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Optimized Parameters for Fluorescence-Based Verification of Ballast Water Exchange by Ships

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Mid-ocean ballast water exchange is mandatory for ships discharging foreign ballast in US territorial waters in order to reduce the risk of biological invasions. However, a reliable tool for determining whether the procedure took place is lacking. We investigated chromophoric dissolved organic matter (CDOM) fluorescence as a tracer of mid-ocean exchange on nine research cruises out of Asia, Europe, and the USA, focusing on challenging source conditions (high salinity, low CDOM). Using parallel factor analysis, we identified nine independent fluorescent components present in varying concentrations in the ocean and in ballast water. One component was sufficient for predicting the coastal vs oceanic source of most ballast water samples. Across nine cruises, thresholds (1.7 and 0.7 ppb quinine sulfate equivalent units) at two fixed wavelength pairs ($\lambda_{ex}/\lambda_{em}$ = 320/414 and 370/496 nm, respectively) discriminated coastal from oceanic ballast water in >95% of samples ($N = 514$). Our results suggest that single- and dual-channel fluorometers could be optimized for verifying ballast water exchange.

Introduction

Ships' ballast water is an important vector transporting aquatic organisms among coasts and across historical barriers to dispersal, sometimes resulting in biological invasions with severe ecological and economic impacts (*1*). To reduce the risk of invasions, the International Maritime Organization (IMO) formulated guidelines in 1991 recommending that ships replace >95% of their original ballast from coastal sources with water from the open ocean. This practice of mid-ocean ballast water exchange (BWE) replaces coastal organisms with oceanic species, which are considered unlikely to colonize coastal habitats (*2*).

BWE requires hours or days to complete, creating obvious constraints for short-duration routes that do not traverse ocean basins, and can present safety risks for some ships and weather conditions. Although a variety of efforts are underway to develop on-board ballast water treatment systems that address such limitations, widespread application is likely many years away (*3*). A general framework has emerged in the US and internationally that includes BWE as treatment technologies are phased in over approximately the next decade (*3*, *4*). Failure to comply with the US Coast Guard's mandatory ballast water management requirements is a Class C felony subject to fines of up to $27,500 USD per day (*5*).

The enforcement of BWE requires a technique for discriminating ballast water sourced from coastal versus oceanic environments. The USCG has implemented a salinity criterion to verify exchange, since oceanic (exchanged) salinities are typically high (>32 ppt) while coastal (unexchanged) salinities are often lowered by freshwater runoff. Under the salinity criterion, water with salinity below 30 ppt is assumed to be predominantly coastal in origin. The utility of this approach is limited simply because many ports have high salinities, indistinguishable from the open ocean, for all or part of the year. Thus, additional tracers are sought that are applicable to a wider range of ports and seasons.

In a recent proof-of-concept study for BWE verification, the effects of BWE on levels of naturally occurring trace metals, radioactive isotopes, and chromophoric dissolved organic matter (CDOM) were measured during four cruises in the Atlantic and Pacific oceans (*6*). Whereas several tracers including CDOM were more successful at tracing BWE than salinity, CDOM was considered particularly promising due to its potential for rapid measurement using handheld devices.

CDOM refers to the fraction of the dissolved organic matter pool that absorbs light and fluoresces in the UV and visible regions of the spectrum. CDOM is produced by the breakdown of terrestrial and aquatic plant matter to humic, fulvic, and amino acids and is found in all natural waters in concentrations that are typically inversely related to salinity (*7*, *8*). CDOM fluorescence of natural waters is often characterized across a range of excitation (240−450 nm) and emission (300−700 nm) wavelengths using excitation−emission matrix spectroscopy (EEMs), resulting in a three-dimensional fluorescence intensity landscape. Within the landscape, distinctive peaks have been found to be informative in distinguishing and tracking various water sources (*7*, *9*).

The interpretation of CDOM fluorescence EEMs, typically characterized using peak heights and ratios, has advanced recently with the application of a chemometrics tool called parallel factor analysis (PARAFAC) (*10*, *11*). PARAFAC decomposes an EEM dataset into the least-squares sum of several mathematically independent components, parametrized by concentrations (loadings) and excitation and emission spectra (pure or combined) and corresponding, ideally, to a chemical analyte or group of strongly covarying analytes. Thus, PARAFAC enables the reduction of a complex three-dimensional data array to a limited number of two-dimensional spectra from mathematically and chemically independent components. Furthermore, since the PARAFAC model is insensitive to random noise (or any nontrilinear variation), it can expose patterns within a dataset that are visually obscure and would be neglected or misinterpreted using traditional peak-picking methods.

Since fluorescence is highly sensitive and excitation−emission matrix spectroscopy enables sample characterization across a range of excitation and emission wavelengths, EEMs have obvious utility for BWE verification. A major drawback is that EEMs are data-intensive, are difficult to parametrize, and are generated using expensive benchtop

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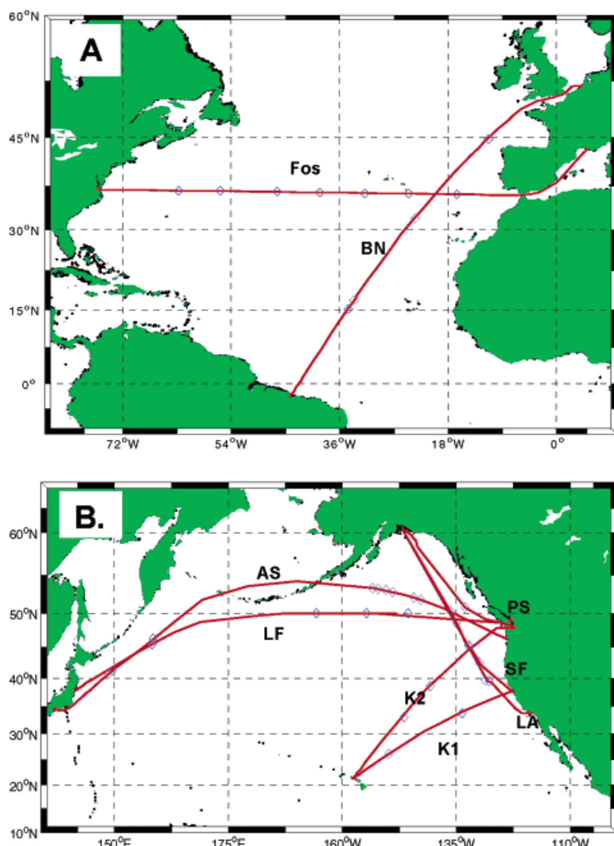

FIGURE 1. Cruise tracks in (A) the Atlantic Ocean and (B) the Pacific Ocean. Ballast water was loaded in the port of origin and exchanged at various locations (◇) en route while the vessel was at least 200 miles from any coastline.

fluorometers, whereas the regulatory needs of any large-scale BWE verification effort favors rapid, in situ measurements by nonscientists using relatively inexpensive instrumentation.

We used CDOM fluorescence signatures, collected as EEMs and characterized using PARAFAC, to identify features that were informative about the coastal versus oceanic origin of ballast water samples. The results were used to identify optimal wavelengths for discriminating ballast water sources. It is intended that this research will inform the potential use of single- and dual-channel fluorometers for verifying ballast water exchange.

Experimental Methods

Ballast water exchange experiments were conducted during nine cruises on commercial ships in the north Pacific and Atlantic oceans (Figure 1). Cruises were selected to maximize the chance that the vessel would ballast high salinity (>30 ppt) port water, since this situation would render useless a salinity-based verification criterion. Furthermore, since CDOM like many terrestrial signals typically decreases with increasing salinity, high salinity port waters necessitate higher detection sensitivity, compounding the challenges for verifying BWE.

Samples were collected from exchanged and unexchanged ballast tanks and, on most cruises, from the ambient ocean along the cruise track. Nontoxic Rhodamine WT dye (Bright-Dyes) was added to ballast tanks at the beginning of two cruises (SF, LA) to enable BWE efficacy to be independently quantified. Dye concentrations were 82 ± 2 μg L^{-1} (cruise SF) and 124 ± 4 μg L^{-1} (cruise LA) in unexchanged tanks. BWE efficacy was determined as >95% in tanks subject to empty−refill (ER) exchange, and 75−93% in tanks subject to 300% flow-through (FT) exchange (*6*).

Ballast water samples were collected from wing tanks (bulk carriers and tanker ships) or double bottom tanks (container ships) using an air driven diaphragm pump attached to tubing that was lowered into the tanks. Ambient ocean (shipside) samples from 5 ± 2 m below the sea surface were collected by tapping the engine cooling pipes near the intake to the ships' sea chests. All samples were filtered (0.7 or 0.45 μm) and then stored frozen (2 weeks to 6 months) until analysis, using protocols described in previous publications (*6*, *12*, *13*). CDOM analysis by excitation−emission matrix spectroscopy (excitation, 240−600 nm in 5-nm intervals; emission, 300−600 nm in 2-nm intervals; 5-nm bandwidths on excitation and emission modes) was performed by a spectrofluorometer at any of three analytical laboratories: (1) University of South Florida, using a SPEX FluoroLog-2 (Cruises SF, LA, PS, Fos, LF, AS) (*7*); (2) University of Maine, USA, using a SPEX FluoroMax-2 (Cruises K1, K2); (3) National Environmental Research Institute, Denmark, using a Varian Cary Eclipse (Cruise BN) (*10*). EEMs were corrected for instrumental differences with user-generated excitation, emission, and lamp intensity correction factors and blank subtraction, and normalized to quinine sulfate equivalent units (QSE) using standard methods (*14*, *15*). Intercalibration of fluorometers, using standard reference materials, was conducted in October 2004. In fully corrected EEMs, the position of the quinine sulfate peak ($\lambda_{ex}/\lambda_{em}$ = 350/450 nm) differed from the technical emission spectrum of Velapoldi and Mielenz (*16*) by less than 4 nm (see Supporting Information).

Reabsorption of emitted radiation in concentrated samples can affect the linearity of the fluorescence response, potentially causing underestimation of CDOM concentrations (inner filter effect). Our samples were relatively dilute with 350/450 ($\lambda_{ex}/\lambda_{em}$) intensities below 8 ppb QSE in >97% of samples; the remainder were below 10.5 ppb QSE. Absorbance (A_{350}) measurements corresponding to ~8 ppb fluorescence were below 0.045 (Shimadzu UV-2401PC spectrophotometer, 10 cm cell; cruise BN). Inner filter effects may have reduced fluorescence by the more concentrated samples in our dataset; however, they are unlikely to have affected fluorescence in samples approaching the <2 ppb BWE thresholds identified in this study.

Sampling frequency varied among cruises (see Supporting Information) depending on voyage duration (3−14 days), ballast exchange method (FT or ER), ballast tank depth (2−20 m), and number of exchange locations (1−4), ballast tanks (2−10), and tank access locations (1−2). Where possible, samples were collected from partially exchanged ballast tanks and from the same tank/treatment combination on multiple occasions. Altogether, this generated a pool of >700 independent samples combined in a nine-cruise ("total") dataset that included fully, partially, and unexchanged ballast water, as well as ambient ocean samples collected en route.

Samples from the total dataset were subdivided into a calibration set (seven cruises: LF, AS, BN, LA, SF, PS, and Fos; N = 687) and a validation set (two cruises: K1 and K2; N = 55) (Table 1). All samples from the calibration set, regardless of source, were used to develop a PARAFAC model and identify suitable wavelengths for verifying BWE. After determining "optimal" wavelengths, the model was discarded and measurements at the chosen wavelengths were extracted from EEMs and used to verify BWE in ballast water samples.

PARAFAC Model. CDOM EEMs from the calibration set were modeled using parallel factor analysis (*11*), which uses an alternating least squares algorithm to minimize the sum of squared residuals across the dataset and, in so doing, estimates the underlying structure of the EEMs. PARAFAC decomposes the data signal into a set of trilinear terms and

TABLE 1. Overview of Research Cruises[a]

	ballast water source	no. of ballast tanks (N)	salinity (ppt) before BWE $\bar{x}$ (±SD)	salinity (ppt) after BWE $\bar{x}$ (±SD)
	Calibration Cruises			
SF	San Francisco 70%/Puget Sound 30% (USA)	3	22.0 (0.8)	32.4 (0)
LA	Longbeach (USA)	2	33.3 (0.1)	32.8 (0)
PS	Cherry Point (USA)	2	29.7 (0.3)	32.0 (0)
Fos	Fos Sur Mer (France)	8	37.6 (0.2)	36.6 (0.1)
LF	Haramachi (Japan)	9	33.2 (0.2)	33.0 (0.3)
	Haramachi/unknown (Korea)	1	33.2 (0.2)	33.0 (0.3)
AS	Yokkaichi (Japan)	2	27.2 (0.6)	32.8 (0.3)
	Inchon (Korea)	4	27.2 (0.6)	32.8 (0.3)
	Kunsan (Korea)	2	27.2 (0.6)	32.8 (0.3)
BN	Rotterdam (Netherlands)	6	32.8 (0.6)	36.2 (0.6)
	Validation Cruises			
K1	Oakland (USA)	3	32.0 (0.7)	35.3 (0.1)
K2	Honolulu (USA)	3	36.3 (0.5)	36.0 (0.2)

[a] See Supporting Information for experimental design.

a residual array:

$$x_{ijk} = \sum_{f=1}^{F} a_{if} b_{jf} c_{kf} + e_{ijk}$$
$$i = 1, ..., I; j = 1, ..., J; k = 1, ..., K; f = 1, ..., F$$

where x_{ijk} is the intensity of the ith sample at the jth variable (emission mode) and at the kth variable (excitation mode); a_{if} is directly proportional to the concentration of the fth analyte at emission wavelength j; b_{jf} is a scaled estimate of the emission spectrum of the fth analyte; c_{kf} is linearly proportional to the specific absorption coefficient (e.g., molar absorptivity) at excitation wavelength k; e_{ijk} is the residual noise, representing the variability not accounted for by the model. PARAFAC algorithms and modeling techniques are reviewed extensively in other publications and are not detailed here (*11* and references within, *17*, *18*).

A nine-component PARAFAC model was generated using the N-WAY toolbox for Matlab (*18*) with nonnegativity constraints applied on all modes (*17*). To reduce the dominance of high-intensity coastal signals over low-intensity oceanic signals, the dataset was first scaled to unit variance in the sample (i.e., concentration) mode. Seven outliers (<1% of total dataset) were identified by jackknifing (*19*) or examination of residual errors and were removed. Nine component spectra were identified and verified by independent modeling of random halves of the dataset (*11*), explaining >99% of the variation within the dataset. To obtain component concentrations, PARAFAC was run on the unscaled data with the component spectra as initial conditions. This recovered relative concentrations that, multiplied by maximum excitation and emission loadings, predicted actual concentrations.

Samples from two cruises out of Japan (LF, AS) showed unexpected fading of fluorescence during frozen storage prior to analysis (half-life approximately 5 months). Expiry of frozen CDOM samples over time frames less than 6 months has not been previously reported and is the subject of a concurrent investigation in our laboratory. While the reason for the fluorescence loss is presently unknown, it did not appear to affect overall spectral properties and could be effectively modeled using an exponential function of days in storage. To enable inclusion of these data, fluorescence intensities on cruises LF and AS were adjusted component-wise to account for fading. The validity of this treatment was ultimately borne out by the consistency of the adjusted results with data from other cruises that did not require adjustment.

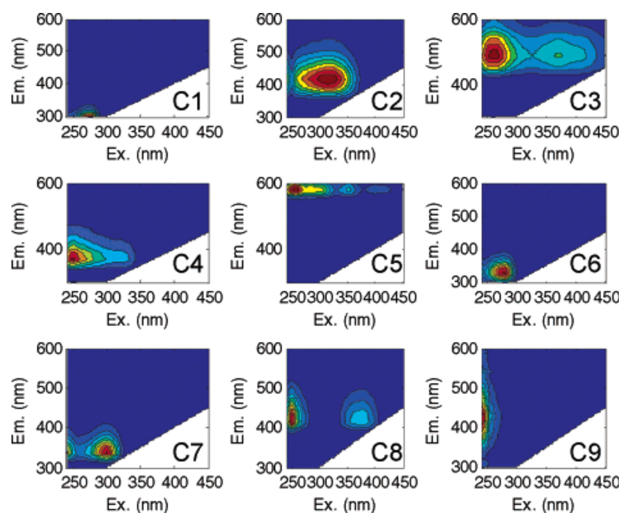

FIGURE 2. Fluorescence signatures of nine independent PARAFAC components (C1−C9) identified in the dataset. See Supporting Information for component spectra and model validation using Rhodamine dye and split-half analysis.

Results and Discussion

Fluorescent Signatures of Ballast Water. Each sample within the calibration dataset could be modeled as a linear combination of nine PARAFAC components with unique fluorescence signatures (Figure 2). Variation explained by each component decreased sequentially for C1−C9. As explained below, characteristic spectra suggest the presence of humic materials (components C2, C3, C8, and C9), protein-like materials (C1, C6, and C7) and a possible polycyclic aromatic hydrocarbon (PAH) contaminant (C4). Spectra corresponding to Rhodamine WT dye (C5), present in <10% of samples, were also retrieved by PARAFAC and provide independent validation of the model. Quantitative dye recovery by PARAFAC was confirmed by linear regression of actual versus modeled Rhodamine fluorescence ($C5_{max} = 0.987F_{255/580}$, $R^2 = 0.99$, $N = 73$; see Supporting Information).

Previous studies have identified several common peaks in CDOM fluorescence, attributed to proteins, humic and fulvic acids (*9* and references within), and their fractions (*20*). Humic-like peaks are usually identified as peak-A and peak-C (UV and visible terrestrial humic) or peak-M (visible and transitional marine humic) (*7*, *9*, *21*). Of the humic-like fluorescence we observed, C2 corresponds closely with previously reported "marine" humic-like signatures (*7*, *9*). A PARAFAC component identical to C3 has been identified in

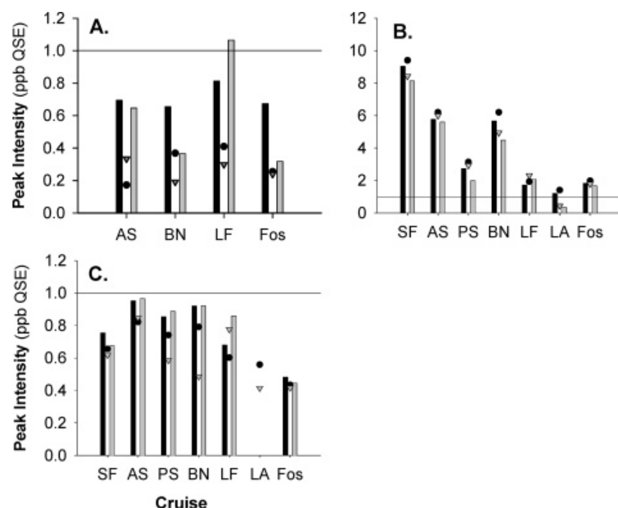

FIGURE 3. Mean peak intensities for components C2 (●) and C3 (▼) and their one-sided 99% confidence limits (CLs) (bars) in samples from seven cruises: (A) open ocean means and upper CLs; (B) unexchanged ballast water means and lower CLs; (C) exchanged ballast water means and upper CLs. Horizontal lines at 1.0 ppb QSE are shown for comparison.

studies from northern Europe (*10*, *22*). The superposition of the primary peaks of components C3 and C8 would yield excitation and emission maxima similar to Coble's peak-A (Ex_{max}/Em_{max} = 260/380−460 nm) (*7*). We did not find evidence of an independent peak-C (Ex_{max}/Em_{max} = 350/420−480 nm); instead, fluorescence in this region was due the combined secondary peaks of C3 and C8. Nonindependence of fluorescence in peak-A and peak-C regions, resulting from fluorophores with double excitation maxima, may explain why peak-C fluorescence is not generally reported in the absence of peak-A fluorescence. However, PARAFAC models of natural waters show that multiple fluorophores have single excitation maxima in the A-peak region (*10*, *22*, this study) and that an independent C-fluorophore may exist (*22*).

Fluorescent intensities of PARAFAC components on calibration cruises varied between samples in response to changing composition of fluorescent materials. Protein-like signals (C1, C6, and C7) were highly variable among replicates and cruises, reflecting both their susceptibility to contamination and their possible production within the ballast tanks. Consequently, they were considered unreliable tracers of ballast water source. Humic-like fluorescence by C8 and C9 explained the least variation within the dataset and along with Rhodamine (C5), which does not occur naturally, are not considered further as tracers of BWE.

The signature of component C4 was similar to pyrene (*23*), suggesting it may be due to PAH contaminants common in fuel oils. If so, its widespread presence in our dataset testifies to the inherent difficulty of obtaining clean samples from ships' pipes and ballast tanks. Since levels of PAH contaminants in ballast water vary unpredictably between ships, component C4 was considered an unreliable tracer of BWE and was eliminated from our target tracer pool.

Tracers of BWE. Intensities of the humic-like components C2 and C3 were considered the best potential tracers of BWE due to low interreplicate variation and, as previously noted, conformity of their signatures with fluorophores identified in other studies. Means and 99% confidence limits for their peak intensities were calculated on a cruise-by-cruise basis for open-ocean seawater and unexchanged ballast water, which represent the end members of the BWE process, and for exchanged ballast water, which is a mixture of the two. Sample sizes were sufficient to develop confidence limits for open-ocean seawater on four cruises (LF, AS, BN, Fos), unexchanged ballast water on seven cruises, and exchanged ballast water on six cruises (Figure 3).

Fully oceanic (>200 miles offshore) shipside samples were collected on four cruises: two each in the Pacific and Atlantic oceans. Upper confidence limits for mean C2 peak intensity in ocean samples were <0.7 ppb QSE (Figure 3A). Component C3 was variable between voyages, with higher fluorescence in the Pacific than Atlantic samples. Of note was the higher fluorescence in ocean samples on cruise LF than AS, although these covered similar ocean tracks in the spring and summer of 2003. This may reflect outliers within a small oceanic dataset on cruise LF (N = 7) rather than true differences between means.

Unexchanged ballast water samples showed consistently higher fluorescence of C2 and C3 than samples from exchanged tanks (Figure 3B). Along the cruise axis, increasing source salinity from left to right (Table 1) corresponded with generally decreasing fluorescence. Concentrations of both components were approximately 2 ppb or greater except on cruise LA (Los Angeles port water), which displayed very low C3 fluorescence.

Mean concentrations of C2 and C3 in exchanged ballast samples were also correlated with port salinity, probably due to the presence of residual coastal ballast water (Figure 3C), as was indicated by elevated intensities in exchanged ballast

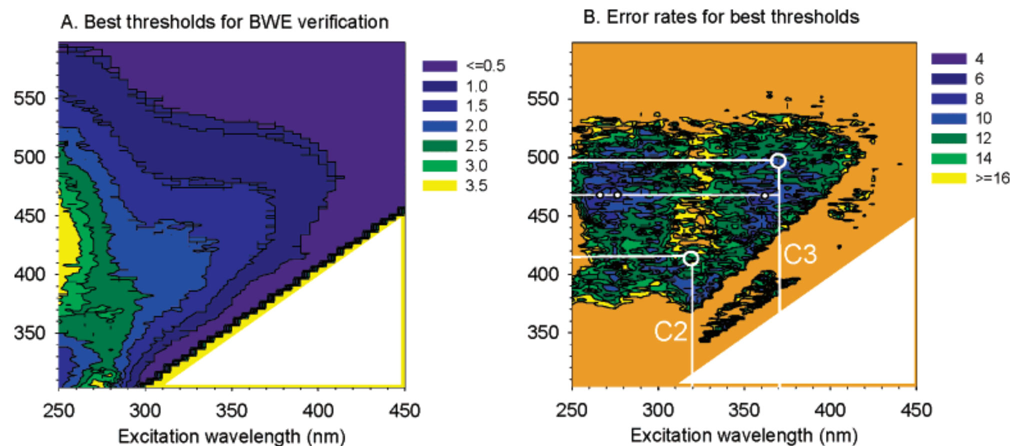

FIGURE 4. Best fluorescence thresholds for BWE verification and their associated misidentification rates. (A) Fluorescence intensity thresholds for BWE (ppb QSE) that minimize misidentification rates across the calibration dataset. (B) "Optimal" wavelength pairs for measuring components C2 and C3 are indicated by hollow circles, while three positions (λ_{em} = 466, λ_{ex} = 265, 275, and 360 nm) with potential for targeting multiple components are indicated by white circles.

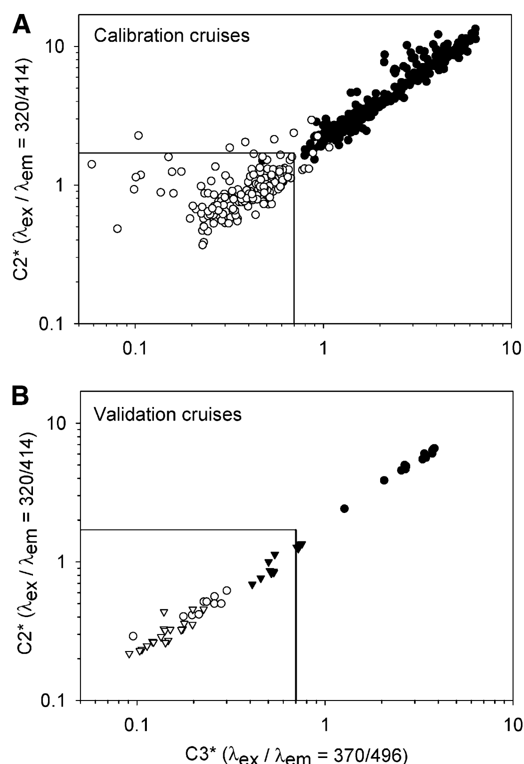

FIGURE 5. Effect of BWE on fluorescence intensity at fixed wavelength pairs C2* and C3* in ballast water samples. Fluorescence intensities are shown for unexchanged (solid symbols) and exchanged (open symbols) samples in (A) the seven-cruise calibration set (*N* = 459; circles) and (B) the two-cruise validation set (K1, circles; K2, triangles; *N* = 55). Straight lines show potential fluorescence thresholds for exchanged ballast water (C2* < 1.7, C3* < 0.7); see Table 2 for associated success rates.

tanks relative to the ocean where BWE occurred. Mean C2 and C3 fluorescence in exchanged tanks was consistently below 1.0 ppb QSE. The clear separation between mean C2 and C3 concentrations in exchanged and unexchanged ballast water samples suggested that it may be possible to verify BWE by isolating these components and setting thresholds on their intensities.

Optimal Thresholds for BWE. Optimal positions and thresholds for distinguishing ballast water source and their associated error rates were determined for each wavelength pair in the calibration dataset (Figure 4; for methodology see S6 of the accompanying Supporting Information). "Optimal" wavelengths for measuring components C2 and C3 were determined on the basis of degree of isolation and rate of successful BWE verification. Components C2 and C3 were relatively isolated in the neighborhood of C2*, $\lambda_{ex}/\lambda_{em}$ = 320/414, and C3*, $\lambda_{ex}/\lambda_{em}$ = 370/496 nm, where their contributions to measured fluorescence, averaged across all samples, were ~67% and ~72%, respectively. Wavelengths capturing fluorescence by multiple components are suitable for verifying BWE as long as total fluorescence varies in a predictable manner following exchange.

Fluorescence intensities at C2* and C3* were highly effective indicators of ballast water source (Figure 5A). In actual (not modeled) EEMs, unexchanged ballast water was identified by C2* fluorescence lower than 1.7 ppb QSE or C3* fluorescence lower than 0.7 ppb QSE, with success rates above 95% (Table 2). Additionally, since >93% of fluorescence at C2* and C3* was attributable to different components, two near-independent determinations of BWE were possible at these positions. This suggests that C2* and C3* may be suitable target wavelengths for a dual-channel fluorometer, enabling BWE verification at one channel to be corroborated at the other.

Excellent discrimination was also possible at some wavelength pairs featuring substantial overlap between C2, C3, C6, C8, and C9, for example, λ_{em} = 466, λ_{ex} = 265/275/360 nm (Figure 5A), indicating that, in our samples, concentrations of the various humic-like components were highly correlated. These positions captured fluorescence from similar components, but in different proportions. Consequently, they may be suitable selections for single-channel fluorometers, where verification via additional independent channels is not required.

To gauge the wider utility of C2* and C3* for discriminating BWE, the above calculated thresholds were applied to EEMs in the validation set (cruises K1 and K2). All exchanged ballast water samples were correctly identified, with C2* and C3* intensities well below all thresholds and lower than intensities in unexchanged samples (Figure 5B). On cruise K1 from Oakland, unexchanged samples exceeded all thresholds, confirming the presence of high salinity port water. On cruise K2 from Honolulu, most unexchanged samples had C2* and C3* intensities below both thresholds, resulting in type II errors (failure to detect unexchanged ballast water). With the exception of unexchanged samples on cruise K2, most misidentifications at positions C2* and C3* were anomalous when compared to results from true replicate samples. Many had been run on the Varian Cary Eclipse at low precision (see Supporting Information). In practice, basing BWE determinations upon means of replicate samples should reduce biases introduced by random error.

Implications of Findings. Using parallel factor analysis on an extensive EEMs dataset obtained on nine cruises in the Pacific and Atlantic oceans, we identified two CDOM fluorescence components (C2 and C3) useful for distinguishing between high salinity (> 30 ppt) ballast water sourced from oceans and ports. By locating wavelength positions where these components were most isolated, and setting thresholds on fluorescence intensities at these positions, we were able to distinguish sources for most (>95%) of our high salinity ballast water samples. Our approach differs substantially from one of "fingerprinting" the CDOM characteristics of specific individual source ports (*24*), as it relies on bulk differences between coastal and ocean waters

TABLE 2. BWE Indicators and Thresholds (C^*_{max}) and Their Rates of Successful Discrimination (%) of Ballast Water Source (C = Port, X = Ocean) on Seven Calibration (Cal) and Two Validation (Val) Cruises

			seven cruises (Cal)		K1 (Val)		K2 (Val)		all nine cruises	
BWE indicator	$\lambda_{ex}/\lambda_{em}$ (nm)	C^*_{max} (ppb QSE)	C $N = 290$	X $N = 169$	C $N = 14$	X $N = 10$	C $N = 12$	X $N = 19$	C $N = 316$	X $N = 198$
C2*	320/414	1.7	98.6	95.3	100	100	0	100	94.9	96.0
C3*	370/496	0.7	99.0	97.0	100	100	33.3	100	96.5	97.5
	265/466	2.4	99.0	98.2	100	100	8.3	100	95.6	98.5
	275/466	1.9	99.0	97.6	100	100	8.3	100	95.6	98.0
	360/466	1.1	99.0	97.6	100	100	0	100	95.3	98.0

and assumes no prior knowledge of the vessel's route or ballasting history. While the method successfully discriminates ocean from port water across a range of environments, examination of the rate of decay of tracer signals with depth/distance from shore is still needed to determine near-shore boundaries beyond which it is no longer possible to discriminate coastal from oceanic water sources.

It was possible to verify BWE for most high salinity ballast water samples in our dataset based only upon fluorescence at the position C3* ($\lambda_{ex}/\lambda_{em} = 370/496$ nm). A second, near-independent determination of BWE was obtained at the position C2* ($\lambda_{ex}/\lambda_{em} = 320/414$). Systematic failures at these wavelengths occurred only under the especially challenging conditions created by full salinity (> 36 ppt), highly oligotrophic port water (cruise K2). Lowering the suggested thresholds would have enabled verification of BWE even on cruise K2, but would simultaneously have increased the number of false positives for ships with residual ballast from relatively low salinity, high-CDOM ports (e.g., cruise AS). Although non-CDOM tracers (e.g., radioactive isotopes) may aid discrimination in such cases, any thresholds set for exchanged ballast water must take into account spatial and temporal variability among potential source ports and offshore locations. Thus thresholds should be high enough and instruments specific enough to avoid false positives from residual port CDOM as well as potential contaminants (e.g., fuel oils) that may be present, even if this reduces the likelihood of detecting unexchanged ballast water sourced from a small number of highly saline, oligotrophic ports.

The high discrimination rates demonstrated here are remarkable considering the many sources of variation encompassed by this study. These included ballast water collected over a wide temporal and geographical range, multiple ships and tanks, samples stored from 1 week to 6 months, and EEMs analysis on three fluorometers. All contribute to the uncertainty surrounding measurements, potentially obscuring subtle treatment effects. Whereas variation due to collection, handling, and storage of samples could be eliminated by measuring fluorescence in situ, developing methods for simple and reliable calibration of field fluorometers will be critical to the success of regulatory efforts.

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Supporting Information Available

S1, experimental design and sampling on nine research cruises during this study; S2, intercalibration of fluorometers used in this study; S3, excitation and emission maxima for nine fluorescent components identified by the PARAFAC model; S4, validation of PARAFAC model using Rhodamine WT dye; S5, validation of PARAFAC model by split-half analysis; S6, methods for determining optimal wavelengths and thresholds for BWE. This material is available free of charge via the Internet at http://pubs.acs.org.

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