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Inter-laboratory calibration of estimated oil equivalent (EOE) concentrations of a water accommodated fraction (WAF) of oil and a chemically enhanced WAF (CEWAF)

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Abstract

Chemical characterization of the presence of oil in environmental samples are performed using methods of varying complexity. Extraction of samples with an organic solvent and analysis by fluorescence spectrometry has been shown to be a rapid and effective screening technique for petroleum in the environment. During experiments, rapid analysis of oil by fluorescence provides the opportunity for researchers to modify the experimental conditions in real time. Estimated Oil Equivalents (EOE) relies on the fluorescence measurement of the aromatic compounds to estimate the oil concentration.

The present intercalibration study was designed to investigate whether different fluorometer instruments can reliably measure EOE and whether the results are intercomparable. Additionally, the need for extraction of oil compounds into an

organic solvent was investigated. Three different fluorometers were used in three different laboratories: a Horiba Aqualog, a Turner Trilogy and a Shimadzu Spectrofluorophotometer RF-1501. Results from these different instruments showed excellent agreement for EOE determinations. A very high correlation was found between the EOE results obtained with Aqualog Horiba and Turner Trilogy ($r^2 = 0.9999$), with no significant differences between the mean EOE results (t-test, $p = 0.30$), and the Aqualog Horiba and Shimadzu ($r^2 = 0.995$) fluorometers, with no statistically difference between the EOE results obtained by the two instruments ($p = 0.40$).

Keywords: Earth sciences, Environmental science, Geochemistry, Geoscience, Hydrology, Natural hazards, Oceanography

1. Introduction

A large research effort in the aftermath of the spill from the Macondo 252 well resulted in a number of journal articles that studied the fate and the effects of the oil under natural and laboratory conditions. Laboratory experiments and field studies were conducted at different levels of organization (molecular, individual, community, etc.) and on different scales (mL's to 100's of liters). One challenge which frequently plagues laboratory studies is the use of experimental conditions that are realistic and relevant to conditions in the field (Aurand and Coelho, 2005; Lee et al., 2013; Sandoval et al., 2017). Continued monitoring of oil concentrations during an experiment are often a prerequisites to ensuring relevant oil concentrations.

Detailed analyses of oil, which rapidly changes composition due to different weathering processes, are, for example, carried out by gas chromatography coupled to mass spectrometry (GC/MS, Overton et al., 2016; Wade et al., 2016) and GC/GC/MS (Hall et al., 2013). These GC/MS or GC/MS/MS methods are essential for the characterization of the components of oils but are not rapid, as they require extraction with a solvent, chromatographic clean up techniques to remove interferences, and analyses using expensive instruments. Many experiments are conducted over short time periods (a few days at the most), and oil analysis using GC-MS makes it very difficult to provide the immediate feedback that allows the modifications of experimental conditions in real-time (Knap et al., 1983).

A common alternative to the use of GC-MS is to use fluorescence, a quicker and less expensive method to estimate oil concentrations in near real time, but these techniques provide no detail on oil composition. A basic fluorescence method was developed in the 1980's for MARPOLMON: Marine Pollution Monitoring of the Intergovernmental Oceanographic Commission as IOC Manuals and Guides 13 (IOC, 1981; IOC, 1984; Knap et al., 1986; Wade et al., 2011). Historically,

fluorescence and GC/MS analyses provided comparable concentration results (Ehrhardt and Knap, 1989).

To replicate field conditions below the oil-slick at the surface, during experiments, it is common to use the water-accommodated fraction of oil (WAF) as the exposure medium (Knap et al., 1983; Wade et al., 2017). WAF replicates the physical state (dissolved, dispersed oil droplets) of the oil-water system under the sea surface during a spill. In order to mimic the application of dispersant to an oil slick, and the consequent increase of oil droplets abundance in the underlying water, chemically enhanced WAF (CEWAF) is used. Different procedures to produce WAF and CEWAF have been published, and standardized procedures to produce WAF and CEWAF with different chemical characteristics are available, though these are dependent on the type of oil and dispersant (e.g. Wade et al., 2017). WAF, if made under high energy (mixing) contains dispersed droplets of oil in addition to dissolved hydrocarbons.

If WAF is made with natural, unfiltered seawater, the presence of plankton, bacteria, and detritus complicates oil determinations. The presence of particles and droplets complicates fluorescence measurements, because particles and droplets create a heterogeneous medium that obstructs the light paths in the fluorescence cell. A simple solution to this problem is the use of organic extracts. The fluorescent method mentioned above (IOC, 1984) has been standardized via a single polycyclic aromatic hydrocarbon, chrysene, a four-benzene ring PAH to make measurements comparable world-wide (Knap et al., 1986). However, mixtures of PAHs or real oil samples are better standards for fluorescence analysis (Mason, 1987). Estimated oil equivalents (EOE) measurements are a very good surrogate for oil concentrations if calibrated with the correct oil (Wade et al., 2011). EOE concentrations also have a linear relationships with oil toxicity (Bera et al., 2018), and are very good surrogates of PAH analysis done by GC-MS, if the correct standards (i.e. spilled oil samples) are used (Burns, 1993).

The Aggregation and Degradation of Dispersants and Oil by Microbial Exopolymers (ADDOMEx) consortium was funded by the Gulf of Mexico Research Initiative (GOMRI) to investigate the mechanism of marine oil snow formation (MOS), as it was discovered that marine snow was an effective transport vehicle for oil to the seafloor (Larson et al., 2018; Passow and Ziervogel, 2016). In order to understand the processes and mechanisms that may lead to the formation of MOS (see Quigg et al., 2016) for review), the ADDOMEx team conducted a diverse series of experiments. As different experimental methods were ranging from large (>100 L) glass mesocosms (Wade et al., 2017) to roller tables (6 L) (Passow et al., 2012) to bottle experiments (1 L and smaller) (Bretherton et al., 2018), the consortium needed a method to reliably determine the concentration of oil in different laboratories. Thus, an inter-laboratory inter-comparison exercise was designed and

performed with the goals to 1) develop a method that allows EOE measurement using the different fluorometers and 2) ensure that these EOE measurements were inter-comparable.

2. Materials and methods

2.1. Experimental design

A total of three intercalibration exercises were conducted. The first experiment was in association with a mesocosm experiment (mesocosm I). The production of WAF for the mesocosms study is described in [Wade et al. \(2017\)](#). In summary, 24 mL Macondo surrogate oil was mixed with 130 L of seawater in a Baffled Recirculating Tank (BRT) and mixed for 14 hours before WAF was transferred to the mesocosm tanks. CEWAF was prepared with the same procedure except that Corexit (dispersant) was premixed with oil at a ratio of 1:20 (V/V) before addition to the BRT. Samples for the intercalibration were collected immediately after WAF and CEWAF production and measured directly i.e. without extraction. A second set of measurements were done on the same source water samples but this time, samples were extracted with dichloromethane (20 mL water sample extracted with 20 mL dichloromethane). The comparison was meant to determine if extraction of samples before fluorescence measurements was a necessary step. A total of eight unextracted and eight extracted samples were measured, each including four oil calibration standards, two replicates of the WAF and two replicates of CEWAF. The fluorescence emissions of each of these samples was measured on all three instruments; a Horiba Aqualog-UV-800, a Turner Trilogy, and a Shimadzu Spectrofluorophotometer RF-1501 (see below).

A second set of intercalibration samples (exercise 2) were prepared by making WAF and CEWAF in deionized water (DI) as well as in freshly collected natural seawater from the Gulf of Mexico. WAFs were made with 0.4–4 mL of Macondo Surrogate oil added to 1.8 L of water in a 2 L aspirator bottle and mixed at 300 rpm for 24 hours ([Bera et al., 2018](#)). CEWAFs were made in a similar process except that Corexit (dispersant) was premixed with Macondo oil at a ratio of 1:20 (V/V). Two concentrations of WAF (WAF1 and WAF2) each in triplicates, and two concentrations of CEWAF (CEWAF1 and CEWAF2) in triplicate each were prepared in DI. One concentration of WAF (WAF3 with triplicates) and one concentration of CEWAF (CEWAF3 with triplicates) were prepared in seawater. All samples were extracted with dichloromethane (10 mL water sample extracted with 10 mL dichloromethane). This second intercalibration exercise was run as a blind test, meaning the nature of the samples were not known to the people measuring the samples. Only the Horiba Aqualog and Turner Trilogy participated in this second intercalibration exercise. Samples were measured immediately after extraction on the Horiba Aqualog whereas extracted samples were shipped overnight, and measured within a week

in the Turner Trilogy. A calibration curve with Macondo Oil (MC 252) extracted in dichloromethane was also prepared on each instrument and used to convert the relative fluorescence units (RFU) of each instrument into EOE oil concentrations. The seven calibration points ranged from 0.1 mg/L to 10 mg/L of oil.

A third set of intercalibration samples were collected during another mesocosm study (mesocosm VI). WAF and CEWAF samples were prepared in the BRT as in the first experiment and a total of ten samples (5 WAF and 5 CEWAF) were measured in both, the Horiba Aqualog and Shimadzu. In this mesocosm study, five replicate WAF and five replicate CEWAF mesocosms were set up and EOE in each measured by both instruments. Five mL of the samples were diluted with 5, 10 or 15 mL dichloromethane to extract the oil before measurement. A calibration curve (see above) was prepared and used to convert fluorescence units to EOE concentrations.

An additional experiment investigated the effectiveness of Corexit to disperse oil when applied in freshwater conditions. EOE of a 1 ppm solution of the single aromatic hydrocarbon, phenanthrene, prepared in both, DI and natural seawater, to which 30 μ L of Corexit (dispersant) was added, was measured on the Horiba Aqualog fluorometer.

Association between variables was tested using Spearman's rank correlation coefficients, and differences in means between pairs of instruments were tested using a paired "t" test. Tests were considered significant if $P \leq 0.05$. All statistical analyses were done in R 3.3.1, a statistical environment (R Core Team, 2015).

2.2. The fluorometers

2.2.1. Horiba Aqualog

The measurement of EOE on the Horiba Aqualog was carried out in two steps. First, the optimal wavelengths was determined by performing a scan from 200 to 800 nm on a mid-range calibration standard (either 2 or 5 mg/L). The maximum signal intensity was observed at an excitation of 260 nm and an emission of 372 nm for the Macondo Surrogate oil (Bera et al., 2018; Knap et al., 2017; Wade et al., 2011, 2017). Then, the calibration standards were measured using these excitation and emission wavelengths to create a calibration curve. Samples were measured at the same wavelengths and the calibration curve was used to determine EOE concentrations in mg L^{-1} in the samples.

2.2.2. Turner Trilogy

During the first intercalibration exercise the Turner Trilogy was used with the crude oil module 7200-063, which has a 365 nm excitation and a 410–600 nm emission

wavelength. As the maximum signal for the Macondo oil, as determined via the Horiba Aqualog, lay at an excitation of about 260 nm and an emission wavelength of 372, chlorophyll a and accessory pigments and colored dissolved organic matter, when present, interfered with the signal. By subtracting the “EOE” of the control mesocosm or blank, measured EOE were corrected for such interferences. During the second intercalibration exercise samples were measured using two different modules of the Turner Triology; the crude oil module 7200-063 described above, and a custom made module with an excitation filter at 260 ± 10 nm and an emission filter at 370 ± 10 nm.

2.2.3. Shimadzu spectrofluorophotometer RF-1501

EOE was measured at the optimum excitation and emission wavelengths of 260 and 358 nm, as determined by the Horiba Aqualog (Wade et al., 2011, 2017).

3. Results and discussion

3.1. Comparison of fluorescence intensity of samples with and without extraction

The results of the first inter-calibration, where the importance of sample extraction was tested are presented in Figs. 1 and 2 and Tables 1 and 2. A comparison between instruments when samples were measured directly, without extraction, are presented in Fig. 1, as relative fluorescence units (RFU) of each instrument. Scatter of the determined concentrations is high, which is reflected in the relatively low Spearman correlation coefficients that nevertheless are statistically significant (Table 1). There was slightly better agreement (spearman correlation coefficient 0.98) between the Turner and Shimadzu fluorometers than between Shimadzu and Horiba (0.81) or Turner and Horiba (0.83).

Extraction with a solvent (Knap et al., 1983; Wade et al., 2011), resulted in more comparable data than measurements in the aqueous phase (Fig. 2, Table 2). Spearman correlation coefficients were >0.96 between the Turner and the other instruments and 1.00 between the Shimadzu and the Horiba and p values were always <0.001 (Table 2). Presumably, the improved results were due to the extraction with DCM, which combines both particulate (droplets) and dissolved oil in a solution eliminating problems caused by oil droplets and particles in a fluorometer cuvette. Presence of oil droplets in WAF and CEWAF was also confirmed by Singer et al. (2000) that showed WAF and CEWAF can have oil droplets of different sizes. Usually CEWAF contains smaller, but more abundant droplets than WAF (Wade et al., 2017). Various organic solvents have been used, but DCM has been shown to be a better solvent than hexane in the presence of particulate material (Law et al., 1987). Extracts in DCM are normally homogenous solutions, and light scattering by

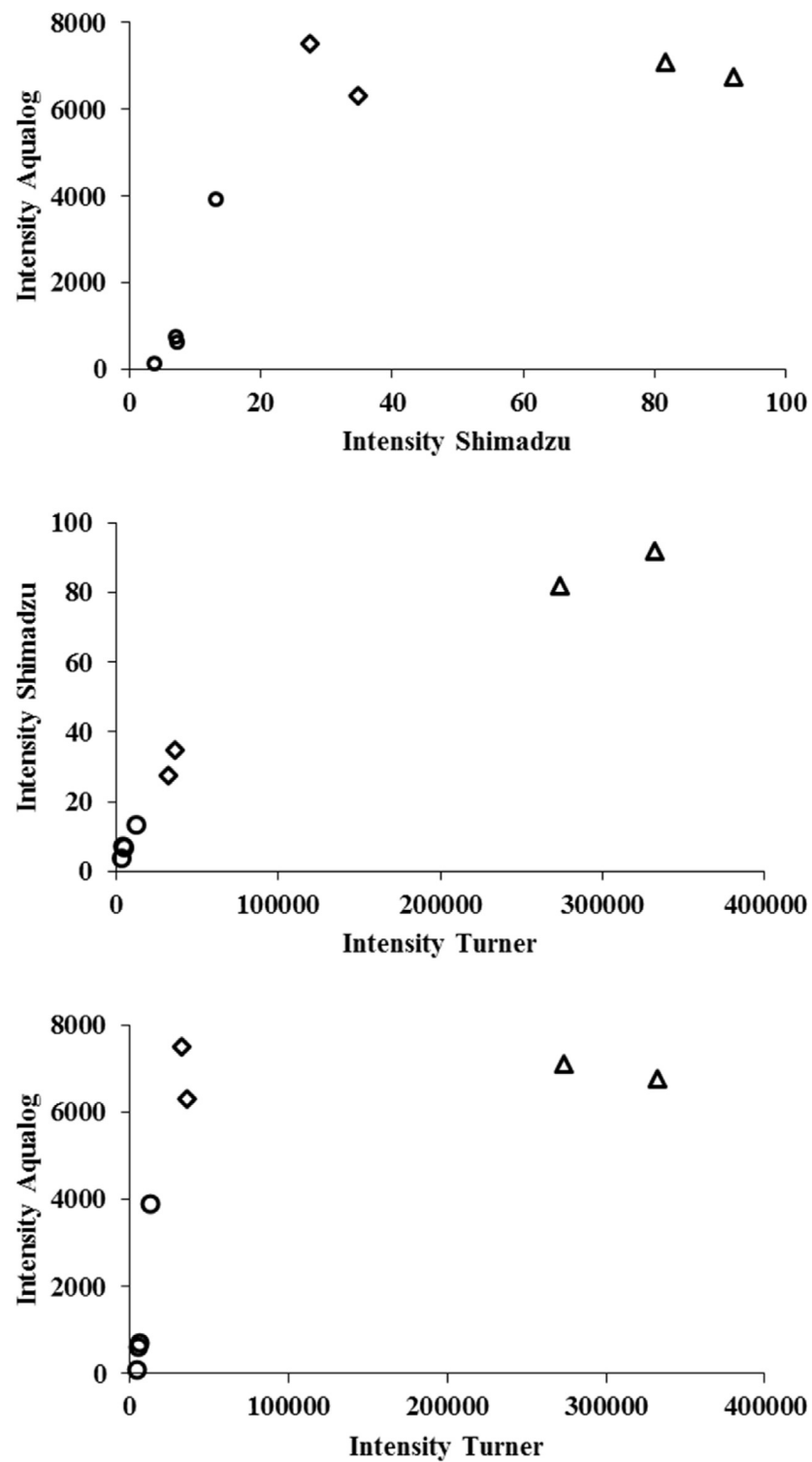


Fig. 1. Comparisons of fluorescence of samples from intercalibration 1 without extraction, expressed as relative fluorescence units using three different fluorimeters. WAF (◇), CEWAF (Δ), and Oil Standard (○). See Table 1 for statistical results.

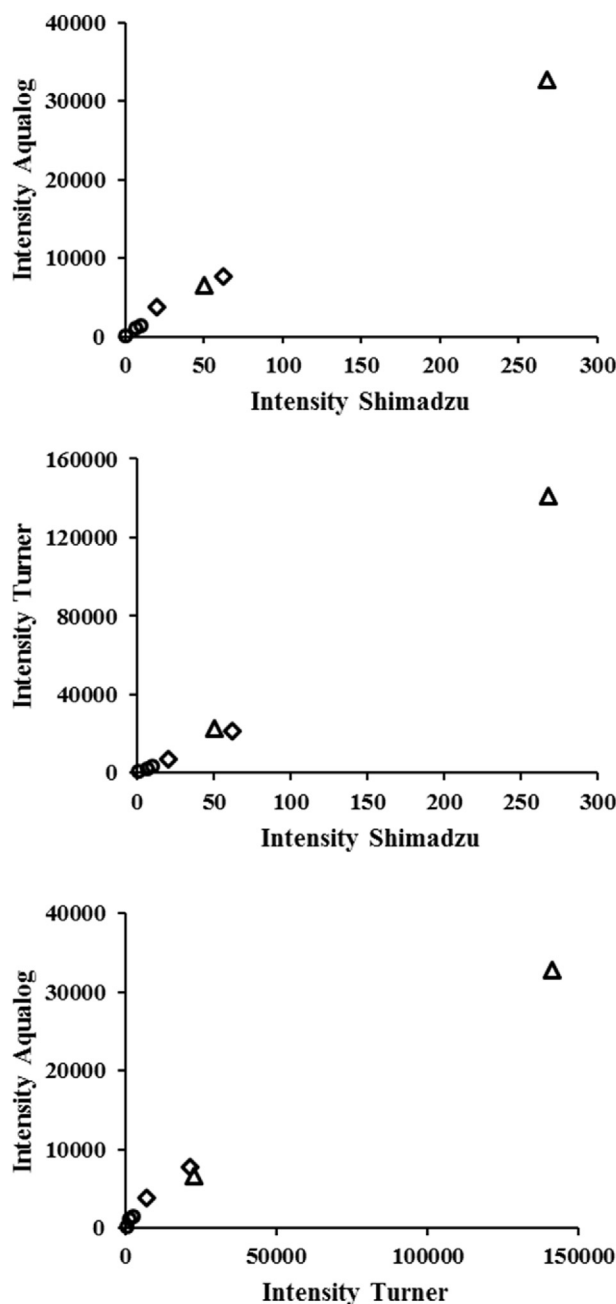


Fig. 2. Comparison of fluorescence intensity of samples using three different fluorometers. Samples were extracted in dichloromethane. WAF ($\diamond$), CEWAF (Δ), and Oil Standard ($\circ$). See Table 2 for statistical results.

particles including microbes is not a problem. Extraction with DCM works efficiently for most types of the water and sediment environmental samples. However, extraction (DCM) of samples with high organic matter content (including Corexit) or samples from wastewater treatment plant can cause emulsion problems during extraction. For small volume samples (1–4 L), repeated (3–4 times) extraction

Table 1. Spearman's correlation coefficients (P-value) for the analysis of WAF and CEWAF samples of intercalibration 1 without solvent extraction. Holme's correction for multiple comparisons to P-values was applied.

	Horiba	Shimadzu
Shimadzu	0.81 (0.02)	-
Turner	0.83 (0.02)	0.98 (0.001)

with DCM, adding salt to the water and filtering the extract through sodium sulphate generally works. However, some petroleum products where oils need to be separated from emulsifiers, different treatments to eliminate these emulsions (e.g. high concentrations of dispersant) may be required (Rajak et al., 2015, 2016).

As this first intercalibration exercise clearly showed that extraction with DCM, improved the quality of the EOE data and made samples measured in different instruments comparable, all further samples were extracted in DCM.

3.2. Comparison of calibration curves and concentrations of EOE

Typical calibration curves using surrogate Macondo oil in DCM are presented for all three instruments in Fig. 3. No attempts were made to increase sensitivities or otherwise improve performance of the instruments. Rather, all instruments were used with the settings as established in each one of the different laboratories. All three instruments successfully produced linear curves (Fig. 3) with a high coefficient of correlation (>0.99). Best fit ($R^2 = 0.9999$) for the calibration curve of the Horiba Aqualog was a polynomial fit (order 2), but the correlation coefficient for the linear fit was 0.9991.

A comparison of the EOE concentrations obtained with the Horiba Aqualog and Turner Trilogy fluorimeters of the WAF and CEWAF samples of intercalibration 2 is presented in Fig. 4 and Table 3. The RFU and EOE values measured with the custom module of the Turner correlated well with those measured with the standard crude oil module of the Turner (Pearson, $r^2 = 0.9992$, $n = 6$). The agreement in data measured with both the Turner Trilogy and the Horiba Aqualog is excellent (Pearson correlation = 0.9999), with no significant differences between the means [$t(6) = 1.15$; $p = 0.30$].

Table 2. Spearman's correlation coefficients (P-values) for the analysis of WAF and CEWAF samples of intercalibration 1 after extraction with dichloromethane. Holme's correction for multiple comparisons to P-values was applied.

	Horiba	Shimadzu
Shimadzu	1 (0.0001)	-
Turner	0.9643 (0.0009)	0.9643 (0.0009)

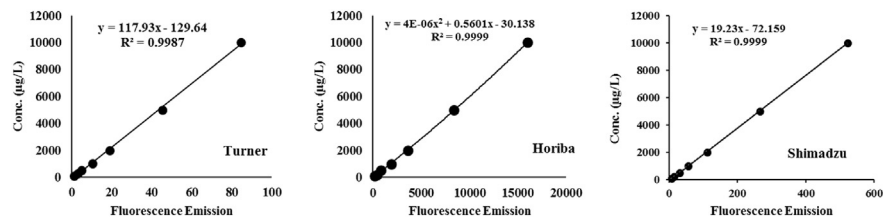


Fig. 3. Calibration curve of surrogate Macondo oil in dichloromethane determined in the Horiba Aqualog, Turner Trilogy (module 7200-063), and Shimadzu fluorometer.

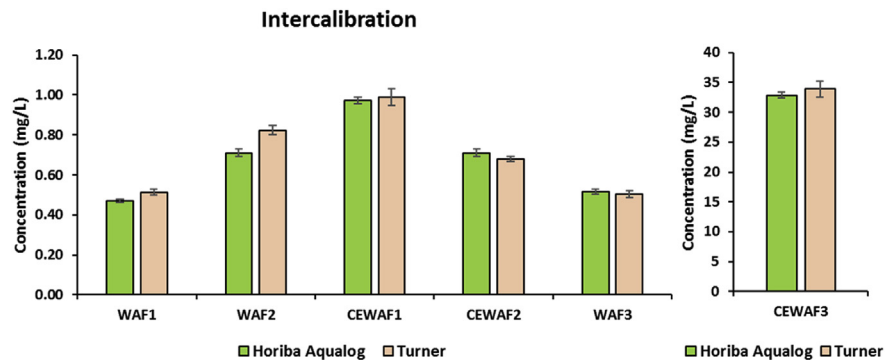


Fig. 4. Equivalent Oil Equivalent (EOE) results for intercalibration samples of exercise 2. Results are given as means $\pm$ standard deviation. See Tables 3 and 4 for error values. Note the change of scale between the two panels.

However, absolute values made it evident that Corexit (dispersant) does not work in DI water. Measured EOE concentrations in CEWAF made in deionized water (zero salinity) did not lead to increased dispersion of oil (Table 3). Addition of Corexit (dispersant) to oil was expected to disperse the oil, forming more, smaller droplets and also increase the dissolved concentration of oil. The solutions of WAF1 and

Table 3. Mean EOE concentrations and standard deviations (triplicates) from intercalibration exercise 2. Three different WAF and three different CEWAFs were prepared and measured by two fluorimeters each.

	Mean EOE Conc. (mg/L)		Standard Deviation	
	Horiba	Turner	Horiba	Turner
Prepared in DI				
WAF1	0.47	0.51	0.01	0.01
WAF2	0.71	0.82	0.02	0.02
CEWAF1	0.97	0.99	0.02	0.04
CEWAF2	0.71	0.68	0.02	0.01
Prepared in seawater				
WAF3	0.52	0.50	0.01	0.02
CEWAF3	32.88	33.91	0.54	1.36

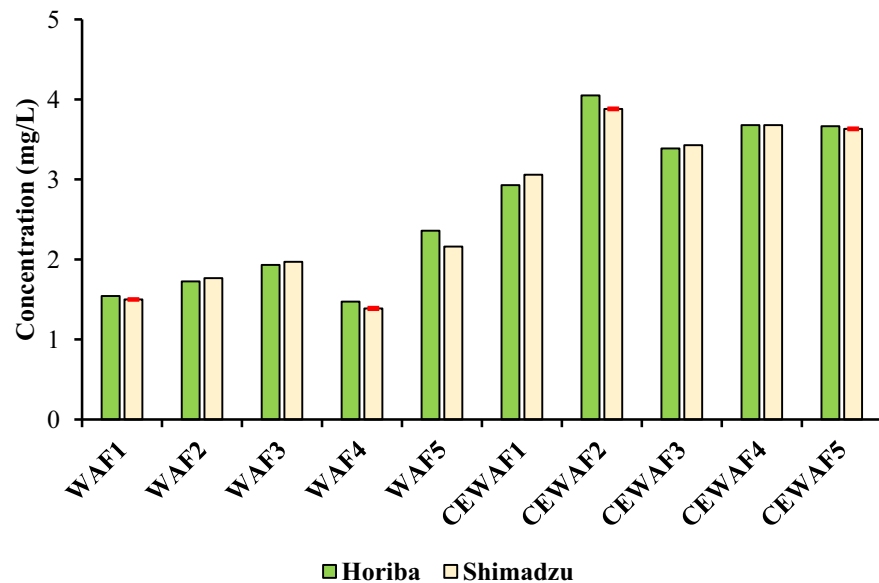


Fig. 5. Equivalent Oil Equivalent (EOE) results from intercalibration exercise 3. Results are given as means $\pm$ standard deviation. See Tables 3 and 4 for error values.

CEWAF1 were both made with DI and contained the same amount of oil. The measured EOE value for CEWAF1 (0.97 mg/L) was only slightly higher than that for WAF1 (0.47 mg/L), indicating very limited dispersion of oil into the water. The CEWAF3 solution, made in natural seawater with the same amount of oil as WAF1 and CEWAF1, had much higher EOE values (32.88 mg/L), confirming that in seawater Corexit (dispersant) is very effective in dispersing oil. An additional experiment with a single hydrocarbon (phenanthrene), where 30 μ L of Corexit

Table 4. Mean EOE concentrations (standard deviation) from intercalibration exercise 3. Samples were from 5 replicate WAF and 5 replicate CEWAF mesocosms and measured by two fluorimeters.

	EOE Conc. (mg/L)	
	Horiba	Shimadzu
WAF1	1.54	1.50 (± 0.004)
WAF2	1.73	1.77 (± 0.008)
WAF3	1.93	1.97 (± 0.006)
WAF4	1.47	1.39 (± 0.006)
WAF5	2.36	2.16 (± 0.007)
CEWAF1	2.93	3.06 (± 0.005)
CEWAF2	4.05	3.88 (± 0.004)
CEWAF3	3.39	3.43 (± 0.002)
CEWAF4	3.68	3.68 (± 0.002)
CEWAF5	3.67	3.63 (± 0.006)

(dispersant) was added to a 1 ppm phenanthrene solution in DI or seawater, also confirmed that dispersability of Corexit (dispersant) is greatly reduced in fresh water. EOE values in fresh water were half of those in seawater.

Intercalibration experiment 3, that compared EOE concentrations obtained with Horiba Aqualog and Shimadzu fluorometers is presented in Fig. 5 and Table 4. Similar to the previous dataset, agreement between EOE concentrations obtained with the Horiba Aqualog and Shimadzu fluorometers is excellent (Pearson correlation = 0.995), with no significant difference [$t(9) = 0.87$; $p = 0.406$] between the mean concentrations of the two instruments. The relative standard deviations (RSD) of all sample measurements are below 1% for Shimadzu.

4. Conclusions

Analysis of WAF and CEWAF samples by fluorescence is a rapid method for obtaining semi-quantitative results of oil concentrations in seawater when calibrated with the correct oil. This method loses reliability if water samples are analyzed directly, rather than extracted, because of increased light scattering due to oil droplets and other particles such micro-organisms (phytoplankton and bacteria). Extraction in dichloromethane eliminates these problems and provides very comparable results using different instruments. The strength of the method are that it is rapid, easy and cheap. Even a small Turner Trilogy instrument can give reliable results. EOE fluorescence provides a good screening technique with good inter-comparability. The use of this technique for rapid feedback during experiments, where the oil added is known and can be used for the calibration is especially useful. Overall, this study shows that EOE is a quick and reliable estimation oil concentration in water samples and can be easily measured by different spectrofluorometer in most laboratories.

Declarations

Author contribution statement

Gopal Bera: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Gerardo Gold-Bouchot: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Uta Passow: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Terry L. Wade, Dawei Shi: Performed the experiments; Analyzed and interpreted the data.

Maya Morales-McDevitt: Performed the experiments.

Noemi Ramirez-Miss: Conceived and designed the experiments; Analyzed and interpreted the data.

Anthony H. Knap, Antonietta Quigg: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

The original data can be found at the Gulf of Mexico Research Initiative Information and Data Cooperative (GRIIDC) at <http://data.gulfresearchinitiative.org> (<https://doi.org/10.7266/N71G0JX8>).

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