

## Verification of mid-ocean ballast water exchange using naturally occurring coastal tracers

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### Abstract

We examined methods for verifying whether or not ships have performed mid-ocean ballast water exchange (BWE) on four commercial vessels operating in the Pacific and Atlantic Oceans. During BWE, a ship replaces the coastal water in its ballast tanks with water drawn from the open ocean, which is considered to harbor fewer organisms capable of establishing in coastal environments. We measured concentrations of several naturally occurring chemical tracers (salinity, six trace elements, colored dissolved organic matter fluorescence and radium isotopes) along ocean transects and in ballast tanks subjected to varying degrees of BWE (0–99%).

Many coastal tracers showed significant concentration changes due to BWE, and our ability to detect differences between exchanged and unexchanged ballast tanks was greatest under multivariate analysis. An expanded dataset, which includes additional geographic regions, is now needed to test the generality of our results.

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### 1. Introduction

Successful invasions of nonindigenous species (NIS) can result in unwanted ecological, economic and human health impacts. For example, a recent study estimated the impact of NIS to exceed \$100 billion dollars annually in the US alone (Pimentel et al., 2000). Furthermore, many analyses suggest that the rate of invasions has increased in recent years in association with human activities (Ruiz et al., 2000). In the US as elsewhere, this has caused considerable public concern and resulted in state, national, and international efforts to reduce the risk of future invasions (McConnell, 2002).

In coastal marine ecosystems, ships are considered the transfer mechanism responsible for most historical and recent invasions (Hewitt et al., 1999; Ruiz et al., 2000). Species are transferred unintentionally in the ballast water and on the hulls of ships, and a portion of these organisms are able to establish upon arrival to a new port. Today, ballast water is considered to be the largest single vector whereby organisms are transported from points of origin and released variously at subsequent ports of call (Ruiz et al., 1997; Anonymous, 2002).

In 1991, the International Maritime Organization (IMO) established voluntary guidelines aimed at minimizing such introductions, requesting that ships perform mid-ocean ballast water exchange (BWE). During BWE, a vessel replaces its original ballast water (taken on board while the vessel was in port or near to the coast) with water from the open ocean. Ballast exchange reduces NIS by (1) discharging a percentage of them into

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the inhospitable environment of the open ocean, and in some cases, (2) by increasing the salinity level within the ballast tank to a level such that many species of freshwater or brackish water origins cannot survive (Taylor et al., 2002).

BWE is now the primary method for reducing the risk of species transfer by ships throughout the world. In the US, BWE is voluntary for most vessels arriving from beyond the exclusive economic zone (EEZ), but federal law requires BWE for such vessels entering the Great Lakes and the Hudson River, north of the George Washington Bridge. Regulations have recently been enacted in several states (California, Oregon, Washington) that require BWE for all vessels intending to discharge foreign ballast in their waters. Moreover, the US Coast Guard intends to extend mandatory regulations for BWE to all vessels delivering foreign ballast to any US port. Although other approved ballast water treatment methods may also be used under federal and state laws, no alternatives to BWE have been approved for use to date (see discussion by Taylor et al., 2002).

The US Coast Guard's current ballast water management regulations specify that BWE is to be performed in water outside the 200 mile EEZ and in depths greater than 2000 m. Ships can choose between two types of exchange: Flow-through or empty–refill, either of which should theoretically result in the removal of >95% of the original ballast water. Flow through (FT) exchange involves simultaneously uploading ocean water while allowing excess ballast water to overflow on to the deck. Due to the mixing of ocean and port water during this process, three tank-volumes of water (300%) are required theoretically to remove >95% of the original water (Hay and Tanis, 1998). Empty–refill (ER) exchange involves emptying the ballast tank completely of port water before uploading ocean water (100%). Since the complete (although temporary) removal of ballast during ER exchanges may be unsafe for particular vessel types or in rough weather, some vessels elect to perform FT in preference to ER ballast water exchanges.

Clearly, a reliable procedure for discriminating between complying and non-complying vessels is a prerequisite for monitoring compliance with voluntary guidelines as well as enforcing mandatory BWE regulations. However, there are currently no established, quantitative tools available that reliably discriminate between exchanged and unexchanged ballast tanks. Overseas, the Australian Quarantine Inspection Service assesses BWE compliance indirectly by reconciling the ship's logs and energy budgets, according to the "Newcastle Verification Method" (Alan Taylor, pers. comm.), on the basis that pump operation during BWE should be reflected in increased energy usage. In its favor, the method is relatively inexpensive and rapid. However, a major disadvantage is that records of energy

budgets can be manipulated by unscrupulous operators, and are not routinely kept on all types of ships (Murphy et al., 2002). Furthermore, since the method audits the attempt at BWE, rather than the outcome, it does not verify that the exchange was successful.

Direct measurement of naturally occurring tracers in the ballast water to deduce whether the tank was ballasted in a coastal or oceanic location, offers a potentially powerful approach to BWE verification. There are two prerequisites for this approach to succeed: first, there is a measurable and consistent difference between tracer levels in ballast water of coastal versus oceanic origin; and second, the implementation of BWE according to the specified guidelines will ensure a predictable water quality outcome. This approach is both quantitative and flexible, since it allows us to set criteria for compliance according to biologically meaningful objectives.

Salinity is a potential indicator of BWE since open ocean salinities are relatively stable and well defined (Pacific ~33 ppt, Atlantic ~35 ppt), whereas coastal salinities are frequently lowered by river discharge and terrestrial runoff. However, salinity alone is an insufficient indicator of ballast water source; whereas the presence of low salinity water is sufficient to show that a ballast tank was not exchanged in mid-ocean, the presence of high-salinity water does not preclude the possibility that the tank contains water from a high-salinity coastal port (e.g. the port of Los Angeles, California).

We implemented research to test whether a suite of chemical water characteristics or tracers can be used to discriminate between exchanged and unexchanged ballast water, regardless of the salinity of the coastal ballast source. Our method deduces the source of ballast water according to the statistical departure of tracer concentrations in the ballast water from baseline concentrations in the open ocean. Our justification for an ocean-referenced approach is that in comparison to coastal water, the surface ocean is a relatively stable environment within which we would expect much less temporal and spatial variation in chemical parameters. Furthermore, reliably identifying the coastal source of a ballast tank can be very difficult, especially when it is a mixture drawn from multiple ports over a long period of time.

We examined two questions with reference to sets of one or more potential tracers.

- Do the tracers, alone or in combination, accurately discriminate between unexchanged, partially exchanged and fully exchanged ballast water?
- Can the tracer(s) be used to detect a vessel which performed BWE in non-compliance with a >200 mile/>2000 m depth ballast exchange requirement? In other words, if BWE were performed less than 200 miles from the nearest coast, how close to the coast might it be performed, before it were possible to tell that it did not occur in fully oceanic water?

## 2. Methods

### 2.1. Experimental design

Verification techniques were tested on four experimental voyages in the North Pacific and North Atlantic Oceans (Table 1). The first voyage, for convenience hereafter referred to as “VSF”, departed from the low salinity, highly invaded San Francisco Bay (Cohen and Carlton, 1995). Because we were specifically interested in tracers capable of discriminating between high salinity coastal water and high salinity ocean water, the remaining three cruises departed from high salinity coastal ports on the US Pacific coast (Los Angeles: “VLA”, Puget Sound: “VPS”) and the Mediterranean Sea (Fos Sur Mer, France: “VFos”). For the latter three voyages, source water salinities were close enough to full oceanic salinities (~33 ppt in the North Eastern Pacific and ~35 ppt in the Northern Atlantic) that a salinity-

based verification criterion would be unreliable, particularly in light of the wide range of ballast exchange efficiency (75–99%) demonstrated by “complying” vessels (Ruiz et al., in preparation; this study). On the Atlantic voyage, extremely low nutrient levels (Dugdale and Wilkerson, 1988; Turley, 1999) and low productivity in the Mediterranean-sourced ballast water made this cruise a particularly difficult and valuable test case for ballast water exchange verification.

Work was implemented in two stages. The first stage focused on experiments in the northern Pacific, and involved three separate vessels that sailed parallel to the coast of North America (Fig. 1). Participating ships were asked to fill tanks at the port of departure, designating 1–2 tanks for ballast water exchange (FT and/or ER) and 1 as an unexchanged control (C). Samples of water for tracer analysis were collected from each tank prior to and following ER exchange, prior to and following partial (100% and 200%) FT exchanges and at

Table 1  
Experimental design and voyage characteristics of the four verification cruises (VSF, VLA, VPS and VFos)

	VSF	VLA	VPS	VFos
<i>Voyage characteristics</i>				
Departure Port	San Francisco (CA)	Los Angeles (CA)	Puget Sound (WA)	Fos Sur Mer (France)
Destination Port	Valdez (AK)	Valdez (AK)	Valdez (AK)	Norfolk (VA)
Date	November 2000	December 2000	May 2001	June 2001
Cruise length (days)	7	7	4	14
Vessel type	Oil carrier	Oil carrier	Oil carrier	Bulk carrier
Cargo	Oil	Oil	Oil	Coal
Source salinity (ppt)	20.6–22.5	33.3	29.2–29.7	37.2–37.6
<i>Experiment characteristics</i>				
Ballast water treatments	C, ER, FT	C, FT	C, ER	C, ER, FT
Tanks per treatment	1	1	1	4
Occasions sampled	4	3	2	4 or 5
<i>Sample characteristics</i>				
Depths				
Surface (m)	1	<1	1	1
Deep (m)	12	11	12	4
Replication				
Surface samples (N)				
CDOM	2	2	4	2
Trace elements	2	2	4	2
Radium	1	2		1
Salinity			2	
Deep samples (N)				
CDOM	2	2	4	2
Trace elements	2	2	4	2
Radium				1
Salinity			2	
Profile depth (m)				
Salinity	0–19	0–20		0–3
<i>Tracer characteristics</i>				
Ballast water tracers	CDOM, trace elements, radium, salinity	CDOM, trace elements, radium, salinity	CDOM, trace elements, salinity	CDOM, trace elements, radium, salinity
Mid-ocean tracers	CDOM, trace elements, radium, salinity	CDOM, trace elements, radium, salinity	none	CDOM, trace elements, salinity

Ballast tanks assigned to three treatments: unexchanged control (C), empty-refill (ER) exchange and flow-through (FT) exchange.

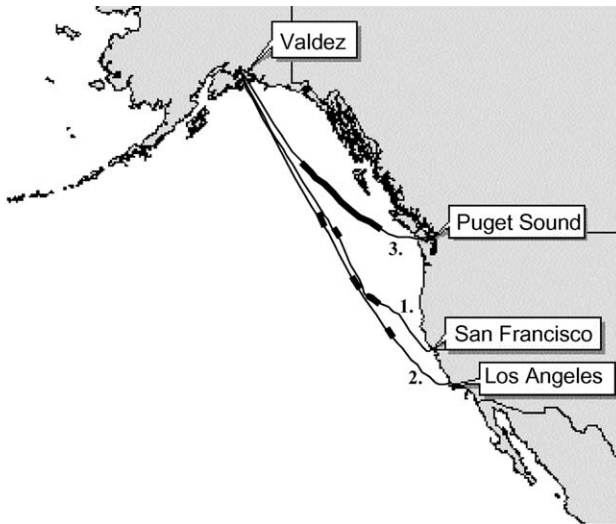


Fig. 1. Vessel tracks during the three Pacific cruises (1: VSF, 2: VLA, 3: VPS). Bold route sections indicate locations of ballast water exchange.

the completion of the voyage. For comparison, 2–6 shipside samples were collected from the ocean in the region of ballast exchange(s). These voyages were important to refine sampling and analysis protocols and streamline measures for subsequent voyages.

The second stage involved experiments aboard a trans-Atlantic voyage (Fig. 2). FT exchange was performed on port (P) or starboard (S) wing ballast tanks (1S, 2P, 3S and 4P), with the opposite wing tanks (1P, 2S, 3P and 4S) left as unexchanged controls until after the final exchange of the treatment tanks. Shortly before arriving in the US, while still >200 miles offshore, these control tanks were subject to a single ER exchange. Shipside samples were taken in the Mediterranean ocean, at ~200 mile intervals across the Atlantic, at ~15 mile intervals beginning 180 miles off the east coast of the USA, and concluding when the ship arrived in Norfolk.

## 2.2. Sample collection and analysis

Trace elements, colored dissolved organic matter (CDOM) and radium were evaluated as verification

tools and are the focus of this paper (Table 2). Samples were collected from ballast tanks and from the ambient ocean according to methods described below. Greater detail on sampling and analysis techniques are available from Murphy et al. (2002).

All ballast water samples were collected using a compressed air driven plastic (polypropylene) diaphragm pump (Wilden: Pro-Flo P.025) attached to 0.25" plastic tubing (Cole-Parmer: Chemfluor 367) installed at fixed depths in the tanks. Prior to each sample collection, the sampling unit was flushed with ballast water for at least 2 min (>10 "dead volumes") to remove stale water in the pump and hoses.

Samples from the ambient ocean (SS) were collected via the ship's fire hose (VSF, VLA) or else by tapping the engine-cooling water pipe at its inlet end (VPS, VFos). While it is difficult to collect uncontaminated samples in this manner, these were the only options available to us, given a vessel speed of 8–14 knots. Both the fire hose and the engine-cooling pipe are supplied with clean ocean water from the side of the ship (intake depth ca. 7 m). The fire hose was flushed at full pressure for at least 30 min prior to sampling. The engine cooling pipe was flushed for 5–60 min before samples were collected.

Procedural blanks were taken at the conclusion of the VFos cruise and used to estimate residual CDOM and trace element levels in the pump and hoses, and hence the potential for cross-contamination due to the sampling apparatus. To obtain procedural blanks, deionized (Milli-Q) water was sampled before and after flushing through the hose and pump system.

All samples were collected by the Smithsonian Environmental Research Center (SERC) and analyzed at three collaborating specialist laboratories in the USA. Trace elements were analyzed at Rutgers Inorganic Analytical Laboratory, Institute of Marine and Coastal Sciences, Rutgers the State University of New Jersey. CDOM samples were analyzed at the Marine Spectrochemistry Laboratory, Department of Marine Science, University of South Florida. Radium samples were analyzed in the Department of Geological Sciences of the University of South Carolina.

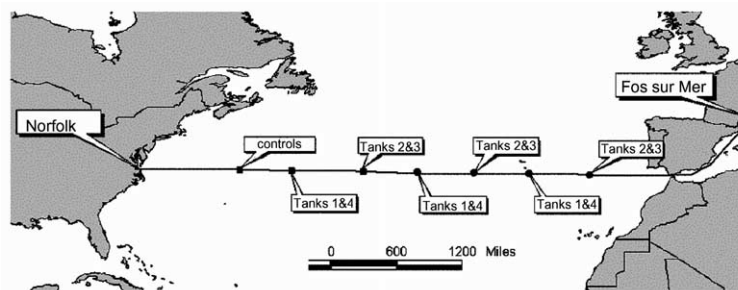


Fig. 2. Cruise track during VFos, showing positions of ballast water exchange. Partial exchanges of treatment tanks (● 100% FT, ■ 100% ER) were conducted at regular intervals during the voyage.

Table 2  
Potential tracers of ballast water exchange tested on commercial voyages

Tracer	Abbr.	Description	Units
Salinity	Sal	Salinity	ppt
Trace elements <sup>a</sup>	Ba	Barium	ppb
	Mn	Manganese	ppb
	Mo	Molybdenum	ppb
	P	Phosphorus	ppb
	U	Uranium	ppb
	V	Vanadium	ppb
Radium/thorium <sup>b</sup>	<sup>223</sup> Ra	223-Radium ( $t_{1/2} = 11.4$ dy)	dpm/100 l
	<sup>226</sup> Ra	226-Radium ( $t_{1/2} = 1600$ yr)	dpm/100 l
	<sup>228</sup> Ra	228-Radium ( $t_{1/2} = 5.7$ yr)	dpm/100 l
	<sup>228</sup> Th	228-Thorium ( $t_{1/2} = 1.9$ yr)	dpm/100 l
CDOM fluorescence <sup>c</sup>	A <sub>qse</sub>	Fluorescence maximum of CDOM "A" peak	QSE
	A <sub>ex</sub>	Wavelength of peak "A" excitation maximum	nm
	A <sub>em</sub>	Wavelength of peak "A" emission maximum	nm
	C <sub>qse</sub>	Fluorescence maximum of CDOM "C" peak	QSE
	C <sub>ex</sub>	Wavelength of peak "C" excitation maximum	nm
	C <sub>em</sub>	Wavelength of peak "C" emission maximum	nm
	A <sub>1</sub>	Fluorescence intensity (exc./em. = 250/450)	QSE
	A <sub>2</sub>	Fluorescence intensity (exc./em. = 260/470)	QSE
	A <sub>3</sub>	Fluorescence intensity (exc./em. = 265/470)	QSE
	C <sub>1</sub>	Fluorescence intensity (exc./em. = 310/420)	QSE
	C <sub>2</sub>	Fluorescence intensity (exc./em. = 350/450)	QSE
	C <sub>3</sub>	Fluorescence intensity (exc./em. = 325/420)	QSE

Fluorescence of colored dissolved organic matter (CDOM) is measured in quinine sulfate equivalents (QSE).

<sup>a</sup> Analyses were performed by collaborators at Rutgers University.

<sup>b</sup> Analyses were performed by collaborators at University of South Carolina.

<sup>c</sup> Analyses were performed by collaborators at University of South Florida.

### 2.2.1. Trace elements

Plastic tubing, syringe filters and centrifuge tubes used for trace element sampling were first acid leached in 1 N HCl and rinsed three times with deionized water. Trace element samples (10–40 ml) were collected in centrifuge tubes and frozen. Samples collected on the first two voyages (VSF and VLA) were not filtered. On the latter two voyages (VPS and VFos), samples were filtered through 25 mm 0.45  $\mu\text{m}$  pore size super polysulfone membranes.

In the laboratory, samples were acidified to 0.2% with 12 N Optima grade (Fisher Scientific) HCl for 24 h before centrifugation. Next, the supernatant was extracted and diluted 10 times with 3% Optima grade HNO<sub>3</sub> acid. The concentrations of five target elements (molybdenum [Mo], barium [Ba], phosphorus [P], vanadium [V], manganese [Mn]) were determined in samples from all cruises, and uranium [U] was determined on the VPS and VFos cruises.

Samples were analyzed on a sector field inductively coupled plasma mass spectrometer (ICP-MS) with high-resolution capability (ELEMENT, Finnigan MAT, Bremen, Germany). Sample concentrations were determined according to published methods (Field et al., 1999).

### 2.2.2. Radium

Radium samples were collected by pumping ballast water (180–200 l) at 1–2 lmin<sup>-1</sup> via a filter (5  $\mu\text{m}$ )

through a plastic column containing a manganese dioxide coated fiber (Mn-fiber, Moore, 1976). Pump rates and sample volumes were monitored using a digital flow meter/accumulator, and on VFos, volumes were standardized using a 55 gallon (~200 l) drum.

Short-lived radium isotopes (<sup>223</sup>Ra, <sup>224</sup>Ra) and <sup>228</sup>Th were measured within 72 h of the vessel's arrival in port, using established methods described in detail by Moore and Arnold (1996). Following completion of the <sup>223</sup>Ra and <sup>224</sup>Ra measurements, the Mn-fiber samples were aged for 2–6 weeks to allow initial excess <sup>224</sup>Ra to equilibrate with natural <sup>228</sup>Th adsorbed to the Mn-fiber. The samples were measured again to determine <sup>228</sup>Th and thus to correct for supported <sup>224</sup>Ra. Concentrations of the long lived radium isotopes <sup>226</sup>Ra and <sup>228</sup>Ra were measured for end-of-voyage VFos samples in August–September 2001 using the methods of Moore (1984). The expected error of the short-lived and long-lived Ra measurements is 10% and 7% respectively.

### 2.2.3. CDOM

Ballast water samples (ca. 120 ml) for quantification of CDOM were collected in sterile, pre-baked amber glass bottles. Water was first filtered on deck through a 47-mm polycarbonate in-line filter holder fitted with pre-baked GF/F filters to extract particles >0.7  $\mu\text{m}$ . Frozen samples were shipped to the University of South

Florida, where CDOM fluorescence and absorbance was determined by emission-excitation matrix spectrometry (EEMs).

Samples were analyzed between January and October 2001. Excitation emission matrices (EEMs) were generated across excitation wavelengths of 220–455 nm and emission wavelengths of 250–710 nm. Readings were corrected for instrument variability and normalized to the standard quinine sulfate dihydrate (presented in quinine sulfate equivalents (QSE)). Further details of methodologies, peak designation and significance are available in published references (Coble, 1996; Coble et al., 1998).

#### 2.2.4. Univariate statistical model

A simple univariate statistical model was used to assess the performance of individual tracers in each tank on each cruise. Given a single variate  $Y_1$ , we wish to know whether it differs from a reference ocean set with mean  $\mu_0$ . If we know that the variance of the ocean set is  $\sigma$ , and if the variable is distributed normally, the test is

$$(Y_1 - \mu_0)/\sigma$$

where null hypothesis:  $H_0 = \text{No difference between ballast water sample and open ocean samples, degrees of freedom: } df = \text{number of ocean observations} - 1$  (2-tailed).

Because a multivariate analysis of variance (MANOVA) test indicated the two oceans were significantly different ( $p < 0.001$ ), all ballast samples from the Pacific voyages were tested against reference set composed of Pacific Ocean samples, while the Mediterranean/Atlantic samples were tested against an Atlantic Ocean reference set. While we examined normality, this was inconclusive because of the small sample size ( $N \leq 15$  for the Atlantic,  $N \leq 10$  for the Pacific). However, we believe that further sampling will demonstrate that most or all of the tracers targeted in this study behave in a conservative manner and are normally distributed in the surface open ocean.

For tanks containing untreated port water (i.e. 0% BWE), successful tracers are considered those that exhibit a highly significant result ( $p < 0.01$ ). Non-significant results represent Type II errors, in other words, acceptance of the false null hypothesis of no difference between open ocean water and unexchanged ballast water. Conversely, where a tank underwent a complete exchange (i.e. 300% FT exchange or 100% ER exchange), successful tracers show a smaller departure from the ocean reference. When tanks are partially exchanged, sensitive tracers should exhibit values intermediate between the results for the untreated and fully exchanged ballast tanks. In other words, as progressively more ocean water is added to a tank, samples from the tank should appear progressively less different from the ocean.

#### 2.2.5. Multivariate statistical model

We compared a vector of discriminating tracers in a ballast tank with the multivariate distribution of the same tracers in the open ocean using the Mahalanobis distance statistic (Johnson and Wichern, 1982). For any individual sample (whether from a ship's ballast tank or from the ambient water along its journey), we can estimate the likelihood that the vector of tracer concentrations falls within the usual range of ocean water. If the likelihood is small, it is inferred that the ballast tank contents were not derived from ocean water.

The square of the Mahalanobis distance statistic,  $D_m$ , is computed as

$$(\mathbf{x}_i - \mathbf{x})' \mathbf{S}^{-1} (\mathbf{x}_i - \mathbf{x})$$

where  $\mathbf{x}_i$  is a column vector of tracers that are being tested,  $\mathbf{x}$  is the column vector of the mean level of tracers in ocean water, and  $\mathbf{S}^{-1}$  is the inverse of the variance-covariance matrix for the tracers in ocean water.

This multivariate test has the potential to be much more powerful than a sequence of univariate tests when the tracers are correlated. However, the opposite can also be true if some tracers are not informative about differentiating ocean water from coastal water.

We ran Mahalanobis tests using salinity, trace element and CDOM samples collected from (1) the ballast tanks, and (2) shipside during the Pacific and Atlantic cruises. First, we developed an "ocean reference set" each for the Pacific and Atlantic oceans, comprising of all shipside samples collected outside the 200 mile EEZ and in depths greater than 2000 m. In the first test, we calculated  $D_m$  and its associated Chi-squared probability for each ballast tank during each sampling occasion, using average tracer concentrations derived from all replicate samples. In the second test performed for the Atlantic ocean only, we calculated  $D_m$  and its associated Chi-squared probability for shipside samples collected within the EEZ, to examine the effect upon  $D_m$  of the proximity of land. Clearly, the two tests relate to different questions regarding BWE compliance: first, whether it is possible to tell if (and to what degree) a ballast tank has been exchanged in mid-ocean, and second, where in the ocean BWE was performed.

As a preliminary effort to identify the strongest performing tracers, we examined the performance of all possible combinations of 2–5 tracers in end-of-voyage samples. We defined the "best average set" as the combination of tracers which maximized the average discrimination factor,  $DF_{av}$ , in end-of-voyage samples, where

$$DF_{av} = 1/[\text{average}(D_{m,\text{exchanged}}/D_{m,\text{control}})]$$

Since the minimum discriminatory ability of a tracer set is arguably as important as its average ability, we defined the "best minimum set" as the combination of

tracers with the highest minimum discrimination factor,  $DF_{\min}$ , in end-of-voyage samples, where

$$DF_{\min} = 1 / [\max(D_{m,\text{exchanged}} / D_{m,\text{control}})]$$

While simplistic, comparisons of average and minimum performance are a useful way to identify tracer sets that perform consistently well across multiple treatments, tanks and voyages. Since we expect that it will be simpler and cheaper to perform verification using tracers derived from a single analytical method, we performed tests separately for combinations drawn from three tracer pools: (1) salinity + trace elements, (2) salinity + CDOM and (3) salinity + trace elements + CDOM. Radium was not included in Mahalanobis analyses since too few radium samples were collected from the mid-ocean to constitute a reliable ocean reference set (Table 1).

### 3. Results

#### 3.1. Overview

Most of the tracers we evaluated (see Table 2) demonstrated potential to detect ballast water exchange on one or more voyages. In general, levels of trace elements, CDOM fluorescence and radium decreased as a result of exchange with ocean water. For many tracers, including Ba, significant decreases in concentration were apparent even after partial ballast water exchanges (Fig. 3). For the majority of tracers and cruises, concentrations changed significantly between initial and final sampling in exchanged tanks, but not in control tanks (Figs. 4 and 5). These results are interpreted as a simple dilution effect of the original ballast water containing terrigenous tracers with offshore water.

ANOVAs on the effect of within-tank sampling depth and location on tracer levels showed that in the vast majority of cases (94% of 549 independent comparisons of tracer levels by ship, time and tank), data from all depths and locations demonstrated good agreement, allowing whole tank concentrations to be estimated from data pooled by depth and location. An examination of the components of variance for the VFos dataset confirmed that within-tank sampling location contributed less than 0.1% of overall variance for most tracers.

Tracer levels in samples drawn from the ships' side during ballast water exchange corresponded well with levels measured in fully exchanged ballast tanks. In a transect of 54 shipside samples taken across the North Atlantic, trace element concentrations and CDOM peak intensities tended to decrease from coastal maxima to oceanic minima (Fig. 6). This trend was considerably more marked in the western Atlantic than in the Mediterranean Sea or on the European continental shelf, illustrating the relative difficulty in determining the

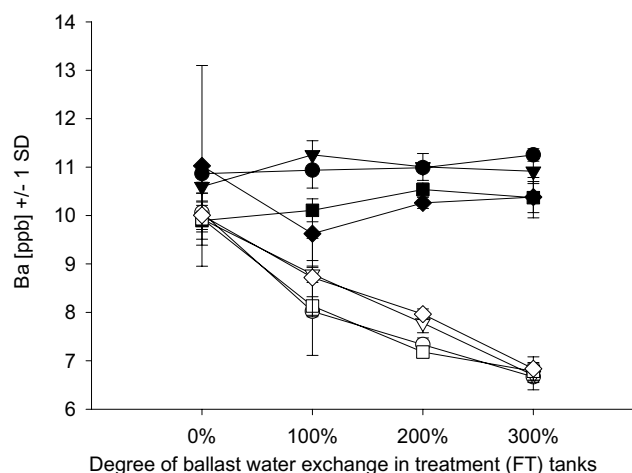


Fig. 3. Barium concentrations in four pairs of ballast tanks on VFos decreased following successive partial ballast water exchanges of the treatment tanks (FT: (1) —○—; (2) —▽—; (3) —□—; (4) —◇—), and remained high in the unexchanged controls (C: (1) —●—; (2) —▼—; (3) —■—; (4) —◆—).

coastal versus oceanic nature of ballast water samples sourced from Mediterranean ports.

In procedural blank samples from the VFos cruise, trace element levels were less than 0.5 ppb. For Ba, U, V and Mo, maximum levels in procedural blanks represented less than 5% of the minimum tracer levels measured in ballast water samples, compared to 25% for Mn. Concentrations of P were higher overall and more variable; in several cases blank concentrations were comparable to ocean water minima (~1.5 ppb). CDOM peak fluorescence intensities in blank samples were similarly variable; in blanks collected from Tank 1 and Tank 3, intensities were 50–100% of the levels measured in fully exchanged ballast water, whereas for the remaining tanks, CDOM intensities were usually less than 20% of levels measured in exchanged ballast water.

Trends for individual tracers and voyages are presented in detail in the relevant subsections below, followed by the results of univariate and multivariate statistical analysis.

#### 3.1.1. Trace elements

Concentrations of the trace elements in unexchanged (control) tanks were either stable (Ba, Mo, U, V) or fluctuated (Mn, P) over time. In exchanged tanks, concentrations were initially similar to controls, but usually decreased following mid-ocean exchange to levels approaching concentrations in the open ocean (Fig. 4). Mo, U and V tended to increase or decrease in concert with salinity. Levels of trace elements in tanks containing eutrophic, low salinity San Francisco Bay water (VSF) were 2–6 times higher than on other voyages, a result which facilitated discrimination between exchanged and unexchanged tanks. Conversely, ballast

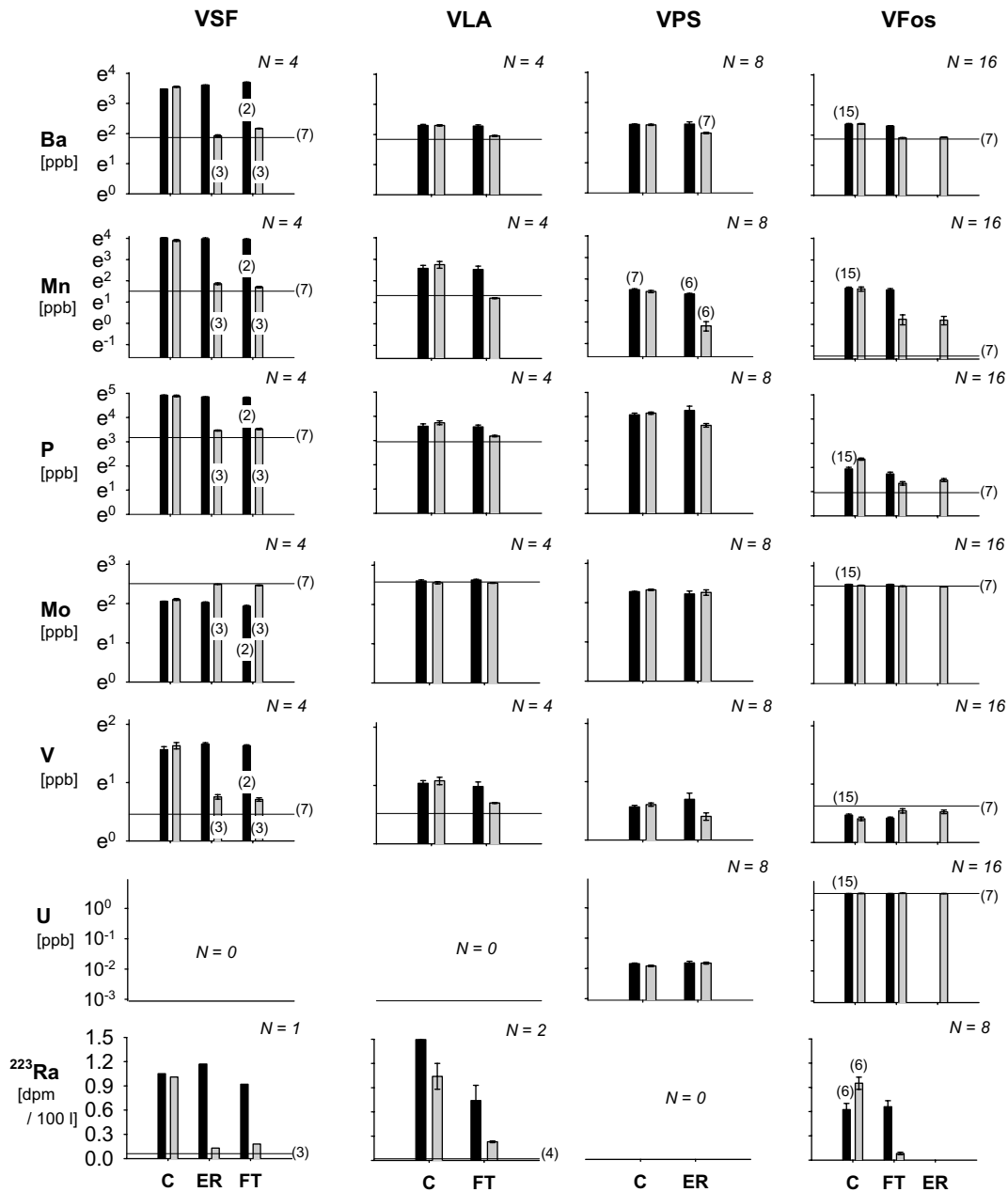


Fig. 4. Concentrations of trace elements (mean  $\pm$  SE) and  $^{223}\text{Ra}$  in ships' ballast tanks on the Pacific and Atlantic cruises. Bars represent concentrations before (■) and following (□) complete exchange of the flow-through (FT) and/or empty-refill (ER) tanks. Control tanks (C) remained unexchanged throughout. Shippside sample means coinciding with the position of ballast water exchange are indicated by horizontal lines. Where sample size ( $N$ ) deviates from numbers indicated at the top right of each graph, these are specified in parentheses.

water from the Mediterranean Sea was relatively oligotrophic and demonstrated subtle changes in tracer levels due to BWE.

Within ballast tanks, trace element data from all depths and locations routinely demonstrated good agreement, indicating reliability of the complete procedure and allowing accurate estimations of whole tank concentrations. Occasional exceptions to this rule usually involved the elements Mn and P, and were often associated with high levels of Fe, Zn and other reactive

metals that were not considered viable tracers in this study (Murphy et al., 2002).

Concentrations of Ba, Mo, U and V in shippside samples were comparable to published ranges for surface northeast Pacific and North Atlantic Ocean samples (Bernat et al., 1972; Chan et al., 1976, 1977; Chen et al., 1986; Collier, 1984; Morris, 1975; Nozaki et al., 2001; Shaw et al., 1998; Sohrin et al., 1987). Mn levels generally exceeded published ranges by a factor of 3–10 (Bruland and Franks, 1983; Landing and Bruland, 1980;

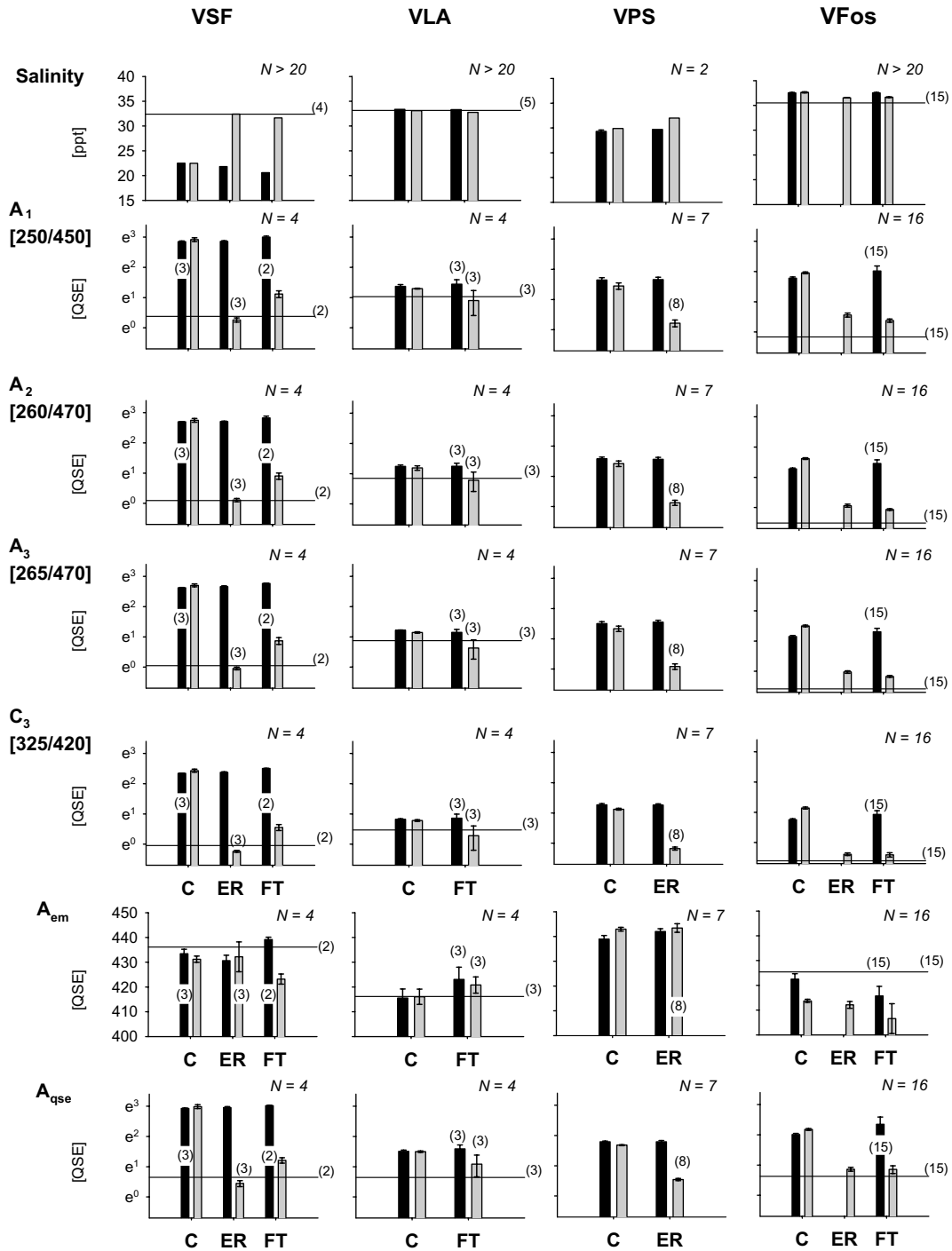


Fig. 5. CDOM concentrations (mean  $\pm$  SE) in ballast water samples during four commercial voyages. Bars represent concentrations before (■) and after (□) complete exchange of the flow-through (FT) or empty-refill (ER) tanks. Control tanks (C) remained unexchanged throughout. Shippide sample means coinciding with the position of ballast water exchange are indicated by horizontal lines. Where sample sizes ( $N$ ) deviate from numbers indicated at the top right of each graph, these are specified in parentheses.

Martin et al., 1985; Statham et al., 1998), while levels of P exceeded expected levels in unfiltered (Pacific) samples but were consistent with published data for (filtered) Atlantic and Mediterranean samples (Karl and Björkman, 2002, and references therein).

Across all four voyages, Ba concentrations ranged from 10 to 30 ppb in unexchanged ballast tanks, but were consistently lower than 7 ppb in fully exchanged tanks. Mn concentrations were 5–55 ppb in unexchanged tanks and 0.4–7 ppb in exchanged tanks. Initial

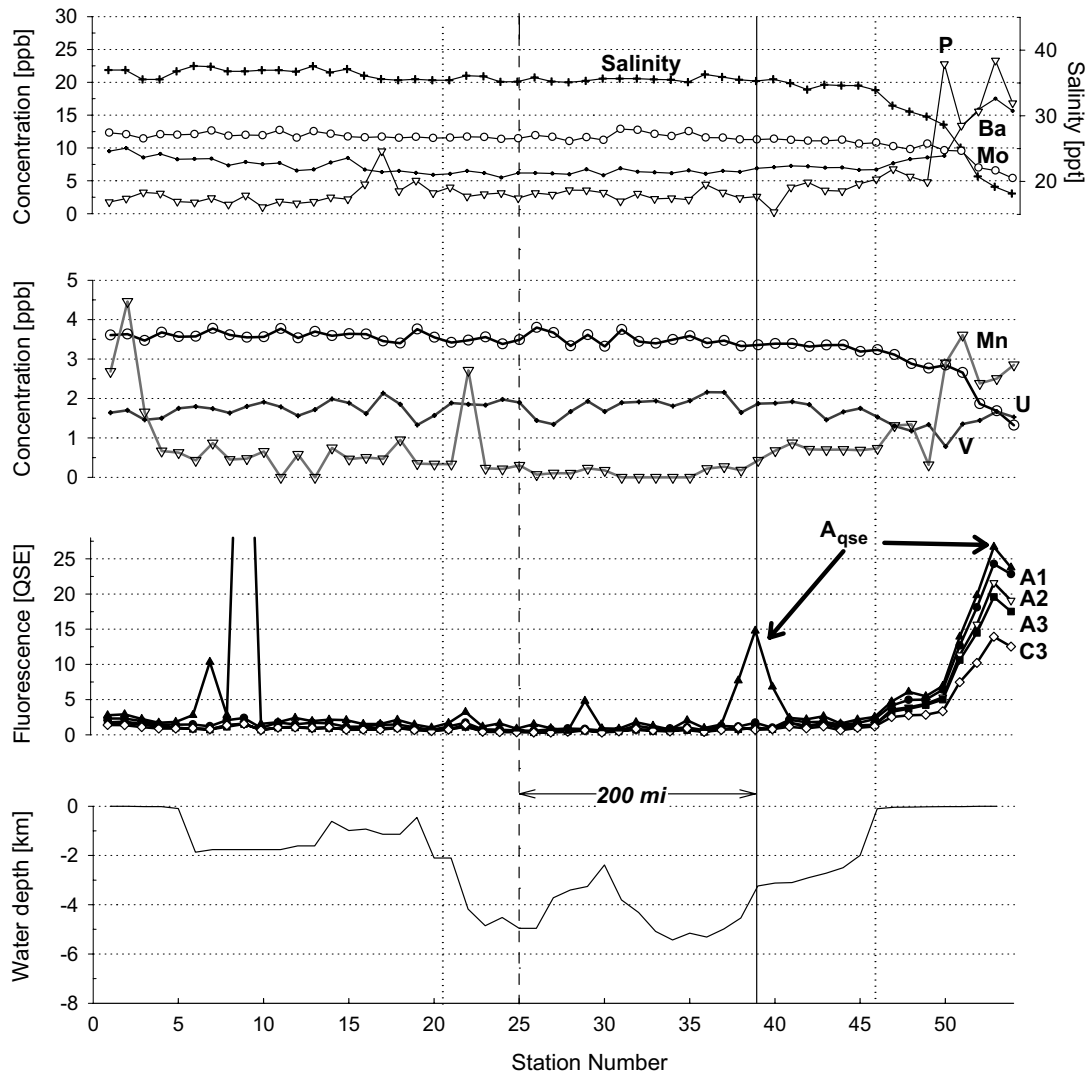


Fig. 6. Salinity, trace element concentration (Ba, Mo, Mn, P, U, V) and CDOM fluorescence intensities ( $A_1$ ,  $A_2$ ,  $A_3$ ,  $C_3$ ) in ship side samples from the North Atlantic on VFos. The boundary of the “open ocean” (>200 miles offshore, >2000 m deep) enclosing ocean reference data is indicated by vertical lines (---), also, 100 miles offshore boundaries (···). Sea floor bathymetry (Smith and Sandwell, 1997) is plotted for comparison.

phosphorus levels were an order of magnitude lower on VFos [ $\sim 6$  ppb] than on the other voyages [38–140 ppb] but decreased in all exchanged tanks on all voyages. Initial levels of V ranged from 1.5 to 5 ppb, and decreased significantly after BWE except on VFos. Levels of Mo were 7–14 ppb on all voyages and varied little as a result of exchange except during VSF, where they increased by approximately 30%. Concentrations of U were comparable in exchanged and unexchanged ballast tanks on both voyages for which this tracer was measured (VFos, VPS), although concentrations were lower overall in the Pacific samples ( $\sim 3.0$  ppb) than in the Atlantic samples ( $\sim 3.5$  ppb). Since Mo and U are conservative with salinity, these results indicate that the Ba, Mn, P and V concentrations were more sensitive indicators than salinity on all voyages.

On the VFos voyage, tracers were measured in replicate ballast tanks. Across all samples of unexchanged ballast water collected on this cruise ( $N = 46$ ), the coefficients of variation (CVs) were less than 6.5% for U and V, and less than 4.5% for Ba and Mo. In contrast, the CVs for Mn and P were 45% and 26% respectively. This variability occurred almost entirely at the level of the tank, thus it reflected real differences between ballast tanks.

Trace element concentrations at each of 54 ocean stations sampled sequentially during the voyage from France to the USA showed slight to moderate offshore gradients (Fig. 6). Concentrations of Mo, U, and V in Mediterranean samples were barely distinguishable from those in samples collected from 200 miles offshore. Ba levels exhibited a gradual decline from around 10

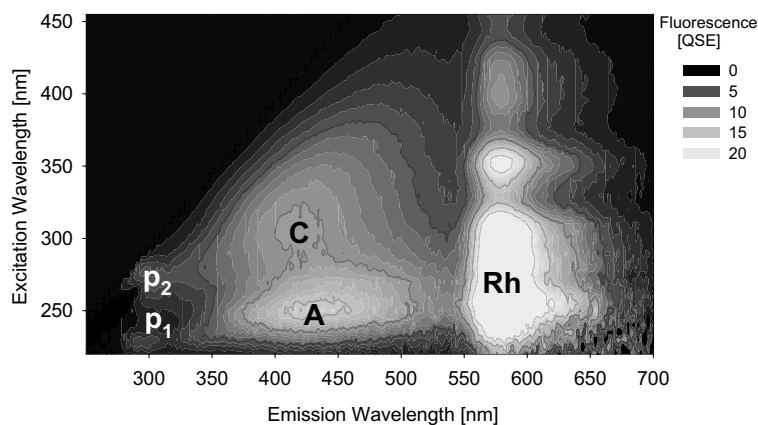


Fig. 7. CDOM signature of a control tank sample from the VSF cruise, showing humic peaks 'A' (exc./em.: 230–265/380–460 nm) and 'C' (exc./em.: 230–265/380–460 nm). This sample also shows fluorescence from rhodamine dye (Rh, exc./em.: 230–450/550–650 nm) which was added to the tanks to provide an independent tracer of ballast exchange efficiency, and protein fluorescence similar to that of tyrosine, with primary ( $p_1$ ,  $1'$ : 230 nm) and secondary ( $p_2$ ,  $2'$ : 270 nm) excitation peaks.

ppb to 6 ppb within the Mediterranean, and persisted near this level until the ship began to approach the US east coast. In the Mediterranean ship side samples, concentrations of P (ca. 2–4 ppb) were lower than in the ballast tanks at the beginning of the voyage, and lower than in the open ocean. Mn levels in shipside samples decreased rapidly while still in the Mediterranean sea, and further decreased to levels below 0.5 ppb in the open ocean. The Mn spike observed at station 22 (63 miles SW of the Cape of St Vincent) may result from an unknown point source originating in Spain or Portugal or else it may be spurious. However, this station did not exhibit elevated levels of iron and heavy metals, as was usually seen with contaminated trace element samples during this project.

At the opposite end of the Atlantic, there were marked changes in trace element concentrations with increasing proximity to the US coastline. Mn levels were the first to rise beginning around 150–200 miles offshore, followed by P (100–150 miles) and Ba (50–100 miles). Levels of the predominantly conservative elements Mo (Paulsen and List, 1997) and U (Andersson et al., 2001) began declining around 50–100 miles offshore in concert with decreasing salinity.

### 3.1.2. CDOM

Two smooth rounded peaks arising from the fluorescence of humic material were common to EEMs plots across all cruises: humic peaks 'A' (excitation/emission: 230–265/380–460 nm) and 'C' (exc./em.: 230–265/380–460 nm) (Fig. 7). Humic peak A was always more intense than peak C and represented the dominant natural peak during all cruises.

Several peaks not attributable to CDOM were also identified in EEMs from ballast water samples. These included rhodamine dye (exc./em.: 230–450/550–650

nm), which was added to the tanks on the first two voyages (VSF, VLA) to provide an independent tracer of ballast exchange efficiency. Protein fluorescence similar to that of tyrosine, with primary ( $1'$ , 230 nm) and secondary ( $2'$ , 270 nm) excitation peaks was observed in both ballast water and shipside samples on multiple voyages. On the VFos cruise, all unexchanged ballast samples and several shipside samples exhibited 1–3 sharp peaks attributed to polycyclic aromatic hydrocarbons (PAHs), some of which overlapped with DOM fluorescence wavelengths.

The maximum intensity of humic peak A ( $A_{qse}$ ), and intensities at several fixed-wavelength pairs within peak 'A' and 'C' ranges (e.g.  $A_1$ ,  $A_2$ ,  $A_3$ ,  $C_3$ ) showed marked responses to BWE (Fig. 5). Peak fluorescence intensities remained stable or increased slightly in control tanks over each voyage, whereas intensities in exchanged ballast tanks approached oceanic minima. The decrease in CDOM fluorescence following exchange was statistically significant except on VLA, for which low replication ( $N = 3$ ) and the apparent contamination of one sample obscured an otherwise consistent trend.

In shipside samples (Fig. 6), CDOM fluorescence was an order of magnitude lower for Mediterranean water (1–2.5 QSE) than water obtained near the US Atlantic coast (12–26 QSE), but generally elevated in comparison to oceanic water (0.25–1.25 QSE).  $A_{qse}$  exhibited occasional spikes due to contamination by non-CDOM fluorophores; similar contamination of  $A_{qse}$  was evident in approximately 10% of ballast tank samples. Contaminants may also have contributed to the result that the emission wavelength ( $A_{em}$ ) corresponding to  $A_{qse}$  did not vary consistently within or across voyages. However, the fixed wavelength peaks  $A_1$ ,  $A_2$ ,  $A_3$  and  $C_3$  exhibited low rates of influence by non-CDOM fluorescence and appeared to be more robust tracers of BWE.

### 3.1.3. Radium

Short-lived radium isotopes  $^{223}\text{Ra}$  and  $^{224}\text{Ra}$  were recovered in measurable levels from the Mn-fibers on all three voyages where radium samples were collected (i.e. VSF, VLA and VFos). We present here only the data for  $^{223}\text{Ra}$ . The very low final activity of  $^{224}\text{Ra}$  on VFos (5–6% of the source concentration) and the presence of its parent,  $^{228}\text{Th}$ , rendered it an unreliable tracer on this voyage; moreover its short half life (3.7 days) would prevent it from being used as a tracer for all voyages longer than  $\sim 1$  week.

Levels of  $^{223}\text{Ra}$  in control tanks decreased on VLA ( $\sim 30\%$ ), increased on VFos ( $\sim 50\%$ ) and remained unchanged on VSF (Fig. 4). Only one ocean sample contained appreciable quantities of  $^{223}\text{Ra}$ . This is consistent with expectation; since the parent of  $^{223}\text{Ra}$ ,  $^{227}\text{Ac}$ , is exceedingly low in open ocean water (Nozaki et al., 1990; Shaw et al., 1998),  $^{223}\text{Ra}$  is presumed to be virtually zero in both the Atlantic and the Pacific.

Levels of the long lived radium isotopes  $^{226}\text{Ra}$  and  $^{228}\text{Ra}$  were measured in VFos end-of-voyage samples. After the final exchange of the treatment tanks on this voyage, levels of both isotopes were significantly lower in the exchanged tanks than in the control tanks, as were  $^{223}\text{Ra}/^{226}\text{Ra}$  and  $^{228}\text{Ra}/^{226}\text{Ra}$  activity ratios (AR) (Table 3). Concentrations of  $^{226}\text{Ra}$  in exchanged tanks were similar to levels reported previously in surface ocean water (Chung and Craig, 1980; Broecker et al., 1976).

On VLA, the decrease in control tanks of  $^{223}\text{Ra}$  is considered an artifact of low replication and sampling difficulties experienced during this voyage. Conversely, the significant increase over time of  $^{223}\text{Ra}$  in the VFos control tanks and its finite concentration in the exchanged tanks following complete mid-ocean exchange indicates the presence of a  $^{223}\text{Ra}$  source within the tanks. A likely source on this voyage was coal, which was transported by this vessel and was undoubtedly present in the water of the source port. Since coal is normally rich in uranium and  $^{223}\text{Ra}$  is produced in the decay chain of  $^{235}\text{U}$ , coal dust in the ballast tanks could have produced  $^{223}\text{Ra}$  over the course of the two week voyage.

Table 3

Radium and thorium isotope concentrations and activity ratios in unexchanged (control) versus fully exchanged (300% FT or 100% ER) ballast water on the VFos cruise ( $N = 8$ )

Isotope	Control $x \pm \text{SE}$	Exchanged $x \pm \text{SE}$
$^{226}\text{Ra}$	$10.13 \pm 0.33$	$6.50 \pm 0.15$
$^{228}\text{Ra}$	$4.70 \pm 0.16$	$2.19 \pm 0.09$
$^{223}\text{Ra}$	$1.02 \pm 0.03$	$0.08 \pm 0.02$
228/226 AR	$0.46 \pm 0.02$	$0.34 \pm 0.01$
223/226 AR	$0.10 \pm 0.01$	$0.01 \pm 0.00$
$^{228}\text{Th}$	$0.32 \pm 0.03$	$0.30 \pm 0.01$

### 3.2. Univariate analyses

We examined the abilities of trace element and CDOM tracers to independently discriminate between ocean water and ballast tanks subject to different degrees of BWE. Each combination of “ship”, “treatment”, “% BWE” and “tracer” was considered a single test case (Tables 4 and 5). Where the average amount of a tracer was significantly different from background ocean levels, this event was tallied next to the corresponding probability statistic (either  $p < 0.05$ ,  $p < 0.01$  or  $p < 0.001$ ). Where the tank concentration was not significantly different to the ocean set, this event was tallied next to the abbreviation “nsd”.

#### 3.2.1. Pacific cruises

For the majority of tracers, the ability to discriminate between untreated tank samples and oceanic (shipside) samples varied between cruises, although Ba and  $^{223}\text{Ra}$  consistently identified unexchanged ballast water across all Pacific cruises. For the VSF cruise samples, salinity, Ba, Mn, Mo, P, V,  $^{223}\text{Ra}$ ,  $^{228}\text{Th}$  and  $A_{\text{qse}}$ , each successfully discriminated untreated port water from ocean water. For the VLA cruise, Ba, Ra, Mn and V and Th were significantly different in the flow-through tank prior to exchange, while on VPS, salinity, Ba, Mn, Mo and P were significantly different in the empty–refill tank prior to exchange. With the occasional exception of  $A_{\text{qse}}$  and  $C_{\text{em}}$ , none of the CDOM parameters independently identified unexchanged tanks on any voyage.

While all FT and ER ballast tanks were eventually exchanged according to IMO guidelines (i.e. 300% FT or 100% ER), our rhodamine dye measurements on VLA and VSF indicated that three FT exchanges were respectively 75% and 93% efficient at replacing the coastal water in the ballast tanks (Murphy et al., 2002). Salinity, Ba, P, V and  $C_{\text{em}}$  levels were significantly elevated following two FT exchanges on VSF. Conversely,  $^{223}\text{Ra}$  was the only tracer to show significant differences following two FT exchanges on VLA.

Ba showed a consistently high level of discrimination between tanks subject to different degrees of exchange. This was demonstrated clearly by the FT tank on VSF, for which the probability statistic increased from  $p < 0.001$  to  $p < 0.01$  to  $p < 0.05$  after three successive FT exchanges. After the final exchange, Ba was still present in significantly higher than ocean levels, even though only 7% San Francisco Bay water remained in this tank. This indicates that Ba was a highly sensitive coastal tracer on this voyage.

#### 3.2.2. Atlantic cruise

The sampling of eight ballast tanks on the Atlantic cruise (VFos) afforded comparisons between replicate tanks subject to similar levels of exchange (Table 5). Overall results reflected the trends seen in the Pacific

Table 4  
Univariate comparison of ballast water samples (C, FT, ER) on the Pacific voyages

Cruise	Treat	Time	% BWE	$p$	Sal	Ba	Mn	Mo	P	V	A <sub>qse</sub>	C <sub>qse</sub>	A <sub>em</sub>	C <sub>em</sub>	A <sub>1</sub>	A <sub>2</sub>	A <sub>3</sub>	C <sub>3</sub>	<sup>223</sup> Ra	<sup>228</sup> Th															
VSF	C	T0-T3	0	0.001	4	4	4	4	4	4	4	4	4	1	4	4	4	1	3	1															
				0.01																	4	3	1	1	1	1	1	1	1	1	1	1	1	1	
				0.05																															
	ER	T0	0	0.001	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1														
				0.01																		1	1	1	1	1	1	1	1	1	1	1	1	1	1
				0.05																															
	FT	T1	98	nsd	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1														
				0.001																															
		T0	0	0.001	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1														
				0.05																															
T1	55	nsd	Undet.	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1															
		0.001																																	
T2	83	0.01	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1															
		0.05																																	
		nsd																																	
T3	93	0.05	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1															
		nsd																																	
VLA	C	T0-T2	0	0.001	3	3	1	3	2	2	3	3	3	3	3	3	3	3	3	3															
				0.01																	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
				0.05																															
	FT	T0	0	0.001	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1															
				0.01																															
T1	60	0.05	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1																
		nsd																																	
T2	75	nsd	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1																
		nsd																																	
VPS	C	T0-T1	0	Undet.	1	2	2	1	2	2	2	2	2	2	2	2	2	2	2	2															
				0.01																	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
				0.05																															
	ER	T0	0	Undet.	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1															
				0.01																															
T1	98	0.05	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1																
		nsd																																	
T1	98	Undet.	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1																
		0.01																																	
T1	98	0.05	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1																
		nsd																																	

Numbers in table are a tally of tanks for which average tracer levels are significantly different to ocean levels at specified probability levels ( $p < 0.05, 0.01, 0.001$ ), not significantly different (nsd) or undetermined (undet.). Results for exchanged tanks (FT, ER) are separated according to levels of BWE ( $n = 1$ ) while results for unexchanged tanks (C, 0% BWE) are summarized over entire cruises ( $n \geq 2$ ).

Table 5  
Univariate comparison of ballast water samples (C, FT, ER) on the Atlantic voyage

Ship	Treat	Time	% BWE	<i>p</i>	Sal	Ba	Mn	Mo	P	U	V	A <sub>em</sub>	A <sub>qse</sub>	A <sub>1</sub>	A <sub>2</sub>	A <sub>3</sub>	C <sub>3</sub>		
VFos	C	T0–T3	0	0.001	16	16	16	16	15	16	16	16	1	16	16	16	16		
				0.01					1										
				0.05															
				nsd															
	ER	T4	>95	0.001	1	4	4	4	1	4	4	4	4	4	1	3	3	1	
				0.01					1										
				0.05					3										
				nsd															
	FT	T0	0	0.001	4	4	4	4	2	4	4	4	4	4	4	4	4	4	
				0.01					2										
				0.05					1										
				nsd															
		T1	~50 (35–60)	0.001	2	4	4	4	4	3	4	4	4	4	4	4	4	4	3
					0.01					1									
					0.05					1									
					nsd														
		T2	~75 (65–87)	0.001	3	2	1	3	1	1	4	4	4	4	4	4	4	4	4
					0.01					1									
					0.05					1									
					nsd														
T3	>95	0.001	2	4	4	4	4	4	4	4	4	4	4	1	3	1	3		
			0.01					2											
			0.05					2											
			nsd					4											

Numbers in table are a tally of tanks for which tracer levels are significantly different to ocean at specified probability levels ( $p < 0.05, 0.01, 0.001$ ) or not significantly different (nsd). Results for exchanged tanks (FT, ER) are separated according to levels of BWE (4 tanks per treatment) while results for unexchanged tanks (C, 0% BWE) are summarized over the entire cruise (4 tanks × 4 times).

cruises, while the magnitude of between-tank variation ranged from small to substantial depending on the tracer. Concentrations of P were quite variable among tanks subject to equivalent treatments, with the result that associated statistical probabilities ranged from sig-

nificantly different to the ocean ( $p < 0.01$ ) to not significantly different ( $p > 0.05$ ) in four replicate tanks each subject to 95% ER exchange. A wide range of probabilities also resulted from tests on CDOM “A” peak intensity (A<sub>qse</sub>)—of the four FT tanks sampled at the

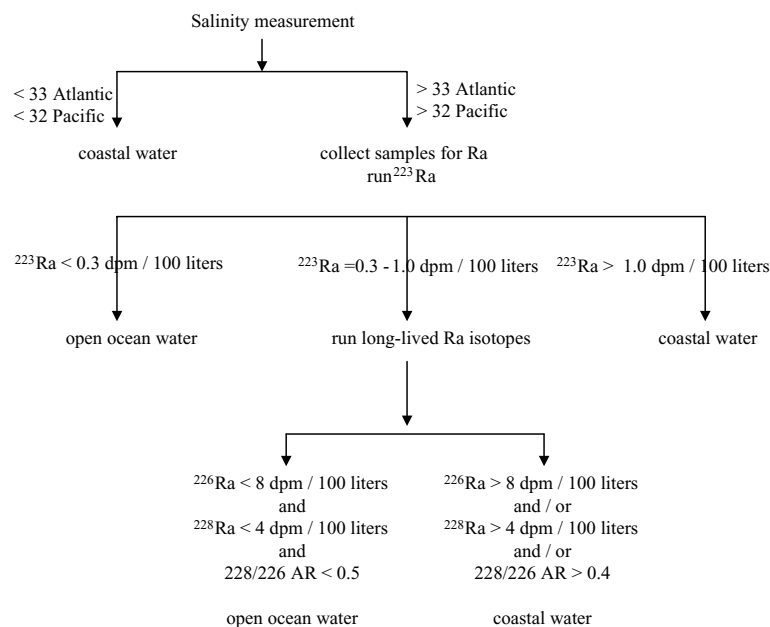


Fig. 8. Proposed hierarchical verification scheme using salinity and radium isotopes.

beginning of the voyage, one corresponded to a probability of  $p < 0.001$ , two to a probability of  $p < 0.05$  and the other did not appear to be significantly different to the ocean samples.

### 3.3. Multivariate analyses

Multivariate analysis using the Mahalanobis distance statistic  $D_m$  was considerably more powerful at detecting ballast water exchange than any single tracer. Some combinations of CDOM fluorescence intensities and trace element concentrations produced highly significant differences between exchanged and unexchanged tanks on all voyages (see next section).

Too few radium samples were collected from the ocean to allow its evaluation by multivariate analysis, however, we were able to develop an hierarchical scheme for verifying BWE according to levels of salinity and radioisotopes measured on VSF, VLA and VFos (Fig. 8). In this scheme, tanks with salinity less than 30 ppt are deemed to be carrying unexchanged ballast water, precluding the need for further testing. Where salinity exceeds 30 ppt, short lived radioisotopes ( $^{223}\text{Ra}$ ) are examined, followed if necessary by long-lived isotopes ( $^{228}\text{Ra}$ ,  $^{226}\text{Ra}$ ) and activity ratios. For the VSF, VLA and VFos cruise data, exchanged and unexchanged ballast tanks separate on the second tier of the scheme. Long-lived isotope data collected in this study (VFos, final samples) confirm these determinations.

#### 3.3.1. Degree of ballast water exchange

Increased discrimination corresponding to the incorporation of multiple tracers in a multivariate statistical analysis is demonstrated clearly by the VFS trace element data (Fig. 9a–c), using three-dimensional plots of  $D_m$  versus tracers and percent exchange (or time, in the case of unexchanged control tanks). Moving from left to right on the tracer axis, one tracer is added to the analysis (i.e. Sal, (Sal + Ba), (Sal + Ba + Mn), etc.). Since  $D_m$  is a difference measure, tanks which are least “oceanic” have the tallest bars and lowest probabilities. Plots for the exchanged tanks on this cruise (Fig. 9a–b) illustrate two trends operating concurrently, first, an increase in  $D_m$  as progressively more tracers are incorporated into the analysis and second, a decrease in  $D_m$  following each partial or complete ballast exchange. Only the first of these trends was apparent in samples taken from the control tank (Fig. 9c), indicating that the evidence of a coastal source for these tanks became stronger as the tracer set was expanded, independent of the timing of sampling. Note that in these figures, the sequence of tracers was chosen for visual clarity and does not reflect a judgement of performance.

To identify the strongest performing tracer sets, we compared difference factors in end-of-voyage samples for all possible sets of 2–5 tracers. On the Pacific voy-

ages, a set of five CDOM measures ( $A_{em}$ ,  $A_{qse}$ ,  $C_{ex}$ ,  $C_2$ ,  $C_3$ ) had the highest overall mean and minimum values of DF (Table 6). On average, there was an 28-fold difference in  $D_m$  for exchanged (ER and FT) and unexchanged end-point samples using this combination of tracers. Trace element measures showed lesser discriminatory power—the best average set (Ba, V) had a  $DF_{av}$  of 8.2 across all voyages, while the best minimum set (Ba, Sal) had a  $DF_{av}$  of 7.6.

On the Atlantic voyage, sets consisting of only two or three tracers were more successful than larger tracer sets.

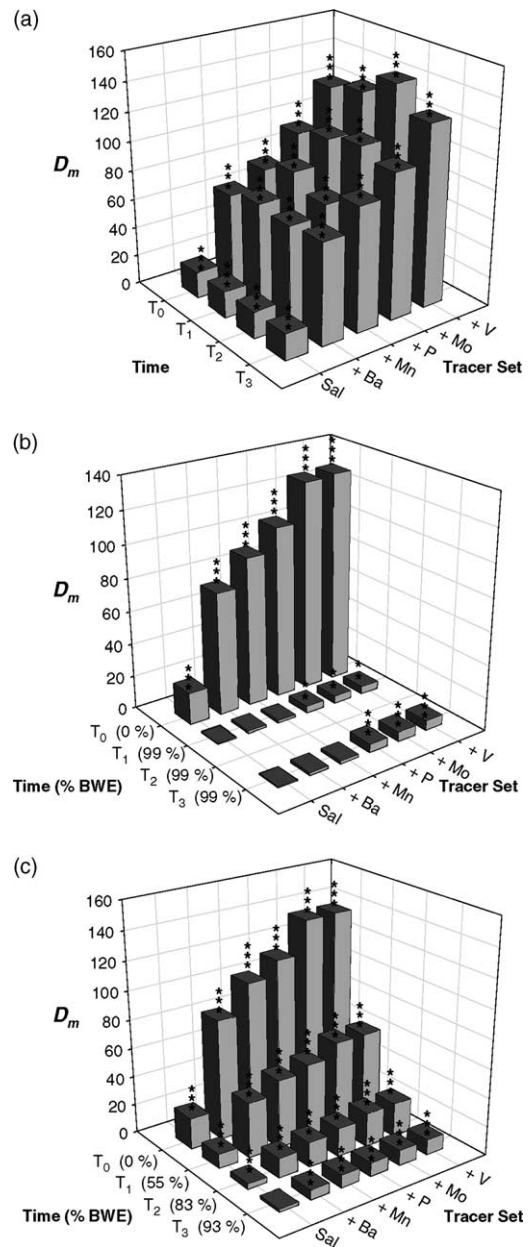


Fig. 9. Mahalanobis distance ( $D_m$ ) of ballast tanks on the VSF cruise out of San Francisco. Tanks with tracer levels that are significantly different to ocean levels are indicated with associated probabilities (\*:  $p < 0.05$ , \*\*:  $p < 0.01$ , \*\*\*:  $p < 0.001$ ). (a) Control, (b) empty-refill exchange, (c) flow-through exchange.

Table 6  
Discrimination factors ( $D_{m,control}/D_{m,exchanged}$ ) for the tracer sets with the highest average discrimination ( $DF_{av}$ ) and highest minimum discrimination ( $DF_{min}$ ) between exchanged and unexchanged tanks across all endpoint samples from the Pacific and Atlantic cruises

Ocean	Tracer types	Best average set Tracers $DF_{av}$ (range of $DF_{av}$ )	Best minimum set Tracers $DF_{min}$ (range of $DF_{min}$ )
Pacific	Salinity/trace element	Ba, V 8.2 (4.2–26.8)	Ba, Sal 7.6 (4.6–45.4)
	Salinity/CDOM	$A_{em}, A_{qse}, C_{ex}, C_2, C_3$ 28.0 (14.3–588.3)	Same as best average set
	Salinity/trace element/CDOM	$A_{em}, A_{qse}, C_{ex}, C_2, C_3$ 28.0 (14.3–588.3)	Same as best average set
Atlantic	Salinity/trace element	Ba, U 8.6 (6.1–14.0)	Same as best average set
	Salinity/CDOM	$A_{qse}, C_3$ 19.9 (13.4–58.4)	Same as best average set
	Salinity/trace element/CDOM	$A_{qse}, C_3, Mo$ 20.4 (12.2–67.2)	$A_{qse}, C_3$ 19.9 (13.4–58.4)

A combination of one trace element (Mo) and two CDOM tracers ( $A_{qse}, C_3$ ) produced on average a 20-fold difference in  $D_m$  for control and exchanged (ER and FT) end-point samples. Although the best CDOM set ( $A_{qse}, C_3$ ) had a marginally lower  $DF_{av}$  than the one including Mo, it had a slightly higher  $DF_{min}$  (13.4 vs 12.2) and is arguably the set with the best overall performance. A scatter plot of  $A_{qse}$  vs  $C_3$  in samples collected from all

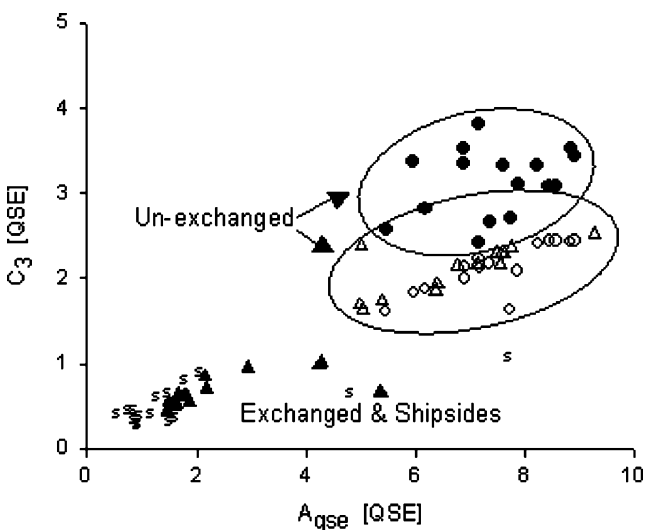


Fig. 10. A scatterplot of two CDOM fluorescence characteristics (humic peak A maximum intensity,  $A_{qse}$ , and humic peak  $C_3$  intensity) shows separation between ballast water samples collected from unexchanged tanks at the beginning (O—control,  $\Delta$ —flow-through) and at the end (●—control) of the VFos cruise, compared to exchanged tanks ( $\blacktriangle$ —flow-through) at the end of the cruise, and to samples collected in the mid-ocean (s).

tanks at the beginning (time =  $T_0$ ) and end (time =  $T_3$ ) of the flow-through experiment, and from the mid-ocean on VFos shows clear separation among treatments (Fig. 10). Again, the best trace element set (Ba, U) had lower

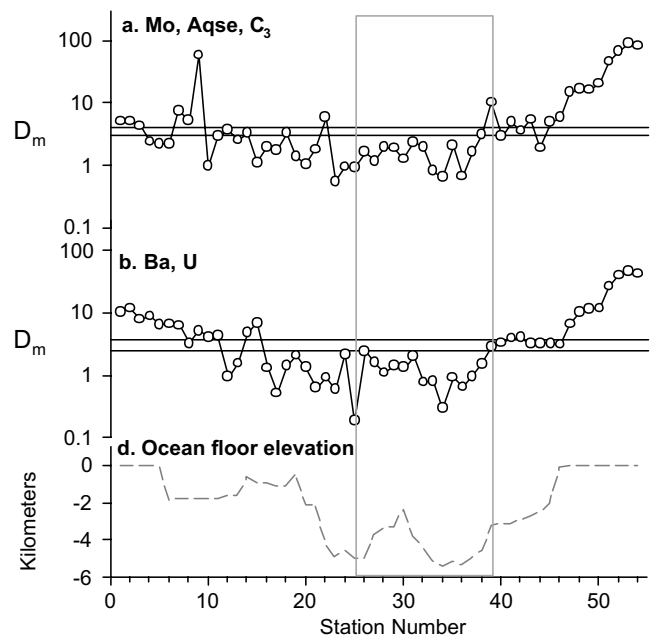


Fig. 11. Mahalanobis distance ( $D_m$ ) of shipside samples transecting the Atlantic (—O—) using (a) best overall (Mo,  $A_{qse}, C_1$ ); and (b) and best trace element (Ba, U) tracer sets. Data plotting above the paired horizontal lines have Chi-Squared probabilities lower than  $p = 0.001$  (upper line) or  $p = 0.05$  (lower line). Data enclosed by box (Stations 25–39) represent fully oceanic samples, i.e. collected more than 200 miles offshore in more than 2000 m water depth. Ocean floor elevation is plotted for comparison (---).

average ( $DF_{av} = 8.6$ ) and minimum ( $DF_{min} = 6.1$ ) discrimination factors than sets that included CDOM.

### 3.3.2. Location of ballast water exchange

In samples collected from an ocean transect, we expect the statistical deviation from “oceanic” characteristics to be highest for samples close to the European and North American continents, and lowest for samples collected in the mid-ocean. For some tracer sets, including the “best minimum” trace element set (Ba, U) in the Atlantic ocean, ocean transect samples showed pronounced onshore–offshore gradients in  $D_m$ , suggesting that it may be possible to tell where in the ocean ballast water exchange is performed (Fig. 11). However, for other tracer sets, including the “best average” Atlantic set (Mo,  $A_{qse}$ ,  $C_3$ ), large fluctuations in  $D_m$  among consecutive samples suggested that factors other than proximity to the coast dominated the response. These results highlight the need to judge tracers not only on their maximum discriminatory abilities, but also on their robustness to potential contaminants.

## 4. Discussion

The goal of this research was to test the potential for various chemical attributes to be used, singly or in combination, to determine (a) whether, and to what extent, a ship's ballast water was subject to mid-ocean exchange, and (b) whether ships performed exchange in the open ocean according to guidelines specifying that the exchange take place 200 miles offshore and in water over 2000 m deep. Sampling of four commercial ships in two ocean basins indicated that several tracers were able to discriminate between fully exchanged, partially exchanged and unexchanged ballast tanks. Furthermore, although preliminary, our results suggest that it may be possible to determine if ballast water was exchanged closer than 200 miles from the nearest coast.

Trace elements, including some metals, demonstrated potential as quantitative measures of BWE—a result which was counter to the a priori expectation expressed by some researchers, who considered contamination by the ship's structure would compromise all metal tracers. Trace element levels were elevated in unexchanged ballast tanks, and in coastal shipside samples, relative to ballast tank samples and oceanic shipside samples. In the Atlantic shipside transect, concentration gradients were much greater near the US coast than in the Mediterranean Sea. Previous work has shown that trace metal and nutrient concentrations are greatest in coastal waters which receive elevated inputs from riverine, eolian and terrestrial sources (Bruland, 1983; Donat and Bruland, 1995; Shiller, 1997). The depressed levels of phosphorus (2–4 ppb) measured in the Mediterranean shipside samples and in tanks at the beginning of the

VFos voyage are consistent with the unique nutrient chemistry of the Mediterranean (Bethoux et al., 1998).

When incorporated into multivariate analyses, we found that CDOM humic fluorescence characteristics may be highly effective indicators of ballast water source. The size and wavelengths of the maximum fluorescence peak in the range of CDOM ( $A_{qse}$ ), combined with the intensity at a fixed wavelength pair ( $C_3$  exc./em. = 325/420), showed large (13–58-fold) differences in the statistic  $D_m$  between exchanged and unexchanged ballast tanks on the Atlantic cruise, despite a salinity differential of only 2 ppt. For the Pacific cruises, these same tracers combined with  $A_{em}$ ,  $C_{ex}$ , and  $C_2$  revealed 14–588-fold differences.

Our data suggest that certain radioisotopes could be used to verify ballast water exchange, including concentrations and ratios of a short-lived isotope of radium ( $^{223}\text{Ra}$ ) and two long-lived isotopes ( $^{226}\text{Ra}$ ,  $^{228}\text{Ra}$ ). For the VFos cruise, which was conducted on a coal-carrier, we hoped to use  $^{228}\text{Th}$  as a proxy to investigate marine particulate sources of  $^{223}\text{Ra}$ , since thorium is largely particle-bound in sea water and is not contained in high quantities in coal. However, there was no difference between concentrations of  $^{228}\text{Th}$  in exchanged and control tanks on VFos. Given the higher sediment load of the coastal water, the lack of variation in  $^{228}\text{Th}$  between control and exchanged tanks is surprising. We suggest that  $^{228}\text{Th}$  retained on the Mn fibers was not a reliable measure of particulate  $^{223}\text{Ra}$  production due to the cancellation of two effects: (1) the dissolved  $^{228}\text{Th}/^{228}\text{Ra}$  AR in the coastal water was a factor of 2 lower than the AR in the open ocean, and (2)  $^{228}\text{Ra}$  was a factor of 2 higher in the coastal water than in the open ocean. More data are required to confirm these results, particularly since our ability to test radium measures was limited due to small sample sizes.

Determination of BWE location requires high sensitivity to small offshore concentration gradients, and is a considerably more difficult task than verifying whether a ballast tank contains water from a coastal port or the mid-ocean. A transect of shipside samples across the Atlantic afforded us the opportunity to examine whether it may be possible to detect when BWE is performed less than 200 miles offshore. Seawater sampled off the US east coast had an obvious coastal signature that was still detectable nearly 200 miles offshore, reflecting the strong salinity signal emanating from the Chesapeake Bay. Near the European coast however, sets of tracers that were highly successful in verifying BWE did not always reliably predict the coastal vs. oceanic nature of shipside samples. While we considered it premature to pursue verification of BWE location extensively during this study, we believe that it may eventually be possible to optimize tracer sets for this capability.

We found little evidence to suggest that tracer concentrations in our ballast tanks varied by depth (1 m vs

12 m) or horizontal location. The important practical implication is that for the tracers of interest, it is possible to obtain an accurate indication of whole-tank tracer concentrations by taking measurements from a single depth and location. This would be untrue for a ballast tank comprised of chemically distinct water masses that have had insufficient opportunity to mix completely (e.g. Taylor and Bruce, 2000). However, this situation is unlikely to arise in ships that have been at sea for more than a few days.

In this study, ballast water samples from tanks that had been subject to complete (>99%) ballast water exchange were significantly different to mid-ocean shipside samples. Both sample types were in contact with the ship's internal structure (engine-cooling pipes and/or fire hoses), thus the differences are likely to arise from either of two causes: First, ballast water exchange is never 100 percent efficient at removing all coastal tracers, since several tons of ballast are commonly trapped in "empty" tanks (Locke et al., 1993; Taylor and Bruce, 2000). Second, entrainment and holding in ballast tanks may affect dissolved tracer concentrations. For example, depending on a tank's configuration and ballasting history, residual sediments may contribute significantly to the characteristics of overlying water.

A plot of CDOM fluorescence intensities  $A_{qse}$  vs peak  $C_3$  at the beginning and end of the VFos cruise provided evidence of a tank holding effect. In this case,  $A_{qse}$  increased over time with the effect that differences between the control and treatment tanks were even greater at the end of the voyage. The tracers Mn and P exhibited complex dynamics in control tanks on the same cruise. Both elements have an organic and particulate source that may demineralize in the tanks and contribute to the dissolved phase. As a result, they are likely to be affected by differing levels of sediment and organic matter in individual ballast tanks. The gradual increase in phosphorus levels over time during the Atlantic voyage was suggestive of P efflux from mildly reducing sediments on the tank bottom, as has been observed for continental slope/shelf sediments (Schenau and De Lange, 2001).

Since every ship is a source of numerous potential contaminants, including metals, oils and chemical coatings, a significant challenge exists in defining the tracer set which contributes the maximum amount of information and minimum amount of noise to a discriminant analysis. In our preliminary analyses, it is revealing that sets consisting of 2–3 tracers in many cases outperformed larger sets. This suggests that the increased signal provided by additional tracers was often overshadowed by the accompanying increase in noise. We suspect that sediments and coal dust entrained on the Atlantic voyage contributed to unpredictable fluctuations of some elements (particularly Mn and P), which reduced their discriminatory power. Similarly,

while  $A_{qse}$  was a powerful tracer in our analyses, we caution that its magnitude is likely to be affected by the presence of fluorescent contaminants, such as polycyclic aromatic hydrocarbons.

Although not a component of this study, there is no doubt that if BWE verification is to be performed routinely in a regulatory framework, practical issues such as sampling time and analysis cost are likely to play an important role in tracer selection. We envisage that a verification testing program could involve multiple tests conducted sequentially according to a hierarchical decision system, in which progressively more costly analyses are performed only if the results of cheaper and/or in situ options are inconclusive.

We have focused on chemical discriminants of ballast water exchange, in most cases using techniques never before applied to this purpose. Although it may also be possible to verify the source of ballast water using biological tracers (e.g. plankton, bacteria), there are several complications to this approach. In particular, the species composition and abundance of ballasted communities are highly variable in space and time, presenting two types of problems. First, the use of a few standard taxonomic indicators would be problematic, necessitating considerable taxonomic expertise for biological verification of BWE on a global scale. Second, and critically, the abundance of coastal indicator organisms from a single port can vary many orders of magnitude over time, creating significant noise in the starting conditions and consequently in expected concentrations following BWE.

Our analyses provide a proof of concept for an approach to verification of ballast water exchange at sea. Using multivariate analysis methods, we were able to identify chemical tracers that performed well as discriminators of BWE across ships, tanks and oceans. Prior to implementation of a ballast water verification program based on these findings, it is necessary to conduct a full examination of the generality of these results under variable starting conditions (including a range of port signatures and vessel types), testing their utility across a range of practical situations. A component of this task would involve expansion of the geographic scope of our ocean reference datasets in order to properly characterize the regions in which ballast exchange may occur. We expect that some specific conclusions of this research, in particular the composition of "best" tracer sets, could change as data are collected encompassing larger temporal and spatial scales. Further important challenges for future research will include the development of simple and reliable sample collection procedures and ways of expediting sample turn-around, so that regulatory authorities can respond quickly to the invasive species threat posed by ships found to be transporting unexchanged ballast water.

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