site 2 Cast 1 (1.2 m)

GOM Site 2 Cast 3 (14.9 m)

GOM Site 2 Cast 5 (20.35 m)

GOM Site 2 Cast 5 (1.3 m)

Fluorescence Intensity (QSE)
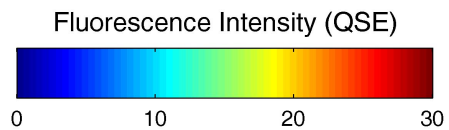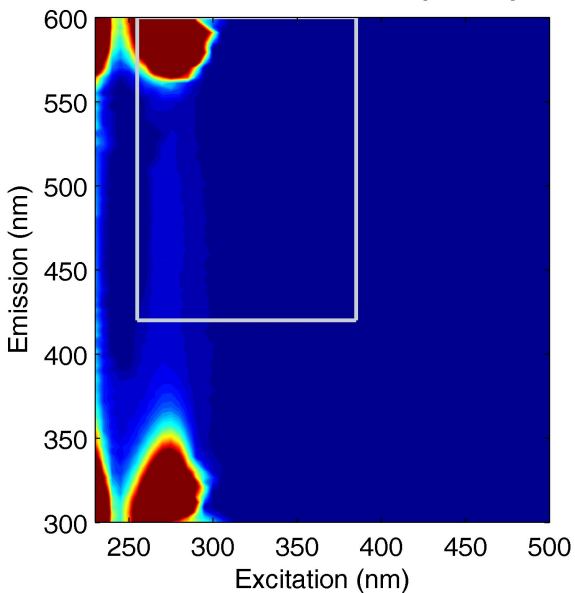
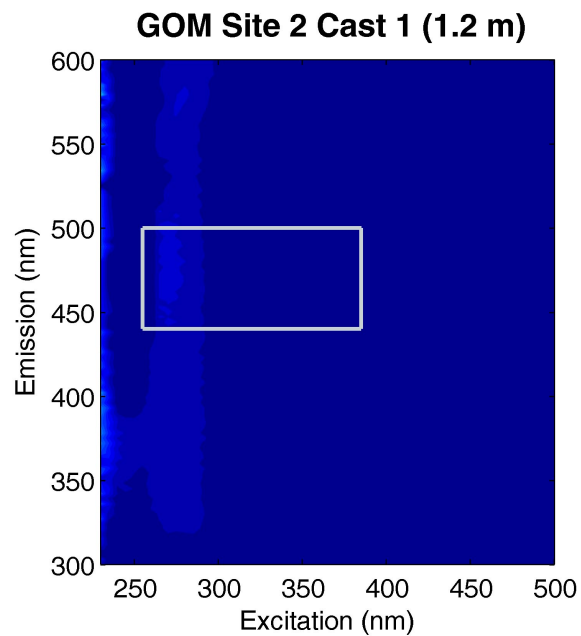**Figure 37.** Representative EEM$_{QSE}$ for Gulf of Mexico hydrocast samples with the optical window for the Turner C3 Crude Oil sensor represented by the grey outline box. No petroleum hydrocarbons were detected by GC-FID.

GOM Site 2 Cast 1 (19.7 m)

GOM Site 2 Cast 1 (1.2 m)

GOM Site 2 Cast 3 (14.9 m)

GOM Site 2 Cast 5 (20.35 m)

GOM Site 2 Cast 5 (1.3 m)

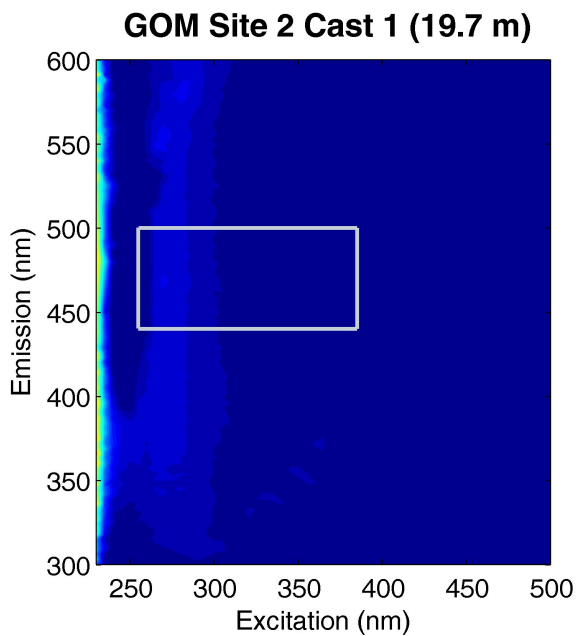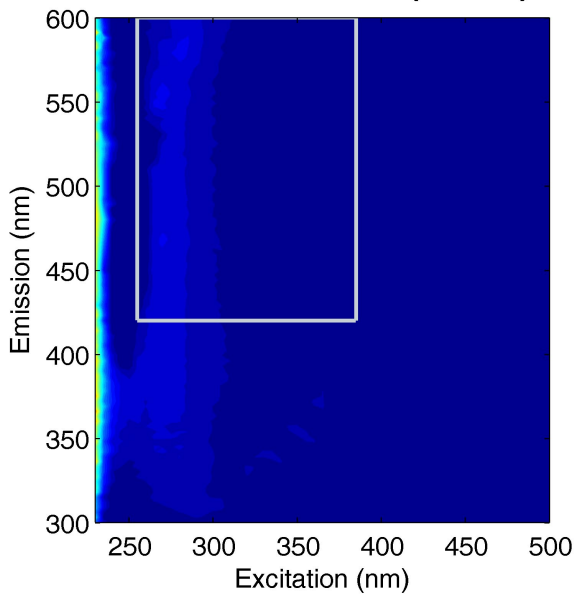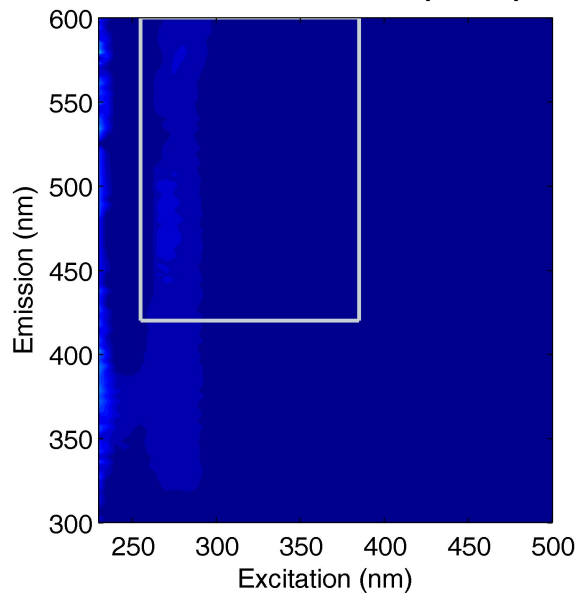Fluorescence Intensity (QSE)
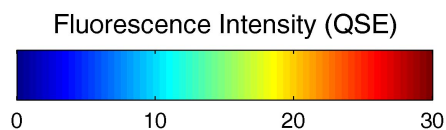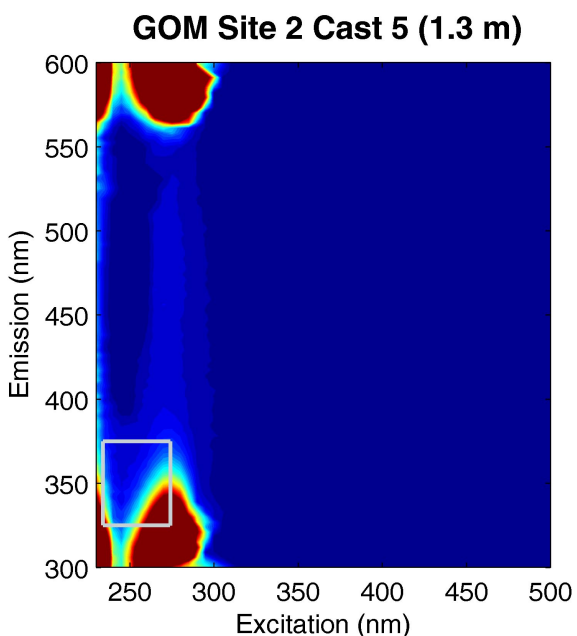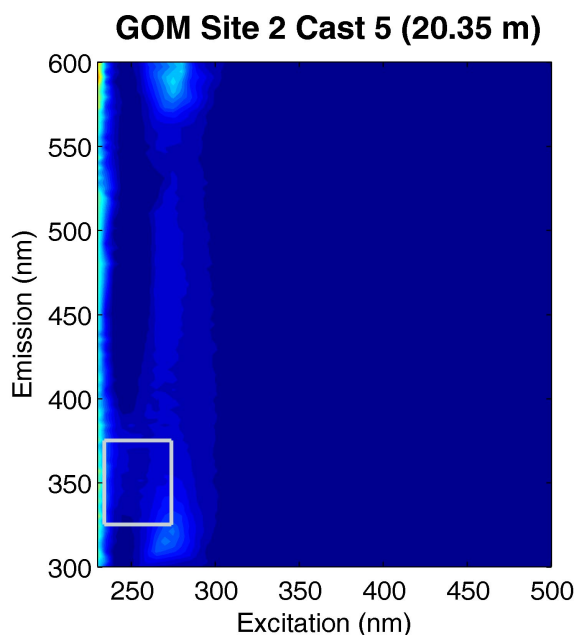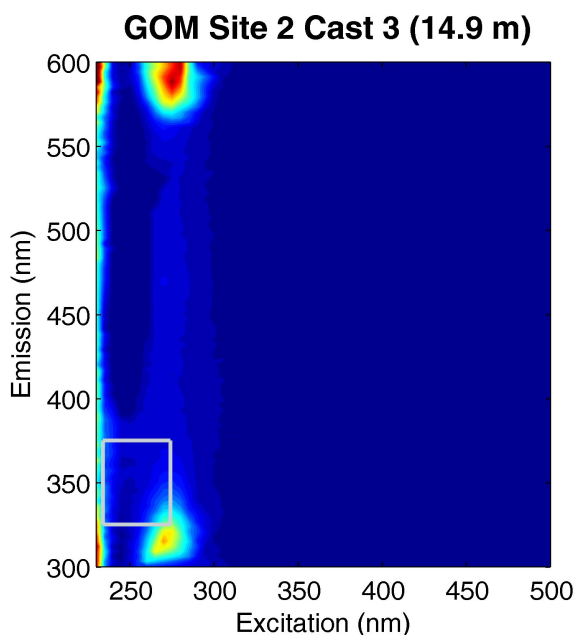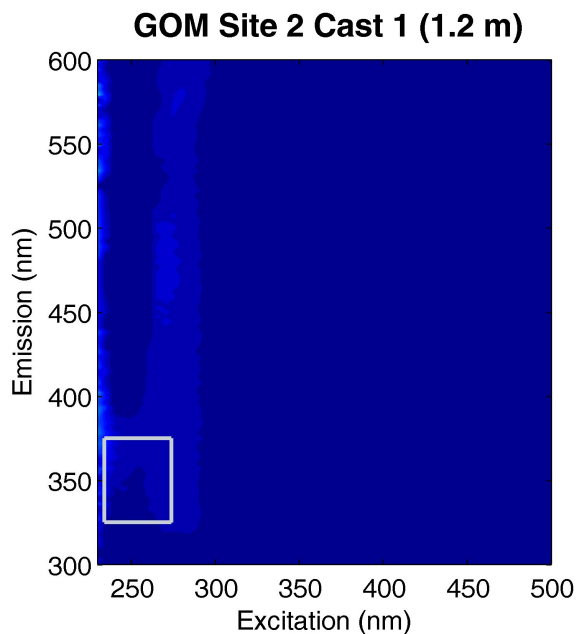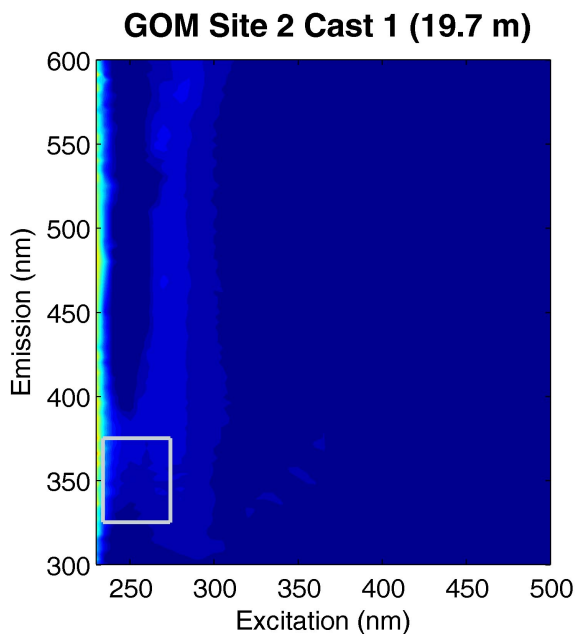
Figure 38. Representative EEM$_{QSE}$ for Gulf of Mexico hydrocast samples with the optical window for the Turner C3 Refined Fuel sensor represented by the grey outline box. No petroleum hydrocarbons were detected by GC-FID.
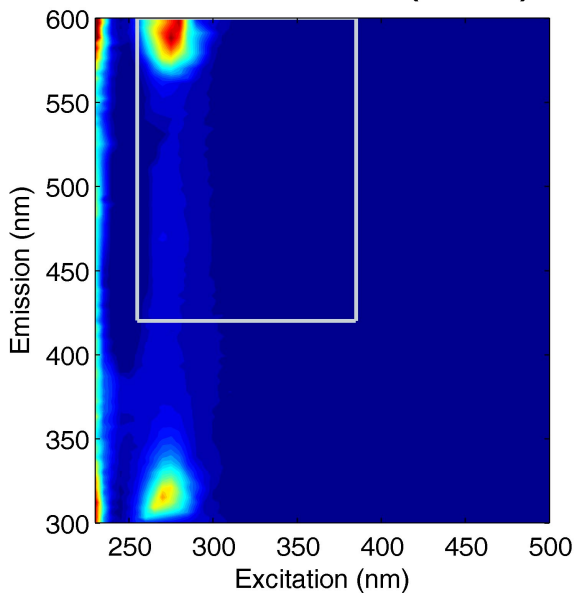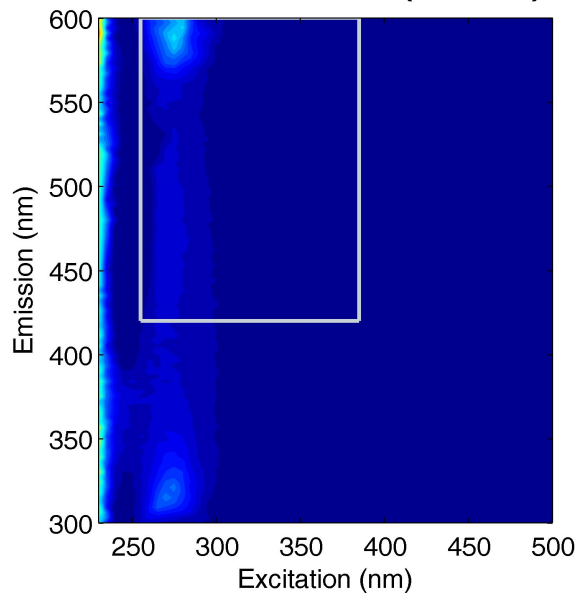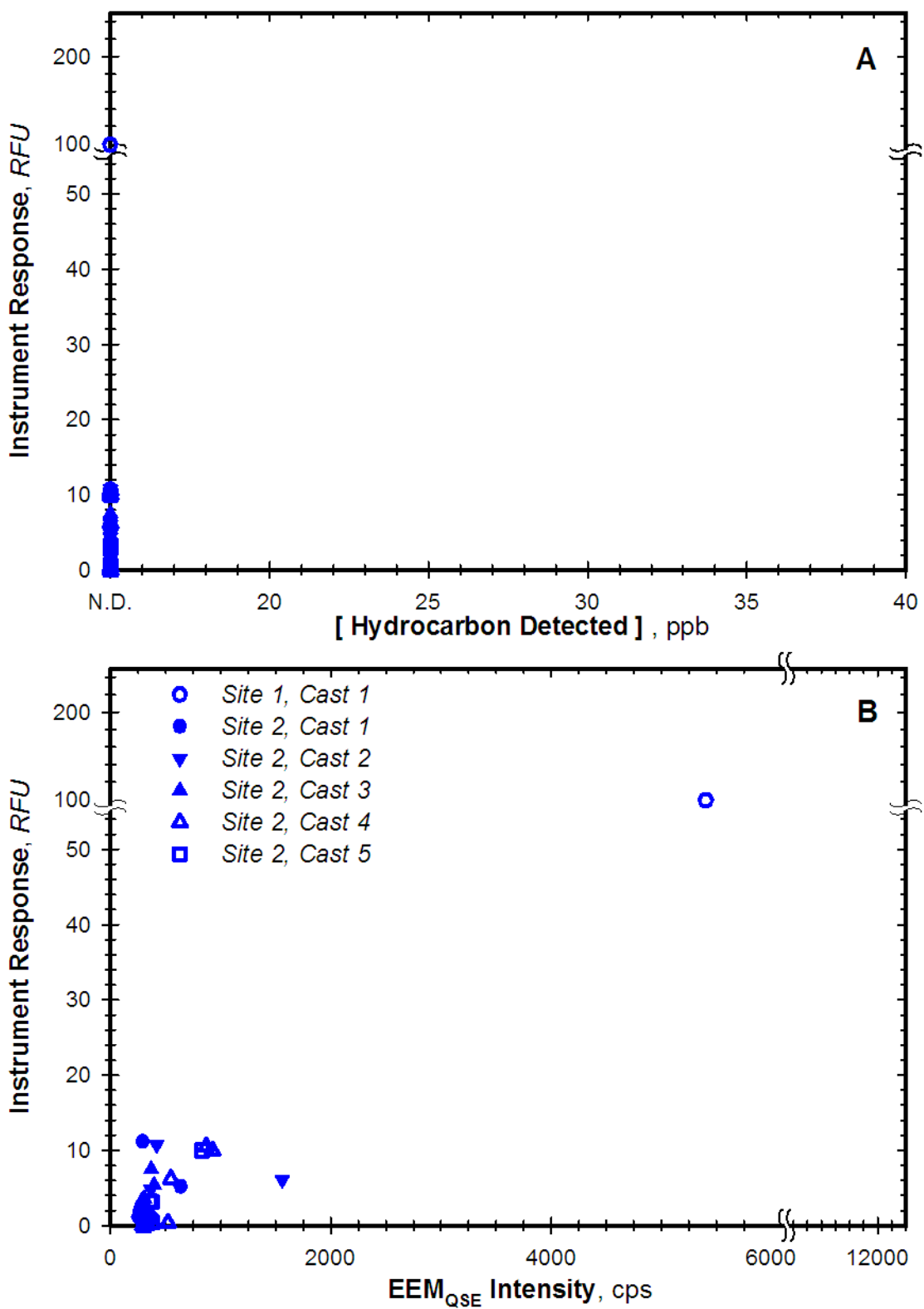
Figure 39. Response of the Turner C3 CDOM sensor to ambient hydrocarbons and water fluorescence properties at the Gulf of Mexico hydrocast sites. (A) Instrument response to TPH detected by GC-FID, no TPH reported for this same batch. (B) Instrument response relative to predicted EEM_{QSE} fluorescent 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.
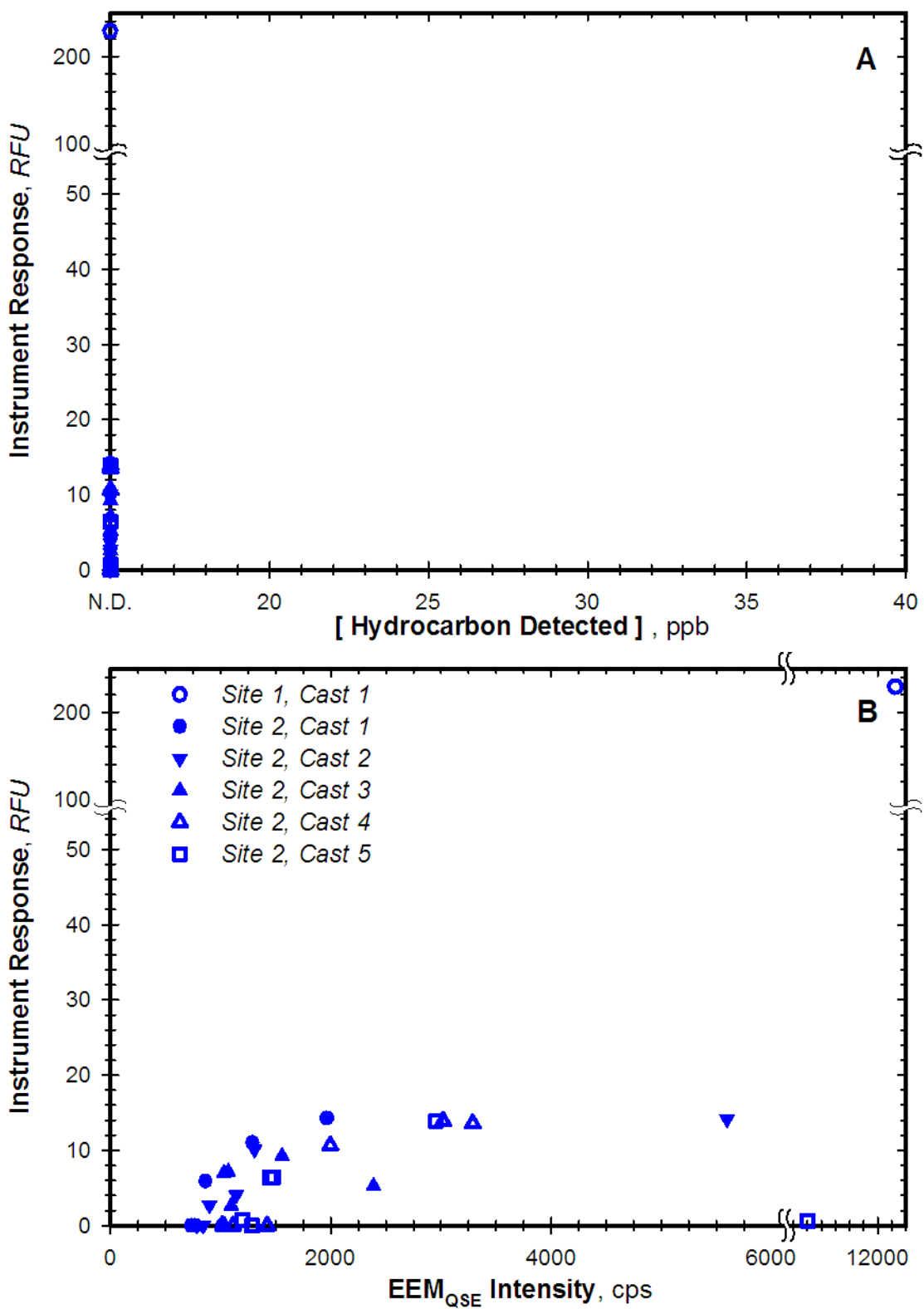
Figure 40. Response of the Turner C3 Crude Oil sensor to ambient hydrocarbons and water fluorescence properties at the Gulf of Mexico hydrocast sites. (A) Instrument response to TPH detected by GC-FID, no TPH reported for this same batch. (B) Instrument response relative to predicted EEM_{QSE} fluorescent 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.
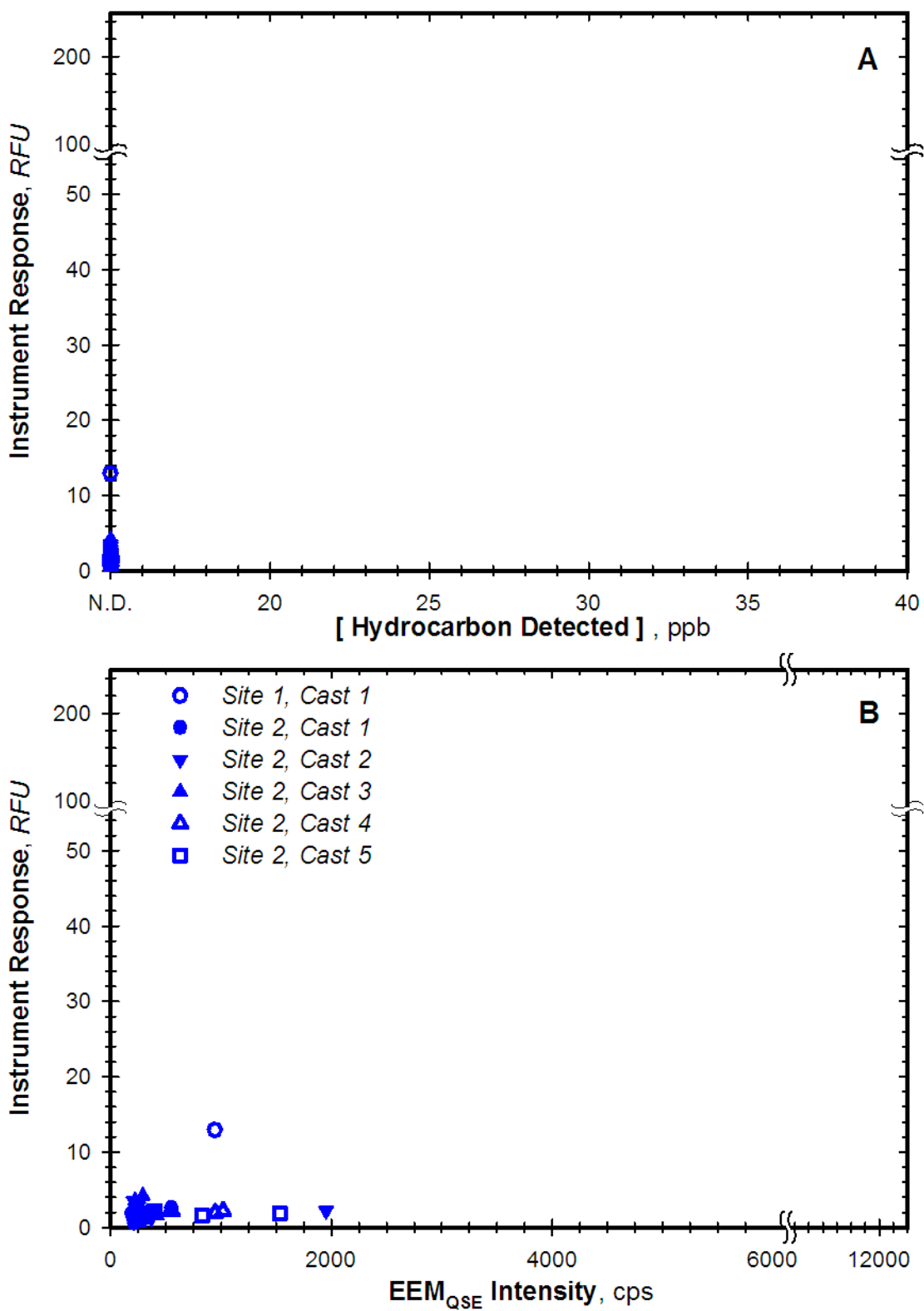
Figure 41. Response of the Turner C3 Refined Fuel sensor to ambient hydrocarbons and water fluorescence properties at the Gulf of Mexico hydrocast sites. (A) Instrument response to TPH detected by GC-FID, no TPH reported for this same batch. (B) Instrument response relative to predicted EEM_{QSE} fluorescent 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.
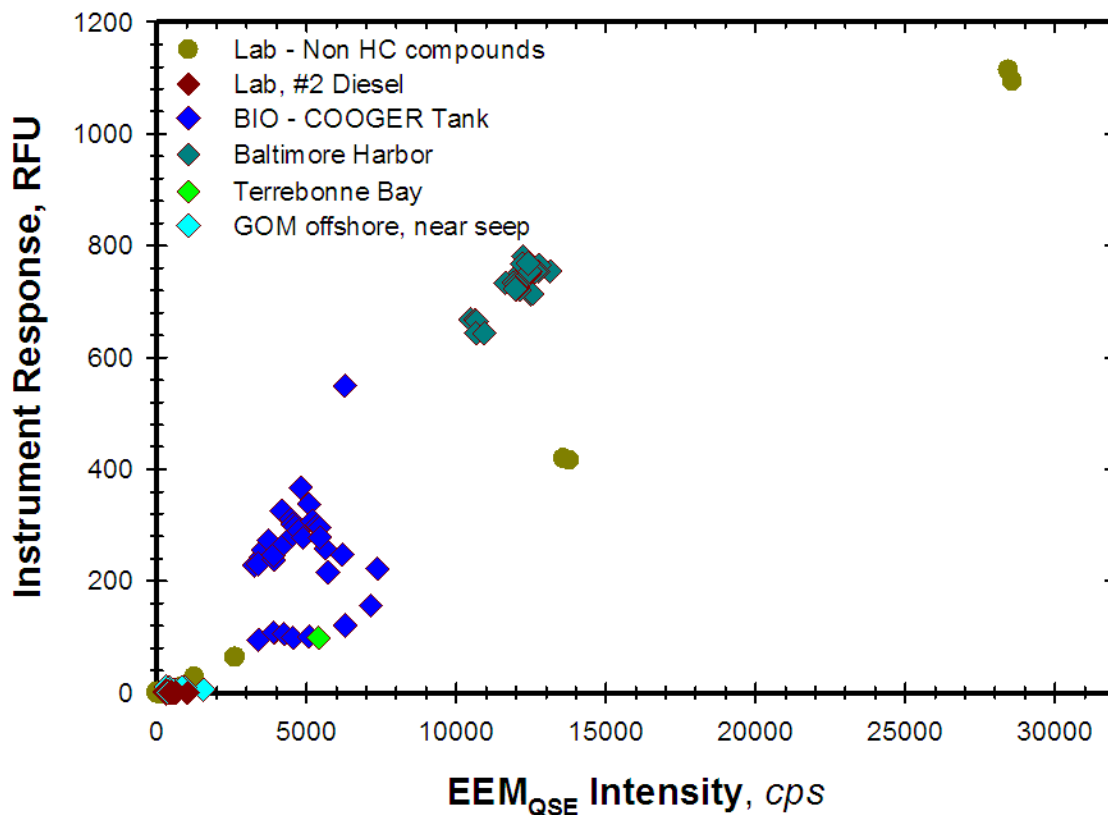
Figure 42. Global response of the Turner C3 CDOM sensor to water fluorescence properties derived from added challenge compounds, defined crude oils physically dispersed in presence or absence of chemical dispersant (BIO-COOGER Tank) or in natural waters with varying turbidity and CDOM loadings (Baltimore 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).
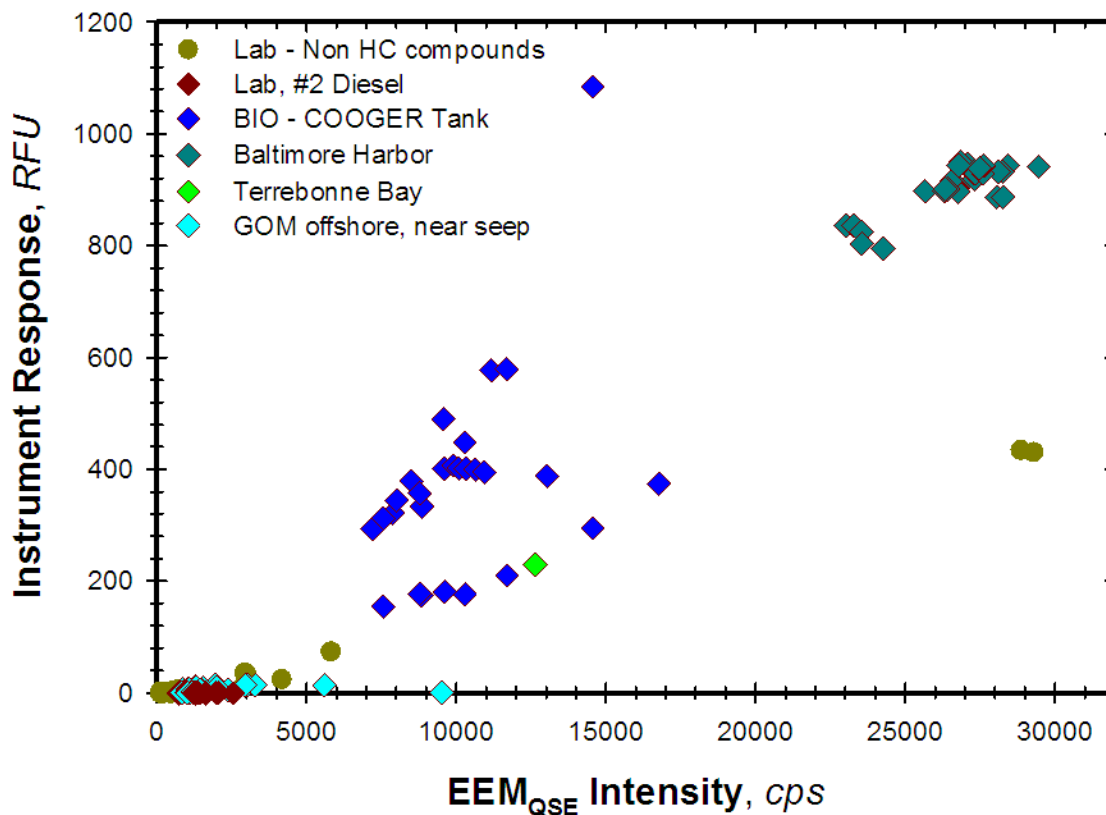
Figure 43. Global response of the Turner C3 Crude Oil sensor to water fluorescence properties derived from added challenge compounds, defined crude oils physically dispersed in presence or absence of chemical dispersant (BIO-COOGER Tank) or in natural waters with varying turbidity and CDOM loadings (Baltimore 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).
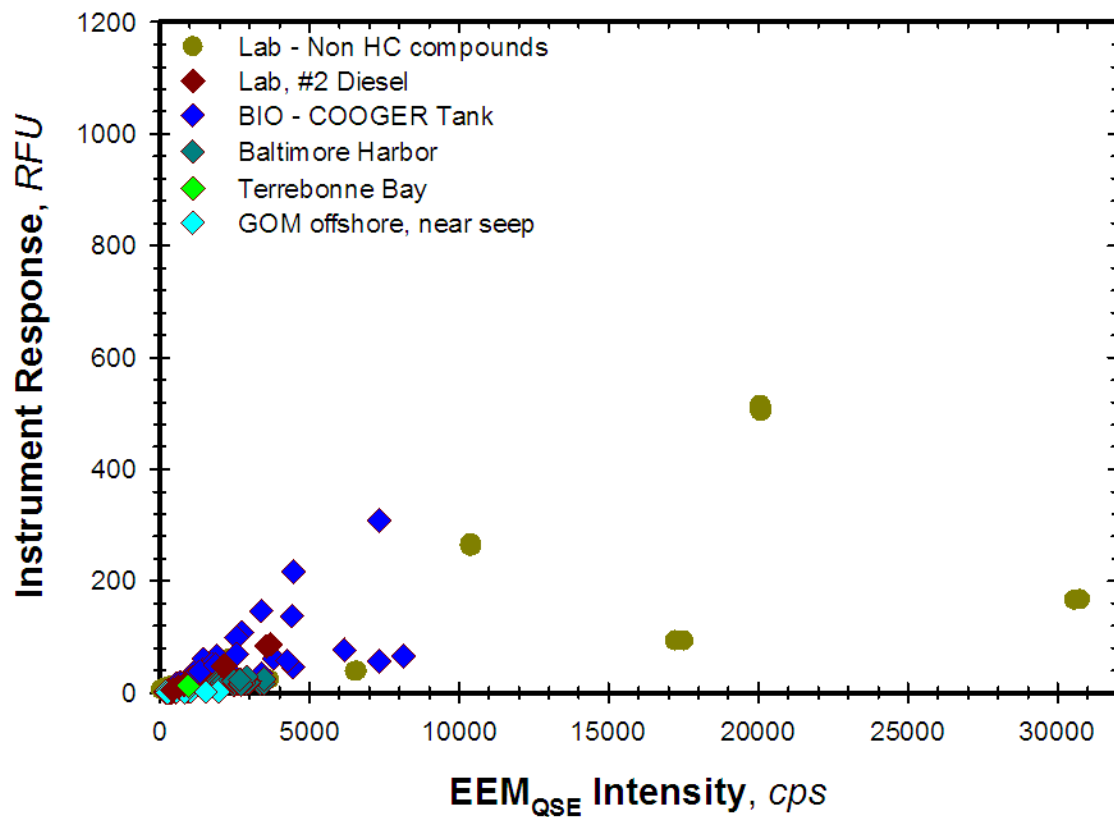
Figure 44. Global response of the Turner C3 Refined Fuel sensor to water fluorescence properties derived from added challenge compounds, defined crude oils physically dispersed in presence or absence of chemical dispersant (BIO-COOGER Tank) or in natural waters with varying turbidity and CDOM loadings (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).

SUMMARY of INSTRUMENT RESPONSE ACROSS ALL TEST APPLICATIONS

The CDOM, Crude Oil, and Refined Fuel sensors on the Turner C3 in situ fluorometer all exhibited the expected linear signal response to challenge analyte concentration with the response slope (sensitivity) mapping to the overlap of the compound's fluorescence properties to the optical configuration of the instrument package (Figs. 42-44). Focusing on EEM_{QSE} intensities corresponding to the 0-1000 ppb range of dissolved analyte, the differential response of the suite of sensors provided in the Turner C3 package is evident. While the CDOM and Crude Oil sensors readily track changes in QS, traditionally used as the calibrant for CDOM, they were insensitive to components of refined fuels (carbazole, NDSA, #2 Diesel Fuel). In contrast, the Refined Fuel sensor exhibited a dampened response to QS but readily detected concentration changes in NDSA, #2 Diesel and crude oils in various dispersion states. Additionally, the Refined Fuel sensor exhibited lower sensitivity to environmental background which was readily detected by the CDOM and Crude Oil sensors albeit with different sensitivities. From the comparison of fluorescence sensor performance with respect to optically relevant EEM_{QSE} space, it should be evident that an understanding of ambient water conditions and choice of calibrant compound are critical for robust interpretation of hydrocarbon-specific fluorescence patterns in natural waters. In general, ***care should be taken in specific interpretation of environmental fluorescence signals in absence of analytical reference samples.***

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 instrument were restored to their initial condition.

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.

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ACKNOWLEDGEMENTS:
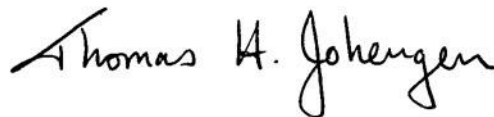
April 25, 2013
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Approved By: Dr. Mario Tamburri
ACT Executive Director

April 25, 2013
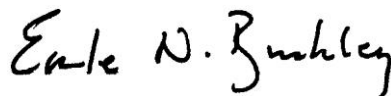_____

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Approved By: Dr. Tom Johengen
ACT Chief Scientist

April 25, 2013
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Approved By: Dr. Earle Buckley
Quality Assurance Supervisor

Three Turner Designs sensors (CDOM, Crude Oil, and Refined Fuels) were evaluated by ACT for response, linearity, accuracy/precision, and reliability. To facilitate testing for all test sites and modes, the three sensors were housed in a C3 Submersible Fluorometer that allowed functions such as calibration, auto-ranging, data logging and streaming, and simultaneous measurement of data from all three sensors.

A few important notes to keep in mind when viewing results from this evaluation: 1) a good amount of research should be done when selecting a calibration standard so as to obtain comparable or meaningful results, 2) there are potential interference factors that convolute the overall response from fluorophores of interest, more so when using fluorimetry for fluorescence detection from UV absorbing material, and 3) because of the various types of materials, all which have different excitation/emission properties eliciting a wide range of fluorescence yields, data collected from these types of sensors are qualitative in nature and can only be approximated at best. Our recommendation regarding the most appropriate way to view these data is looking at absence/presence and trend analysis of fluorescence response in an effort to determine relative changes in a fluorophore of interest rather than ground truth concentrations of specific compounds.

Response/Linearity
The necessity for testing linearity is more so for the purpose of establishing linear detection limits or saturation points for certain compounds or applications, which we feel is important to research and understand prior to sampling. Based on results from this evaluation, we would expect the Crude Oil sensor to be more sensitive than the CDOM sensor to the presence of oil in water and equivalent in sensitivity when no oil is present. The three sensors' response to actual fuel compound confirmed both the ability for our refined fuel's sensor to detect fuel compounds rendered undetectable by the CDOM or Crude Oil sensors and the minimal to no interference observed from CDOM or Crude Oil sensors when fuel is present in significant concentrations. Turbidity rejection in all three sensors was excellent; no scattering detected at ~8.0 NTU equivalent, BB3 concentration.

Accuracy/Precision
Precision of measured responses were more a focus of this evaluation, rather than accuracy as the ability to estimate concentrations accurately. Measurements of specific concentrations for multiple challenge compounds taken for a total of 10 minutes showed low signal variation allowing a clear separation among concentrations above detection limits.

Range
Detection limits (full range of detection, minimum detection limit, saturation) for CDOM, Crude Oils, and Refined Fuels are compound dependant and will also vary with water types. For example, the maximum total hydrocarbon detected during the moored sampling mode was ~35 ppb, well below our specified detection limit, but the potential for this fuel compound to have a high fluorescence yield may have attributed to the observed response. The variability among the compounds found in natural environments makes it a difficult task to specify with certainty instrument capabilities, with respect to limits and detection ranges, therefore we do our best to specify limits for lab standards which we believe can be easily obtained by users for the purpose of verifying functionality of the instrument rather than definite calibrations for estimating concentrations.

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