Developing thresholds for tracing ships' ballast water: An Australian case study

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ABSTRACT

To limit the spread of nonindigenous marine species, ships can be legally required to conduct mid-ocean ballast water exchange (BWE) prior to discharging ballast water. It has been proposed to verify BWE by measuring concentrations of coastal tracers in ballast tanks, which should track their removal. Using three Australian ports as case studies (Port Botany, Port Curtis, and Port Phillip Bay), each representative of a different BWE verification difficulty level, the spatial and temporal variability of chromophoric dissolved organic matter (CDOM) and three trace elements (manganese, barium, and phosphorus (Mn, Ba and P, respectively), were measured to assess their utility as tracers of coastal (unexchanged) ballast water. CDOM fluorescence at $\lambda_{ex}/\lambda_{ex}$ = 320/414 nm (C2*) and 370/494 nm (C3*) and Mn concentrations were significantly higher in ports than in the adjacent Tasman Sea, except near port entrances and at a few sites in Port Botany. Barium concentrations demonstrated least power to discriminate coastal sources, but P easily discriminated water from mesotrophic Port Phillip Bay. In general, tracers showed greater variation between and within ports, rather than between seasons. Conservative BWE thresholds were calculated to be 1.6 quinine sulfate equivalents for C2*, 0.9 quinine sulfate equivalents for C3*, 1.4 μgL^{-1} for Mn and 6.9 μgL^{-1} for Ba. Overall, these thresholds would allow water sourced from eastern Australian ports to be identified as coastal at 92%, 69% and 74% of sites examined using C3*, Mn and Ba, respectively, requiring 71 ± 26%, 54 ± 40% and 59 ± 38% replacement with mid-ocean water to be within ocean baseline concentration ranges.

INTRODUCTION

Propagule supply is a key process in the dynamics of marine assemblages, providing the pool of potential new recruits (Underwood and Keough 2001). Density-dependent processes such as reproduction also have profound impacts on population establishment through the Allee-effect, where fitness is reduced at low con-specific densities (Taylor & Hastings 2005). Indeed, the number of arrivals to new habitats has been consistently correlated with non-indigenous species establishment (Hayes & Barry 2008), indicating 'propagule pressure' (Williamson 1996) is a fundamental driver of anthropogenically-mediated biological invasions (Verling et al. 2005).

Not surprisingly, decreasing propagule pressure to aquatic environments underpins local, national and international policies to manage risk of invasive species (IMO 2004, MAF 2005, EPA Victoria 2004). For marine systems, ships' ballast water is unarguably the largest vector for transport of planktonic lifestages and microorganisms (Drake et al. 2007), with billions of tonnes of water being delivered to ports annually. The International Maritime Organisation's (IMO) International Convention for the Control and Management of Ships Ballast Water and Sediments D-2 regulation (IMO 2004) aims to reduce the concentration of organisms in ballast water by several orders of magnitude. While significant research and development is underway to achieve this through treatment technologies, ballast water exchange (BWE) at sea is currently the major practice by which ships reduce the delivery of propagules to foreign and domestic ports, and current regulations require that the prescribed management measure—typically 95% replacement of coastal with oceanic seawater (2004, IMO 2004, CSLC 2006)—has been undertaken. Despite the phasing out of BWE by 2016, it will be the means of ballast treatment for at least another seven years, and is likely to be used as a

46 backup by ships that can not implement their adopted technology for one reason or another,
47 such as process failure.

48 Studies in the North Atlantic and Pacific Oceans have identified several chemical tracers that
49 can track the performance of ballast water exchange in removing port water, including
50 salinity, chromophoric dissolved organic matter (CDOM), manganese, barium and
51 phosphorus (Mn, Ba and P, respectively) (Hunt et al. 2007, Murphy et al. 2004, 2006, 2008a).
52 Concentrations of all tracers other than salinity are typically elevated in ports relative to the
53 mid-ocean where BWE takes place, due to their closer proximity to terrestrial sources and
54 human activities. This is particularly the case when ports are positioned at river mouths,
55 where large quantities of terrestrial materials are exported to the ocean (e.g., Macquarie
56 Harbour, Tasmania, Australia; Carpenter et al. 1991).

57 Little is known about the spatial and temporal variability of potential ballast water tracers in
58 southern Pacific waters (cf Hunt et al., 2007; Murphy et al. in press). Australian ports are
59 expected to be amongst be the most challenging ports globally for BWE verification due to a
60 combination of lowest percentage of rainfall as runoff (average of 12% compared to 33% for
61 North America), highest spatial and temporal variability in rainfall and runoff, low relief and
62 nutrient poor soils (Arthington & Pusey 2003, Davis and Koop 2006), resulting in potentially
63 low and variable terrestrial signals. Of particular concern is the number of Australian ports
64 containing large populations of invasive species (CRIMP 1996, Aquenal 2001, Hewitt et al.
65 2004). These populations are at risk of secondary dispersal via ballast water, both
66 domestically, and to foreign ports. For BWE verification to be used as a management tool, it
67 is therefore critical to evaluate the effectiveness of proposed tracers for Australian ports.

A difficult and potentially controversial aspect of verifying BWE using tracer methods is deciding upon appropriate thresholds for discriminating between exchanged and unexchanged ballast water. Tentative BWE thresholds have been proposed for CDOM (Murphy et al. 2006) and for Ba and Mn (Murphy et al. 2008a), based upon tracers measured in the ballast tanks of ships operating in the North Pacific and Atlantic oceans. A limitation of that approach was the efficiency of ballast water exchange (and therefore, the proportion of port water retained in "exchanged" ballast tanks) was not accurately known for most ships, and this uncertainty was propagated into the calculation of BWE thresholds. This limitation may be overcome by calculating the mixing between endpoints with known chemical quantities: i.e. the original port water, and the ambient ocean where ballast water is exchanged. Assuming replacement of port with oceanic seawater during BWE, tracer concentrations in ballast tanks should diminish linearly with increasing exchange efficiency (**Figure 1**). When no coastal ballast water remains, tracer concentrations in ballast tanks should be at oceanic levels. Current regulations specify a minimum of 95% volumetric replacement of port water during BWE, thus the BWE threshold can be calculated as the concentration of tracer which would be achieved if a ballast tank were to contain 95% port water and 5% oceanic seawater.

Like elsewhere, Australian ports differ in their configuration (e.g. port volume and flushing rate), freshwater (and other) inflows as well as seasonal runoff patterns, providing a range of potential source waters for ships' ballast (**Table 1**). As a first order estimate, open ports with oceanic salinities (>30 psu) are classed as "difficult" test cases, since low river inputs correlate with low concentrations of CDOM and other terrestrial markers (Boehme et al. in preparation, Blough & Del Vecchio 2002, Ferrari & Dowell 1998). It is apparent that the majority of Australian ports are likely to represent moderate to high-difficulty cases for BWE

verification (**Table 1**). Using three locations as test cases, we assessed the spatial and temporal variability of chemical tracers in ports representative of different estimated BWE verification difficulty levels: (1) Moderate (Port Phillip Bay); (2) Difficult (Port Curtis); and (3) Very Difficult (Port Botany). The objectives were to assess whether chemical tracer concentrations in these representative ports are consistently different to those in the adjacent open-ocean, to propose BWE thresholds appropriate for these ports, and to evaluate their effectiveness given within- and between-port variability and differing ballast water exchange efficiencies, on verifying BWE on ships carrying ballast from Australian ports.

MATERIALS AND METHODS

Port assessment

Given it would take significant resources to sample all 73 ports within Australia, they were first evaluated for their importance as ballasting locations. An earlier analysis by Hayes et al. (2005) using Lloyds Maritime Intelligence Unit data together with an environmental matching analysis, revealed the top 20 locations in Australia where pest incursions are most likely, based on the translocation of ships' ballast water and known port infestation status (**Table 1**; Hayes et al. 2005). While these locations are those importing (rather than exporting) the largest volumes of ballast water, this list has been used as a way of prioritizing the existing ports in Australia to evaluate how easy it would be to verify them as coastal ballast sources. From this subset, ports were then assessed for the presence of nearby freshwater inputs (including whether they were located within an estuary, the estuary width and area), their openness to the ocean (i.e., distance from shipping berths to the ocean) and regional climate (e.g. average annual precipitation, rainfall seasonality). These parameters were combined to assess the overall ease of using salinity and other tracers as a BWE verification measure and ports were ranked accordingly (with rank 1 being easiest to verify).

Where possible, the ranking was cross-checked with *in situ* salinity data from published and other credible sources. Although Port Phillip Bay and Port Curtis were ranked 6th and 7th, they were given a different BWE verification status (Moderate and Difficult, respectively) because of Port Phillip Bay's relatively long residence time (Harris et al. 1996) which would allow terrestrial markers to accumulate. Port Botany with its close proximity to the coast and relative openness to the ocean was deemed Very Difficult.

Port Surveys

Port Botany, Port Curtis and Port Phillip Bay (approx. latitude 34 ºS, 23 ºS and 38 ºS, respectively; **Figure 2**), representing Very Difficult, Difficult and Moderate BWE verification difficulty, respectively, were sampled for their spatial and temporal variability of tracers. Seasonal variability was assessed for Port Curtis and Port Phillip Bay, as these ports have distinct precipitation patterns (summer monsoon vs winter-dominated rainfall, respectively). All surveys were deliberately conducted during periods of low rainfall so that port water would be most challenging to identify as coastal. Sampling of Port Botany was conducted in autumn on April 18 – 19, 2007 (Port Botany fall). Water samples were collected at 24 sites throughout Botany Bay (Figure 2). The first field survey of Port Phillip Bay was conducted in autumn on April 24 – 26, 2007 (Port Phillip fall). Samples were collected at 24 sites encompassing the Port of Geelong (6 sites), the Port of Melbourne (9 sites) and the wider Bay region (9 sites; **Figure 3**). End-member samples were also collected from the Yarra River at Studley Park boathouse (above Dights Falls, the uppermost extent of the tidal excursion), which is the major source of freshwater to the Port of Melbourne (Harris et al., 1996). The second survey of Port Phillip Bay (Port Phillip spring) took place in spring on October 25 – 26, 2007. Sampling locations during the second survey were as close as possible to the sites visited in the first survey. Two surveys were conducted in Port Curtis

near Gladstone. Approximately half the sites were chosen to coincide with sites previously surveyed by the former Center for Research on Introduced Marine Pests (CRIMP) and the University of Central Queensland (UCQ) during the baseline surveys of introduced marine species in Australian ports (Lewis et al., 2001). The remainder were selected principally to provide coverage of the Calliope river and point sources to the port. The first survey was conducted in winter on August 21 – 22, 2007 (Port Curtis winter; **Figure 4**). A second survey (Port Curtis fall) was conducted in autumn on April 2 – 4, 2008 (**Figure 4**).

During each port survey, water was collected for analysis of CDOM and trace metals (Mo, Ba, Mn, P) at each site, encompassing a marine end-member collected near the port entrance, sites near features such as shipping terminals, active berths and shipping channels, and an upstream site to represent a freshwater source.

Sample collection

Water samples for laboratory analysis of trace elements and CDOM (n = 3 each) were collected at 5 m depth (or 1 m off the bottom at shallow sites) using a peristaltic pump (Cole Parmer 7533-60 12vDC) fitted with rigid Teflon tubing and flexible Masterflex tubing and connected to a high-capacity inline 0.45µm capsule filter (GE Memtrex MP). Five meters was the chosen sampling depth since this is a typical location of ships ballast water intake vents. The apparatus was flushed with at least 3 L of water prior to sampling, and filters were changed 3-5 times during each survey depending on turbidity levels in the port. All tubing and filters were acid-washed before use. Additional air samples to assess trace element baselines (n = 3) were also collected at locations where aerosol input was thought to be significant, such as the Sydney Airport runway approach in Botany Bay (**Figure 2**). Filtered water was collected in acid-cleaned 50 ml plastic centrifuge tubes for trace element analyses,

or ashed amber glass bottles for CDOM analyses. Following collection, samples were stored on the boat in a cooler, then refrigerated and ultimately frozen at -20 ºC before being shipped to the US for analysis.

Physico-chemical characteristics

Physico-chemical profiles were measured *in situ* every 1 to 5 m depth (deep sites) or to 0.5 m off the bottom at shallow sites. Salinity, conductivity, temperature, and dissolved oxygen were measured using one or both of a YSI-85 and YSI 650MDS datasonde equipped with a 65 probe. Instruments were calibrated with a NIST-traceable seawater conductivity standard (YSI 3169, 50 000 μScm^{-1}) or with a freshly-made solution of 0.5 M KCl (conductivity = 58.640 mS cm^{-1}) prior to each cruise. During the Port Botany and winter Port Curtis surveys, the precision of field measurements of salinity was determined to be approximately ± 2 psu (6%) at 35 psu, which was lower than desired. Consequently in later surveys, both instruments were recalibrated just before use and salinities measured in the field were cross-checked against laboratory measurements conducted on samples sent to the Smithsonian Environmental Research Laboratory (SERC). The precision of salinity measurements in all other surveys was determined to be approximately ± 0.5 psu (1.5%) at 35 psu.

Sample Analysis

Samples were shipped by international express post to the US for analysis to be consistent with previous studies (Murphy et al. 2004; Murphy et al. 2009). Trace element samples were analyzed at Rutgers Inorganic Analytical Laboratory, Institute of Marine and Coastal Sciences, Rutgers the State University of New Jersey. Samples were analyzed on a sector field inductively coupled plasma mass spectrometer (ICP-MS) with high-resolution capability (ELEMENT, Finnigan MAT, Bremen, Germany). Total dissolved concentrations of four trace elements (barium [Ba], manganese [Mn] and molybdenum [Mo] and phosphorus [P])

were determined according to published methods (Field et al., 2007). All three trace element replicates were analyzed from each site, except during the second survey of Port Curtis, for which two replicates per site were analyzed.

CDOM analysis was performed by excitation-emission matrix spectroscopy (EEMS) at the Smithsonian Environmental Research Centre (SERC), USA, using a JY Horiba Spex Fluorolog-3 (Edison, New Jersey, USA). Samples were analyzed in a 1-cm quartz cell maintained at 20 °C with a temperature-controlled cell holder and processed in ratio mode with a 0.5 second integration time and a 5 nm bandwidth for both excitation and emission. The experimental wavelength range was 220-455 nm in 5-nm intervals on excitation and 290-700 nanometers (nm) in 4-nm intervals on emission. Fluorescence excitation-emission matrices (EEMs) were corrected for instrument and lamp variability and normalized to parts per billion quinine sulfate equivalent units (QSE) as previously described (Coble et al 1993, method 1) using an in-house program written for Matlab (Ver. 7). Following Murphy et al. (2006), the excitation/emission ratios of C2* = 320/414 and C3* = 320/494 are presented. These wavelengths are used as indicators for two mathematically independent humic-like fluorescence components present in ship's ballast (components C2 and C3 in Murphy et al. 2006), for which BWE thresholds have previously been developed. Component C2 is believed to represent microbially derived organic matter, whereas C3 is believed to represent degraded plant matter (Murphy et al., 2008). Although six other natural fluorescent components have been identified in ballast water, they have not proved as reliable for tracking ballast water sources (Murphy et al. 2006).

One source of measurement error for CDOM is the inner filter effect, where reabsorption of emitted and/or incident radiation by concentrated samples causes a reduction in measured light per molecule in solution. In another study in this region (Murphy et al. in press), inner

212 filter effects were found to be insignificant (5%) for C2* below approximately 17 ppb QSE.
213 Samples from this study were relatively dilute with C2* intensities below 15.5 QSE at all but
214 the freshwater end-member sites distant from the ports. Thus inner filter effects may have
215 resulted in underestimation of intensities at these end-member sites, but would not have had a
216 significant effect upon determined concentrations at sites within the ports.

217 Across the entire study, 271 CDOM samples and 243 trace element samples were analyzed.
218 Analysis occurred within 6 weeks of sample collection for CDOM and within 8 months of
219 sample collection for trace elements. Previous studies in both laboratories have found no
220 significant impacts on data quality due to storing samples for this length of time.

221 **Data quality control and statistical analysis**

222 In general, the coefficients of variation (SD/mean) for replicate samples collected from each
223 site were small (2 – 8%), indicating that measurements were precise despite limited
224 replication. A small proportion of samples (2.9%) were identified as severe outliers relative
225 to two other replicates and were omitted from the dataset. An additional 2.6% of samples
226 produced inconsistent data (i.e. showed higher or lower levels relative to their replicates) for
227 2 or fewer elements, usually Mn and P. For these samples, data for the inconsistent elements
228 were removed but data for the remaining elements were retained.

229 Analysis of Variance (ANOVA) was used to test differences in tracer distribution between
230 ports and between seasons. To test spatial differences, data from surveys conducted in
231 autumn only (Table 2) were compared, to limit confounding due to temporal variability. To
232 test temporal differences, only surveys from Port Phillip Bay and Port Curtis were used, as
233 these locations were sampled in two different seasons (Table 2). In all cases, data was
234 (natural) log transformed to meet the criterion for homogeneity of variances before ANOVA

was performed (SPPS version 15.0). However, differences in variability of tracers (even when river samples were excluded from the analysis), meant that ANOVA couldn't be used to evaluate port differences for C3* or Ba, or to evaluate differences between seasons for Ba. Instead for these comparisons, a non-parametric Kruskal-Wallis test was used.

Assessment of tracers for development of BWE thresholds

Tracer concentrations in ports were compared with those in the adjacent Tasman Sea to see what resolution they offered for discriminating coastal water and how consistent this was spatially and temporally. Considering all the port data (but excluding freshwater endmembers) a theoretical mixing line was calculated between the maximum observed port tracer concentration and the Tasman Sea background (Figure 1). The tracer concentration at 5% port water and 95% ocean water was then proposed as a conservative BWE threshold. The appropriateness of this threshold was then assessed for different sites within ports, with results expressed as the BWE efficiency at which the ballast tracer concentration would be within two standard deviations of the mean Tasman Sea concentration. This provided a measure of the probability of a false negative determination — i.e. the failure to detect a non complying ballast tank. The probability of a false positive determination —i.e. incorrectly assessing a tank as being non-compliant, was also determined by calculating the port tracer concentration that would be indistinguishable from the ocean baseline (i.e. less than 2 standard deviations above the mean) if only 5% of port water were retained in a ballast tank.

RESULTS

Physico-chemical parameters and conservative tracers

Vertical profiles of physico-chemical parameters confirmed the water column at most sites was relatively mixed in the upper 5 m and that our samples were representative of the surface

layer. Salinity within all ports was ≥ 32 psu, with sites closest to port/bay entrances generally having highest salinities and those closest to freshwater inputs having lowest (Figure 3). Salinity at Port Botany showed the largest range (between 32 and 38.5 psu), but in other surveys, the total salinity range was small - only about 3 psu, excluding freshwater endmembers. Despite difficulties with the salinity probes during the Port Botany and first Port Curtis surveys (which consistently over-estimated actual salinities by up to 2 psu), the results show that background salinities in Port Curtis varied seasonally, with lower salinities in autumn compared to winter, reflecting the region's summer-dominated rainfall ($F = 56.6$, $p < 0.001$). Salinity was also different seasonally in Port Phillip Bay, but with higher salinities in autumn compared to spring ($F = 2.542$, $p = 0.05$, respectively).

The conservative trace element molybdenum (Palmer & Edmond 1993, Sohrin et al 1998), was less variable than salinity (data not shown), with low concentrations for both tracers coinciding in freshwater. This trace element only showed significant seasonal differences between surveys in Port Phillip Bay with average (± SD) values in April 2007 being ~15% higher than in October 2007 (11.9 ± 0.4 μg L^{-1} compared to 9.9 ± 0.8 μg L^{-1}; $F = 9.256$, $p = 0.004$). Overall, molybdenum and salinity were weakly correlated ($n = 100$; $R^2 = 0.652$) and because Mo showed no greater resolution than salinity, it is excluded from further analysis.

CDOM

CDOM C2* (320 nm / 414 nm) ranged from 0.63 to 52.31 QSE and was highly correlated with C3* (370 nm / 494 nm) across the entire range of measurements ($n = 99$, $r^2 = 0.99$, $p = <0.001$). Due to near-perfect correlation between C2* and C3* and that C3* appears to be derived from exclusively terrestrial material (Murphy et al. 2008b), only data for C3* are presented. However, C2* intensities can be estimated by multiplying C3* by 1.68 and adding

0.55, except for sites with low C2* concentrations such as Port Botany and at the entrance to Port Phillip Bay where C2* is better estimated by 1.68*(C3*).

CDOM showed larger gradients between sites compared to salinity, with C3* concentrations ranging between 0.38 and 8.38 QSE, excluding freshwater endmembers (Error! Reference source not found.A). As expected, CDOM concentrations were lowest at sites close to port entrances and increased at sites adjacent to freshwater inputs, but were similar across Port Botany and Port Phillip Bay as well as one Port Curtis survey (Table 2). Interestingly, CDOM concentrations showed little seasonality in Port Phillip Bay whereas in Port Curtis, there were lower concentrations in winter compared to fall (significant port*season interaction; $n = 73$, $F = 8.77$, $p < 0.01$).

Trace elements

The air blanks showed very low concentrations of all trace elements (0.12 µg L^{-1} Mo, 0.03 µg L^{-1} Ba, 0.003 µg L^{-1} Mn, 0.08 µg L^{-1} P) indicating minimal baseline concentrations.

*Manganese***.** Average manganese concentrations were similar in Port Botany and Port Curtis (~1.8 µg L^{-1}) but were at least 50% higher in Port Phillip Bay ($n = 66$, $F = 19.27$, $p < 0.001$). Like CDOM, concentrations were lowest at port entrances and highest close to freshwater inputs, with a significant outlier at one upstream site in the Calliope River, where the Mn concentration was 21.4 µ gL^{-1} at a salinity of 33 psu. Of the total Mn samples, 79% exceeded the upper 95th percentile (mean + 2 standard deviations) of Mn concentrations in the Tasman Sea. Interestingly, Mn concentrations showed significant seasonality in the two Port Phillip Bay surveys, but not in Port Curtis (Table 2). However when the Yarra River freshwater endmembers and Calliope River outlier were excluded from the ANOVA, there was no difference in Mn between ports or seasons and no significant interaction term.

Barium. Barium concentrations averaged between 6.8 and 9.8 µg L^{-1} (Table 2) but were as high as 15.9 µg L^{-1} upstream from Port Botany in the Georges River estuary, and up to 53.9 µg L^{-1} at the upstream site in the Calliope River draining into Port Curtis (Figure 5C). Unequal variances between ports precluded the use of ANOVA to assess spatial and seasonal variation, but non-parametric tests revealed significant between port differences in Ba concentration. Overall, there was evidence of some seasonality in Ba at Port Curtis with concentrations remaining fairly stable in Port Phillip Bay (Table 2). Similar to Mn, 80% of samples had higher barium concentrations than the upper 95th percentile of Mn concentrations in the Tasman Sea (Figure 5C).

Phosphorus. Concentrations of the element phosphorus were significantly different between ports ($n = 65$, $F = 460$, $p < 0.001$), with P in Port Phillip Bay approximately an order of

magnitude higher P than in Port Curtis (Table 2), even at sites close to the port entrance. Port Botany had intermediate P concentrations (Figure 3). ANOVA indicated no difference between seasons in both Port Phillip Bay and Port Curtis. Phosphorus was within the upper 95th percentile of the ocean background concentration at Port Curtis and at a few sites close to the entrance of Port Botany as well as freshwater endmembers connected to Port Phillip Bay. Otherwise, P concentrations in port were well above Tasman Sea concentrations.

Overall, coefficients of variation (CV = SD/mean) for replicate samples from each port survey ranged from 1.9 to 13.6%. The CV indicates precision of the sampling + analytical process, so is increased by natural variability in tracer concentrations at sites, sample contamination, analytical error or any combination of these factors. Control samples (blanks) to assess sample contamination all had very low trace concentrations, and our QAQC process confirmed consistency between replicates, so the likely source of variation contributing to high CVs was actual variability in tracer concentrations between sites. Considering the entire dataset, coefficients of variation were greatest for CDOM and P during the Port Botany fall and Port Phillip fall surveys (approximately 8% and 12%, respectively), probably due to outward sediment fluxes of dissolved organic matter and phosphorus during sampling at shallow sites.

Assessment of tracers for development of BWE thresholds

In almost all cases, coastal tracers in ports exceeded the upper 95th percentile of adjacent Tasman Sea concentrations (). CDOM had greatest discriminatory power, with 99% of sites sampled having higher C3* concentrations than the Tasman Sea, compared to manganese (79%), barium (80%) and phosphorus (58%). Given this relatively high degree of separation between port and ocean tracer concentrations for C3*, Mn, and Ba, BWE thresholds were developed and are presented separately for each tracer (Figure 6). In developing thresholds,

there were two considerations acting in opposite directions to elevate or reduce thresholds, respectively: minimizing the potential for false positive determinations and being able to detect water derived from low-tracer ports.

A conservative ballast water threshold for C3* was therefore determined using the maximum concentration of C3* measured within ports (8.38 QSE at a riverine site in Port Botany), and the upper 95^{th} percentile value of 0.51 QSE in the Tasman Sea (Table 2; Murphy et al. in press). The C3* threshold of 0.90 QSE was calculated as 0.05*8.38 + 0.95*0.51 (i.e. 95% replacement of port water with ocean water) and its utility for diagnosing BWE of ballast water from the three ports is depicted in Figure 4. Ranges of C3* concentrations measured within ports and directly at shipping berths are shown to diminish when mixed with Tasman Sea water, and the BWE efficiency needed to reduce port concentrations in ballast tanks to below threshold (i.e., compliant) levels is summarized in **Table 3**. For ballast water from berths in Port Curtis, BWE efficiencies of approximately 78% (winter low) to 90 % (fall high) would be required. Similarly, for ballast water from berths in Port Phillip Bay, maximum BWE efficiencies of approximately 81% (spring) and 78% (fall) would be required. While the conservative threshold holds reasonably well for ballast water originating at berths in Port Phillip Bay and Port Curtis, it is less successful at determining BWE status of ballast water loaded in Port Botany. At the least coloured of the four berths sampled, C3* was 0.52 QSE and hence already below the threshold, while ballast from the most highly coloured berth would require only 65% replacement with oceanic seawater (**Table 3**). The threshold would also be less successful in determining the BWE status of ballast sourced from a number of non-berth sites in each of the ports, particularly those located close to the port entrance (PC_{min}, PB_{min} and PP_{min}), with these sites at worst indistinguishable from the

362 open ocean (PC_{min}) or requiring significantly less than 95% exchange to bring them into
363 compliance (49% for PP_{min} and 38% for PC_{min}).

364 A manganese threshold of 1.4 µg L^{-1} was determined using the maximum concentration of
365 Mn measured within ports (8.25 µg L^{-1} in Port Phillip Bay), and the upper 95th percentile
366 value of 1.08 µg L^{-1} in the Tasman Sea (**Table 2**; Murphy et al. in press). Its utility for
367 diagnosing BWE of ballast water from the three ports is shown in. This threshold was
368 moderately successful in determining the BWE status of ballast sourced from sites within
369 Port Phillip Bay (but not the entrance; PP_{min}), with water from berths requiring 72 – 92%
370 BWE in spring and 77 – 94% in fall (Table 3). In contrast, water from Port Botany and Port
371 Curtis, including sites at berths, in shipping channels and the wider port area required far less
372 exchange to bring Mn concentrations below the threshold. Generally low manganese
373 concentrations in Port Botany meant that water from berths required no exchange to be
374 compliant. In Port Curtis, water from berths required 0 – 16% BWE in winter and 0 – 86 %
375 BWE in fall, but across the entire location, average BWE requirements were ~ 32% in both
376 seasons.

377 The barium threshold of 6.9 µg L^{-1} was calculated using the maximum concentration of Ba
378 measured within ports (15.9 µg L^{-1} in the Georges River estuary, Port Botany), and the
379 upper 95th percentile value of 6.41 µg L^{-1} in the Tasman Sea (Table 2; Murphy et al. in
380 press). Similar to manganese, barium showed smaller differentials between port and ocean
381 concentrations compared to CDOM, leading to 17 of 24 sites (71%) in Port Botany and 6 of 9
382 sites (67%) in the Port Curtis winter survey having Ba concentrations below the calculated
383 threshold. As a result, water sourced from these ports would be challenging to track using
384 barium. The threshold had greatest utility in Port Phillip Bay, where water from berths would
385 require 81 - 88% (fall) or 57 - 88% (spring) BWE to bring ballast into compliance. In

386 contrast, there was strong seasonality in BWE compliance for ballast water sourced from Port
387 Curtis, requiring BWE efficiencies of 73 - 91 % in fall, but only 0 =- 37% in winter (**Table**
388 **3**).

389 **DISCUSSION**

390 While there has been steady progress in identifying and quantifying the distribution of
391 chemical tracers suitable for verifying ballast water exchange (see Murphy et al. 2004, 2006,
392 2008a, in press), until now a consistent and intuitive framework for developing tracer
393 thresholds to assess BWE compliance has been lacking. This study proposes and tests such a
394 framework, using data from three ports in Australia representative of a range of conditions
395 that could be encountered in unexchanged ballast tanks. Australian ports typically have very
396 high salinities (Table 1), which normally correlate with low CDOM and relatively low
397 dissolved trace element concentrations (Blough & Del Vecchio 2002, Ferrari & Dowell 1998,
398 Hatje et al. 2003) that may be relatively difficult to distinguish from high salinity, low-tracer
399 oceanic samples. For this reason, Australian ports represent especially challenging and
400 informative test cases for developing and testing BWE verification thresholds.

401 **Ballast water tracer thresholds**

402 In developing BWE thresholds, the probability of two possible erroneous determinations
403 needs to be considered: the false positive (i.e. the initial tracer concentration in ballast is too
404 high to achieve a final concentration below the threshold even when diluted with 95% ocean
405 water) and the false negative (i.e. the tracer concentration in ballast is below the threshold
406 even when diluted with less than 95% of oceanic seawater). As **Figure 1** depicts, there is a
407 trade-off between setting thresholds high enough to minimize the potential for false positive
408 determinations, and retaining enough sensitivity to detect unexchanged ballast water derived

409 from low-tracer ports. In this study, to minimize the rate of false positive determinations (i.e.
410 reduce the likelihood of imposing a penalty when 95% BWE was carried out), the threshold
411 was calculated using the maximum tracer concentrations observed both in ports and in the
412 adjacent open ocean. This came at the cost of detection sensitivity, particularly for water
413 sourced from Port Botany, which was located in a relatively open embayment.

414 The threshold proposed for CDOM (C3*) was considerably more robust than those for the
415 trace elements manganese and barium. Larger differentials for C3* between port
416 concentrations and the Tasman Sea (with ≥74% sites across all ports requiring exchange to be
417 compliant) resulted in relatively high BWE requirements which were more consistent across
418 seasons, particularly in Port Phillip Bay (Table 3). In general, water sourced from port
419 entrances required the least exchange (0 - 79%), followed by water from the shipping channel
420 (0 – 89%), at berths (0 – 94%), the wider port area (18 – 93%) and riverine sites (73 – 95%).
421 Water sourced from Port Curtis in fall had the highest BWE requirement (86 ± 13%),
422 whereas water from Port Botany had the lowest (52 ± 36%; Table 3).

423 In comparison, thresholds for manganese and barium offered less utility for discriminating
424 between coastal and oceanic seawater samples. Trace element concentrations in Port Botany
425 and Port Curtis were both closer to ocean concentrations (yielding lower resolution to track
426 coastal water) and more seasonally variable, leading to greater seasonality in the degree of
427 BWE required to reach oceanic tracer levels. Water sourced from Port Curtis required 72 ±
428 28% exchange in fall but only 28 ± 35% in winter. No threshold could be established for P
429 concentrations that would apply for all ports, however P in samples from Port Phillip Bay
430 always exceeded 20 μgL^{-1} and hence significantly exceeded background concentrations in the
431 Tasman Sea of 12.1 μgL^{-1} (Table 2; Murphy et al. in press).

Table 4 shows how the thresholds calculated for these eastern Australian ports compare to those previously identified to discriminate ballast water exchanged in the northern Pacific and Atlantic Oceans and the Tasman Sea. Barium thresholds are most consistent across regions (6.9 - 7.0 μgL^{-1}), followed by C3* (0.7 – 1.0 QSE), whereas Mn thresholds varied two-fold (1.0 - 2.4 μgL^{-1}). For C3* and Ba, the thresholds developed in this study are comparable to, or marginally lower than, those identified by Murphy et al. (in press) to separate exchanged and unexchanged ballast water sourced from south-eastern Australia. This is consistent with the observation that tracer concentrations in ballast tanks are often slightly elevated in ballast tanks relative to the ocean where ballasting occurred (Murphy et al. 2008a), a result that may be attributable to a combination of factors including the prevalence of relatively low ballast water exchange efficiencies among the existing shipping fleet.

The potential for identifying coastal ballast water from eastern Australian ports depends on the relevant threshold concentration. Using the CDOM C3* Pacific/Atlantic threshold (0.7 QSE), 96% of port sites would be identified as coastal compared to 92% of sites using this study's threshold (0.9 QSE) and 91% of sites using the Tasman Sea threshold (1.0 QSE). For Mn, the proportion of sites verifiable as coastal ballast increases from 49% to 56% to 81% as the threshold level decreases from 2.6 to 2.0 to 1.0 μgL^{-1} using the Tasman Sea, Atlantic, and Pacific thresholds respectively. Decreasing the threshold therefore increases the sensitivity of detecting coastal water but also increases the probability that ballast from some ports would need more than 95% replacement with oceanic seawater in order to become compliant. In such situations however, ports with high tracer concentrations are likely to also have lower salinity (Figure 3; Blough & Del Vecchio 2002, Ferrari & Dowell 1998), such that salinity itself could be used to verify coastal ballast origin.

Utility of tracers in BWE verification

Published data on Ba and Mn concentrations in ports from the Southern Hemisphere are limited, and existing data for CDOM are almost exclusively measured by absorbance rather than fluorescence emission (Clementson et al. 2004), while P is typically measured as filterable reactive P not total dissolved P. This study of coastal tracers in SE Australian ports therefore adds to the global database, and provides regulators with an evidence-based approach for BWE verification.

Spatial and seasonal variability in ports is a practical complication to the use of chemical tracers for verifying BWE. Ports experiencing greater exchange with coastal waters and lower freshwater inflows would be expected to have lower tracer concentrations, making them potentially more difficult to verify as ballast water sources. Significant variability in tracer concentrations would arise from episodic or seasonal freshwater inflows and long water residence times that allow tracers to accumulate. Port Botany, due to a combination of the port size as well as relatively strong signals emanating from the nearby Georges River estuary, showed steep gradients in CDOM concentrations, whereas tracer signals throughout Port Phillip Bay were relatively stable (Figure 3). The long water residence time in the Bay (~ one year; Harris et al. 1996) and relatively restricted harbor entrance is likely an important determinant of tracer variability, and is in marked contrast to more rapid flushing rates in the relatively open waters of Port Botany and Port Curtis. Overall, the data collected in eastern Australian ports showed overlapping tracer concentrations between ports and seasons (Figure 3), indicating good spatial and temporal stability for use in characterizing ballast sources.

CDOM stood out as the best performing tracer in this study because it demonstrated steepest concentration gradients from ports to the adjacent ocean. The trace elements Mn, Ba and P provided little, or inconsistent, additional resolution for identifying coastal ballast. Despite

this, we caution against the use of a single criterion to verify BWE. Instead, we recommend a tiered approach, where one would test ballast water for salinity < 30 psu, indicating an unambiguous coastal source; then test for CDOM that exceeds the range of ocean values derived from the geographic region of interest. Evidence of non-compliance resulting from tracer testing should then be interpreted in light of the ship's record of ballast water origin and management. Tests for the trace elements Mn, Ba or P would be reserved for situations in which further evidence of non-compliance is considered a high priority, for example, if the ballast water is suspected to have been sourced from a high risk ballast source such as Port Phillip Bay (Hewitt et al. 2004; i.e. test for P).

Future research

Although this study has contributed significantly to the global database, further data on CDOM variability in the world's ports and along oceanic shipping routes are needed for accurate prediction of the origin of water in ships' ballast tanks, and to define thresholds that strike the appropriate balance between sensitivity for detecting unexchanged ballast and robustness to natural variability in port and ocean concentrations. Advances in technology (e.g. optical instrumentation) and remote sensing are likely to increase the feasibility of generating large databases for this purpose. Similarly, technologies and decision support systems that increase the opportunity for real-time verification of BWE compliance are a high priority future research, including the development and testing of sturdy and affordable in situ instruments that can be lowered into ballast tanks. CDOM was the most sensitive tracer of water origin in this study, and along with P is the most amenable to in situ measurement.

Conclusions

Ballast water exchange by ships will continue to at least 2016 during the phasing in of treatment technology (IMO 2004), and assessments of whether ships have conducted BWE

503 are likely to be undertaken for at least the next 7 years. Surveys of CDOM and trace element
504 (Ba, P, Mn) concentrations in three saline ports in eastern Australia indicated these tracers
505 were in most cases significantly elevated above background ocean levels. CDOM was a
506 particularly sensitive tracer of Australian port waters, usually providing far greater sensitivity
507 for discriminating potential ballast water sources than salinity or the trace elements. This
508 supports previous research suggesting it would be possible to verify BWE by measuring
509 concentrations of naturally occurring chemical tracers in ballast tanks. This study has further
510 provided a framework for defining accurate concentration thresholds that delineate between
511 exchanged and unexchanged ballast water. Thresholds must be applicable over large
512 geographic regions and robust to spatial and temporal variability of tracer concentrations
513 within and between ports. Based upon the highest concentrations observed in Australian ports
514 and baseline levels offshore in the Tasman Sea, ballast tanks with tracer concentrations
515 exceeding 1.6 QSE at $\lambda_{ex}/\lambda_{em}$ = 320/414 nm (C2*) or 0.9 QSE for CDOM at $\lambda_{ex}/\lambda_{em}$ =
516 320/414 nm (C3*) would be assumed to have been derived wholly from coastal
517 environments, or else incompletely exchanged at sea. Similarly, tanks with Mn exceeding 1.4
518 µg L^{-1} and Ba exceeding 6.9 µg L^{-1} would be assessed as non-compliant. While these
519 thresholds are likely to discriminate most unexchanged ballast water from coastal ports,
520 partially-exchanged ballast tanks from high-salinity/low tracer ports, or from locations near
521 or external to port entrances, may be erroneously judged as compliant according to these
522 criteria.
523

REFERENCES

(2004) US Coast Guard. Mandatory Ballast Water Management Program for U.S. Waters: Final Rule, 33 CFR 151, Subpart D, p 44952-44961

Aquenal (2001) Exotic Marine Pests Survey, Port of Launceston, Tasmania, for Port of Launceston Pty Ltd, 104 pp.

Blough NV, Del Vecchio R (2002) Chromophoric DOM in the coastal environment. In: Hansell DA, Carlson CA (eds) Biogeochemistry of marine dissolved organic matter. Academic Press, p 509-546

Burford MA, Alonghi DM, McKinnon AD, Trott LA (2008) Primary production and nutrients in a tropical macrotidal estuary, Darwin Harbour, Australia. Est Coastal Shelf Sci 79(3):440-448

Carpenter PD, Butler ECV, Higgins HW, Mackey DJ, Nichols PD (1991) Chemistry of trace-elements, humic substances and sedimentary organic matter in Macquarie Harbor, Tasmania. Aust J Mar Freshw Res 42(6): 625-654

Clementson LA, Parslow JS, Turnbull AR, Bonham PI (2004) Properties of light absorption in a highly coloured estuarine system in south-east Australia which is prone to blooms of the toxic dinoflagellate Gymnodinium catenatum. Est Coastal Shelf Sci 60(1): 101-112

CRIMP (1996) Introduced species survey, Port of Devonport, Tasmania. CSIRO Division of Fisheries, 51 pp.

CSLC (2006) Ballast water regulations for vessels arriving at California ports or places after departing from ports or places within the Pacific Coast region. California State Lands Commission rule: Title 2, CCR Division 3, Chapter 1, Article 4.6 (effective March 22, 2006)

548 Davis JR, Koop K (2006) Eutrophication in Australian rivers, reservoirs and estuaries - a
549 southern hemisphere perspective on the science and its implications. Hydrobiologia
550 559: 23-76.
551 Drake LA, Doblin MA and Dobbs FC (2007) Potential microbial bioinvasions via ships'
552 ballast water, sediment, and biofilms. Mar Poll Bull 55: 333-341.
553 EPA Victoria (2004) Waste management policy (Ships' Ballast Water), Victoria government
554 gazette No.S 100, The Craftsman Press Pty Ltd
555 Ferrari GM, Dowell MD (1998) CDOM absorption characteristics with relation to
556 fluorescence and salinity in coastal areas of the southern Baltic Sea. Est Coast Shelf
557 Sci 47:91-105
558 Field MP, LaVigne M, Murphy KR, Ruiz GM and Sherrell RM (2007) Direct determination
559 of P, V, Mn, As, Mo, Ba and U in seawater by SF-ICP-MS. Journal of Analytical
560 Atomic Spectrometry, 22: 1145-1151
561 Harris G, Batley G, Fox D, Hall D, Jernakoff P, Molloy R, Murray A, Newell B, Parslow J,
562 Skyring G, Walker S (1996) Port Phillip Bay Environmental Study Final Report,
563 CSIRO, Canberra.
564 Hatje V, Apte SC, Hales LT, Birch GF (2003) Dissolved trace metal distributions in Port
565 Jackson estuary (Sydney Harbour), Australia. Marine Pollution Bulletin 46:719-730
566 Hayes KR, Barry SC (2008) Are there any consistent predictors of invasion success? Biol
567 Invasions 10: 483-506
568 Hayes KR, Sliwa C, Migus S, McEnnulty F and Dunstan P (2005), National priority pests -
569 Part II Ranking of Australian marine pests. Final report for the Australian
570 Government Department of Environment and Heritage, CSIRO Division of Marine
571 Research, Hobart, Australia, 99 pp.

572 Hewitt CL, Campbell ML, Thresher RE, Martin RB, Boyd S, Cohen BF, Currie DR, Gomon
573 MF, Keough MJ, Lewis JA, Lockett MM, Mays N, McArthur MA, O'Hara TD, Poore
574 GCB, Ross DJ, Storey MJ, Watson JE, Wilson RS (2004) Introduced and cryptogenic
575 species in Port Phillip Bay, Victoria, Australia. Marine Biology 144(1): 183-202
576 Hunt CD, Tanis D, Bruce E, Taylor M (2007) Optical signatures of seawater and potential
577 use for verification of mid-ocean ballast water exchange. Marine Ecology Progress
578 Series 331:35-47
579 IMO (International Maritime Organisation) (2004) International Convention for the Control
580 and Management of Ships Ballast Water and Sediments. BWM/CONF/36, 16
581 February 2004 (ISBN: 9789280100334). International Maritime Organisation London
582 IMO (International Maritime Organisation) (2006) Guidelines for ballast water exchange
583 design and construction standards (G11). Resolution MEPC149(55). Adopted13
584 October 2006
585 Kostoglidis A, Pattiaratchi CB, Hamilton DP (2005) CDOM and its contribution to the
586 underwater light climate of a shallow, microtidal estuary in south-western Australia.
587 Est Coast Shelf Sci 63:469-477
588 Lewis S, Hewitt C, Melzer A (2001) Port Survey for Introduced Marine Species – Port
589 Curtis. Final Report, Centre for Environmental Management, Centre for Land and
590 Water Resource Management, Central Queensland University, Gladstone
591 Longmore AR (2008) Port Phillip Bay Environmental Management Plan: Monitoring the
592 state of Bay nitrogen cycling (2006-2007), Marine and Freshwater Fisheries Research
593 Institute, Queenscliff, Victoria, Australia
594 MAF (Ministry of Fisheries) (2005) Import health standard for ships' ballast water from all
595 countries. Rule issued pursuant to Section 22 of the Biosecurity Act 1993 on 13 June
596 2005

597 MAF (Ministry of Fisheries) (2007) Managing and controlling the risk to the marine
598 environment from ballast water discharges: New Zealand's response to the
599 international convention for the control and management of ship's ballast water and
600 sediments. MAF Biosecurity New Zealand Discussion Paper No: 2007/04
601 Murphy K, Boehme J, Coble P, Cullen J, Field P, Moore W, Perry E, Sherrell R and Ruiz G
602 (2004) Verification of mid-ocean ballast water exchange using naturally occurring
603 coastal tracers. Marine Pollution Bulletin 48(7): 711-730
604 Murphy KR, Ruiz GM, Dunsmuir WTM, Waite TD (2006) Optimized parameters for
605 fluorescence-based verification of ballast water exchange by ships. Environmental
606 Science and Technology, 40(7): 2357-2362
607 Murphy KR, Boehme JR and Ruiz GM (2007) Verification of Mid-ocean Ballast Water
608 Exchange by Commercial Ships Arriving to New Zealand. Final report to the New
609 Zealand Ministry of Agriculture and Forestry, Smithsonian Environmental Research
610 Center, Edgewater
611 Murphy KR, Field MP, Waite TD, Ruiz GM (2008a) Trace elements in ships' ballast water as
612 tracers of mid-ocean exchange. Science of The Total Environment, 393(1): 11-26
613 Murphy KR, Stedmon CA, Waite TD, Ruiz GM (2008b) Distinguishing between terrestrial
614 and authochthonous organic matter sources in marine environments using
615 fluorescence spectroscopy. Marine Chemistry 108(1-2): 40-58
616 Murphy KR, Boehme JR, Noble M, Smith G, Ruiz GM (2009) Deducing ballast water
617 sources in ships arriving to New Zealand from southeastern Australia. Mar Ecol Prog
618 Ser 390:39-53
619 Palmer MR and Edmond JM (1993) Uranium in river water. Geochim Cosmochim Acta
620 57:4947-4955.

621 Sohrin Y, Fujishima Y, Ueda K, Akiyama S, Mori K, Hasegawa H, Matsui M (1998)
622 Dissolved niobium and tantalum in the North Pacific. Geophys Res Lett 25:999-1002.
623 Taylor CM, Hastings A (2005) Allee effects in biological invasions. Ecology Letters, 8: 895–
624 908
625 Underwood AJ, Keough MJ (2001) Supply-side ecology: The nature and consequences of
626 variations in recruitment of intertidal organisms. In: Bertness MD, Gaines SD, Hay
627 ME (eds) Marine Community Ecology. Sinauer Associates Inc., MA, USA
628 Verling E, Ruiz GM, Smith DL, Galil B, Miller AW, Murphy, KR (2005) Supply-side
629 invasion ecology: characterizing propagule pressure in coastal ecosystems.
630 *Proceedings of the Royal Society B* 272: 1249-1256
631 Williamson M (1996) Biological Invasions. Chapman and Hall, London.

632 **TABLES**

633 Table 1: The top twenty ports in Australia likely to be infested with an introduced species, ranked in increasing order of their predicted difficulty
634 in verifying as a coastal ballast source (1 being the easiest to verify). Estuary data was sourced from Geosciences Australia
635 (www.ozcoasts.org.au), and annual rainfall data from the Australian Bureau of Meterology (www. bom.gov.au; calculated using data from 1961-
636 1990). Estuary width and water area together with rainfall provide an indication of potential freshwater input and hence the strength of tracer
637 signals in ports, whereas distance of berths from ocean provides an indication of the potential marine influence within ports.
638

Ranking	Port	Estuary associated with port	Width of estuary entrance (km)	Estuary water area (km2)	Distance of berths from ocean	Average annual rainfall (mm)	Salinity (psu)	Reference
1	Darwin, NT	Darwin harbour	6.45	161.34	Situated within Darwin Harbour > 10 km from ocean	1600-2000 Tropical Savannah - Wet Autumn - Uniform temp	31.07 ± 0.83 (Oct 2004) 35.56 ± 0.1 (Feb 2006)	Burford et al. 2008
2	Brisbane, QLD	Brisbane River	1.75	18.67	Port at mouth of river in Moreton Bay, but > 30 km from ocean	1200-1600 Temperate uniform rainfall - hot summer	33.6 ± 2.1	Compiled from monthly measurements made at mouth of Brisbane River from 1990-2002 by QLD Dept Envt & Resource Management (DERM, formerly EPA)
3	Sydney, NSW	Port Jackson/Parramatta River	1.58	50.47	Port situated in Sydney Harbour but < 5 km from ocean	1000-1200 Temperate uniform rainfall - hot summer	34.1 - 35.5	R. Hill, unpublished data; measured in Middle Harbour; Aug to Sept 2008
4	Launceston,	Tamar River	2.87	91.71	Port situated in	600-800	26.8 to 34.8	Exotic marine pest survey,

	TAS				lower reaches of the Tamar estuary <5 km from ocean	Temperate uniform rainfall - warm summer		Port of Launceston; Aquenal 2001
5	Newcastle, NSW	Hunter River	0.55	28.42	Port at mouth of Hunter River < 6 km from ocean	1000-1200 Temperate uniform rainfall - hot summer	predicted to be marine except during flood	
6	Melbourne, VIC	Yarra River	0.9	3.48	Ports of Melbourne and Geelong sit within Port Phillip Bay at mouth of Yarra River, ~50 km from ocean	600-800 mm Temperate uniform rainfall - warm summer	34 – 38	Measured in Hobsons Bay between June 2006 and July 2007; Longmore, 2008
7	Gladstone, QLD	Calliope River	0.63	5.1	Some berths located < 2 km from mouth of river and > 20 km from ocean	800-1000 Temperate uniform rainfall - hot summer	32.7 - 35.9	This study
8	Devonport, TAS	Mersey River	0.73	4.61	Port in estuary < 2 km from ocean	1200-1600 Temperate uniform rainfall - warm summer	30 to 34.7	Summarized from all depths at 8 sites; Introduced species survey, Port of Devonport, CRIMP 1996
9	Fremantle, WA	Swan River	0.39	37.65	Port at mouth of Swan River < 2 km from ocean	600-800 Temperate dry hot summer	27 – 37	Kostoglidis et al. 2005
10	Botany Bay, NSW	Cooks River	0.16	1.07	Port situated in Botany Bay but < 1 km from ocean	1000-1200 Temperate uniform rainfall - hot summer	35.33 ± 0.58	P. York, unpublished data; measured at Bona Point between Sept 2003 to Jan 2004

11	Port Kembla, NSW	Port Kembla harbour	0.37	2.24	Harbour directly adjacent to ocean	1200-1600 Temperate uniform rainfall - warm summer	34.8 – 35.5	E. Watson, D. Spadaro, L. Barwell, unpublished data; measured in Jan 2006
12	Adelaide, SA	Not located on an estuary			Port at mouth of river/inlet < 3 km from ocean	600-800 Temperate dry warm summer	36.0 - 39.7	Murphy et al., in press
13	Burnie, TAS	Not located on an estuary			Directly adjacent to ocean	1000-1200 Temperate uniform rainfall - warm summer	predicted to be marine	
14	Portland, VIC	Not located on an estuary			Directly adjacent to ocean	600-800 Temperate dry warm summer	predicted to be marine	
15	Bunbury, WA	Not located on an estuary			Directly adjacent to ocean	600-800 mm Temperate dry hot summer	predicted to be marine	
16	Townsville, QLD	Not located on an estuary			Directly adjacent to ocean and < 2 km close to Ross River mouth	800-1000 Tropical Savannah - Wet Autumn	predicted to be marine except during flood	
17	Mackay, QLD	Not located on an estuary			Directly adjacent to ocean	1200-1600 Temperate dry winter - hot summer (summer dominated rainfall)	predicted to be marine except during flood	
18	Port Stanvac, SA	Not located on an estuary			Directly adjacent to ocean	200-300 Temperate dry warm summer	predicted to be marine	

19	Thevenard, SA	Not located on an estuary			Directly adjacent to ocean	200-300 Temperate dry warm summer	predicted to be marine	
20	Port Hedland, WA	Not located on an estuary			Directly adjacent to ocean	300-400 Dry hot desert - Summer drought	predicted to be marine	

639

640 Table 2: Tracer concentrations found in ports during different seasons. Data are means ± SD
641 or measured range, and are in units of QSE for C2* and C3* (CDOM), μgL^{-1} for Ba, Mn and
642 P (trace elements) and psu for salinity. Background tracer levels in the Tasman Sea were
643 derived from [a] Murphy et al., in press; [b] Murphy et al., 2007.
644

Tracer	**Tasman Sea**	**Port Botany Fall**	**Port Curtis Winter**	**Port Curtis Fall**	**Port Phillip Fall**	**Port Phillip Spring**
Date		18-Apr-07	21-Aug-07	03-Apr-08	24-Apr-07	25-Oct-07
n		24	9	17	25	25
C2*	0.57 ± 0.15 [a]	3.8 ± 3.6	4.9 ± 0.5	8.1 ± 2.9	4.5 ± 1.3	4.7 ± 1.4
C3*	0.35 ± 0.08 [a]	2.3 ± 2.1	2.3 ± 0.2	4.2 ± 1.3	2.3 ± 0.7	2.4 ± 0.7
Mn	0.5 ± 0.29 [a]	1.7 ± 1.5	1.9 ± 1.2	1.9 ± 1.6	5.2 ± 2.4	3.4 ± 1.4
Ba	4.85 ± 0.78 [a]	6.8 ± 2.7	7.0 ± 0.4	9.0 ± 1.8	9.8 ± 1	9.3 ± 1.6
P	12.1 ± 3.0 [a]	17 ± 4.2	6.8 ± 1	6.9 ± 1.3	81.2 ± 15.9	63.8 ± 16.9
Salinity	34.3 - 37.8 [b]	37.3 ± 1.9	35.8 ± 0.1	32.8 ± 0.7	41.2 ± 0.7	37.5 ± 0.8

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646

Table 3: Summary of the range of % BWE efficiencies required for ballast water from different sites within each port to meet the proposed BWE threshold of 0.90 QSE (C3*), 1.4 μgL^{-1} (Mn) and 6.9 μgL^{-1} (Ba). Dash denotes no data and zeros represent sites with tracer concentrations that require no exchange.

Site type	**Port Botany fall**			**Port Curtis winter**			**Port Curtis fall**			**Port Phillip fall**			**Port Phillip spring**		
	C3*	Mn	Ba	C3*	Mn	Ba	C3*	Mn	Ba	C3*	Mn	Ba	C3*	Mn	Ba
Entrance	0 - 62	0	0	-	-	-	38 - 79	0	0	0	0	0	0	0	0
Shipping channel	0 - 49	0	0	76	0	0	88 - 89	0	72 - 80	57 - 82	0 - 93	80 - 88	49 - 83	17 - 90	0 - 88
Berth	0 - 65	0	0	76 - 81	0 - 16	0 - 37	88 - 94	0 - 86	73 - 91	70 - 84	77 - 94	81 - 88	70 - 83	72 - 92	57 - 88
Wider port area	26 - 91	27 - 90	0 - 81	79	43	0	88 - 93	22 - 90	70 - 90	62 - 86	56 - 95	79 - 89	18 - 87	0 - 90	0 - 90
Riverine	88 - 95	81 - 92	86 - 95	77 - 95	43 - 98	22 - 99	89 - 92	57 - 93	84 - 88	73 - 84	55 - 97	82 - 98	74 - 84	34 - 97	76 - 96
Overall average	52 ± 36	28 ± 38	23 ± 37	80 ± 6	32 ± 39	28 ± 35	86 ± 13	35 ± 38	72 ± 28	73 ± 17	80 ± 27	82 ± 18	73 ± 22	75 ± 29	74 ± 29
% sites > threshold	74	37	29	100	56	33	100	65	88	96	92	96	96	92	87

651 Table 4: Potential univariate threshold levels for BWE verification using CDOM (QSE units)
652 and trace elements (μgL^{-1}) identified in this and previous studies. Ballast water with values
653 above these thresholds retains significant coastal influences. Data sources are [a] Murphy et
654 al. 2004; [b] Murphy et al. 2006; [c] Murphy et al. 2008; [d] Murphy et al., in press.
655

Tracer	Pacific	Atlantic	Tasman Sea	This study
C2*		1.7 [b]	1.8 [d]	1.6
C3*		0.7 [b]	1.1 [d]	0.9
Ba	-	7 [a, c]	6.8 [d]	6.9
Mn	1 [c]	2 [c]	2.6 [d]	1.4

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662 **FIGURE LEGENDS**

663 Figure 1: Schematic showing theoretical tracer concentration ranges in three ports and how
664 their concentration would change with ballast water exchange. The bold horizontal line
665 (potential BWE threshold) is the trace concentration which would be achieved if port water
666 containing the maximum tracer concentration was replaced with ocean water at 95% ballast
667 water exchange (BWE) efficiency. The hatched grey band at the bottom of the plot represents
668 the range of tracer concentration in the ocean (average ± 2SD). In this scheme, Port A
669 contains ballast water that would have a moderate BWE verification difficulty, whereas water
670 sourced from Port B and Port C would have difficult and very difficult status, respectively.
671
672 Figure 2: Sampling sites in Port Curtis during the winter and fall surveys (A), Port Botany
673 during fall (B), and Port Phillip Bay during the fall and winter surveys (C).
674
675 Figure 3: Salinity and C3* CDOM (A), manganese (B), barium (C) and phosphorus (D)
676 distribution within and between ports. Data are means at average depth sampled. The dashed
677 line represents the upper 95th percentile of tracers in the Tasman Sea (source: Murphy et al.,
678 in press and Murphy et al. 2007), providing a benchmark for comparison.
679
680 Figure 4: Schematic showing CDOM C3* (A), manganese (B) and barium (C) tracer ranges
681 in each port and how their concentration would decrease with ballast water exchange. The
682 dashed horizontal line (BWE threshold) is the concentration of the tracer which would be
683 achieved if port water containing the maximum tracer concentration was replaced with ocean
684 water at 95% ballast water exchange (BWE) efficiency. The grey band represents the tracer
685 concentration (mean ± 2sd) in the ocean water adjacent to the source port water (in this case,

686 Tasman Sea; Murphy et al. in press). The values presented in the left hand portion of the
687 figure are tracer concentrations measured in each port, as compared with the full range of
688 tracer concentrations in each location. PB_{max} and PB_{min} are the maximum and minimum
689 tracer values in Port Botany, respectively; PC = Port Curtis, PPB = Port Phillip Bay.
690 Symbols: ✖ = Port Botany fall; ○ = Port Curtis winter; ● = Port Curtis fall; △ = Port Phillip
691 Bay spring; ▲ = Port Phillip Bay fall.
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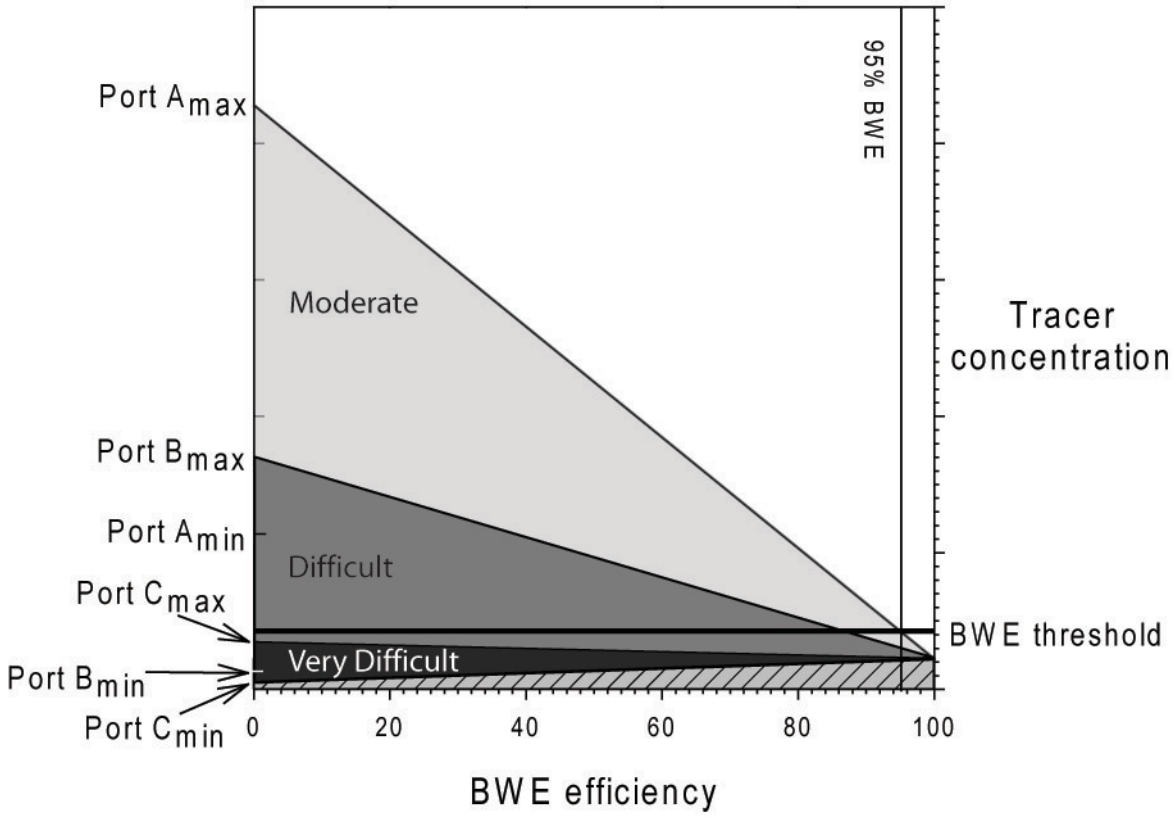
693

694 **FIGURES**

695

696



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698 Figure 1
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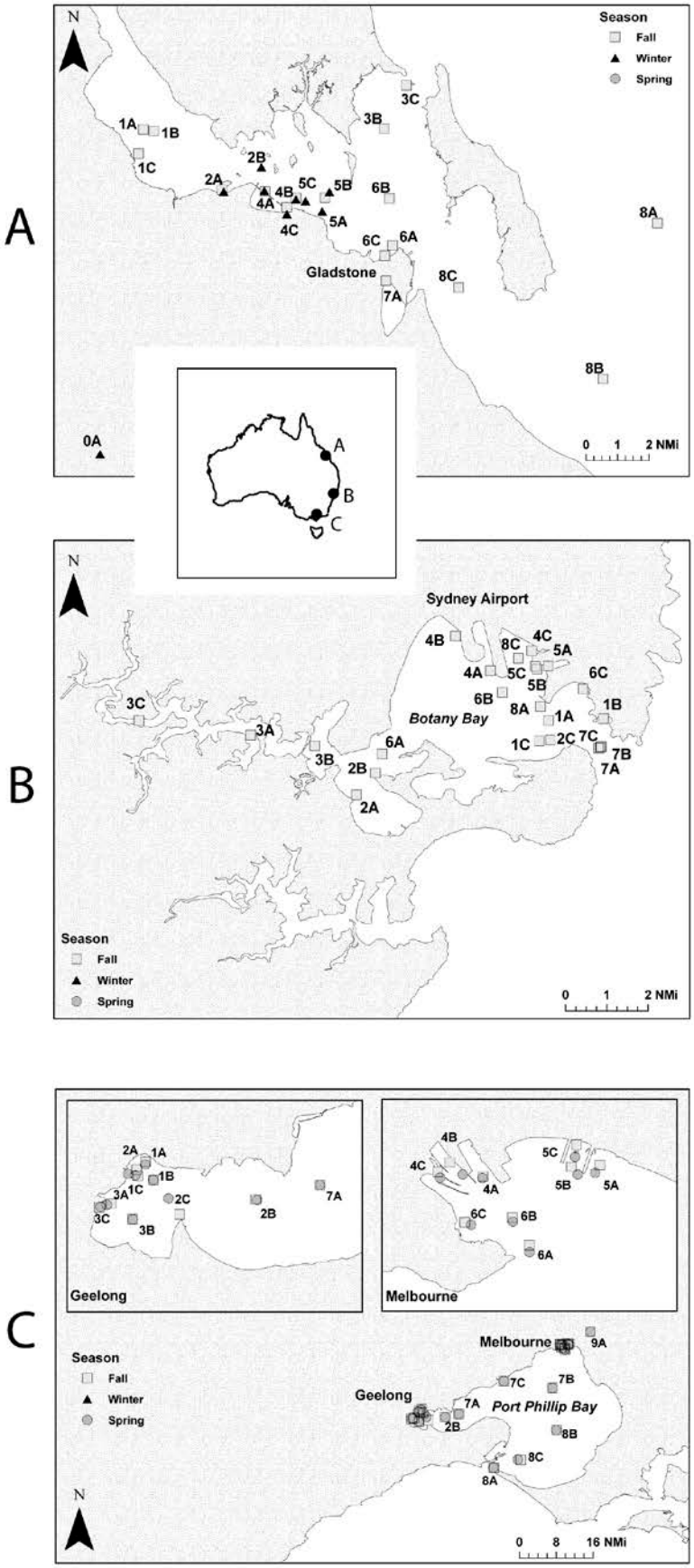
A
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Botany Bay
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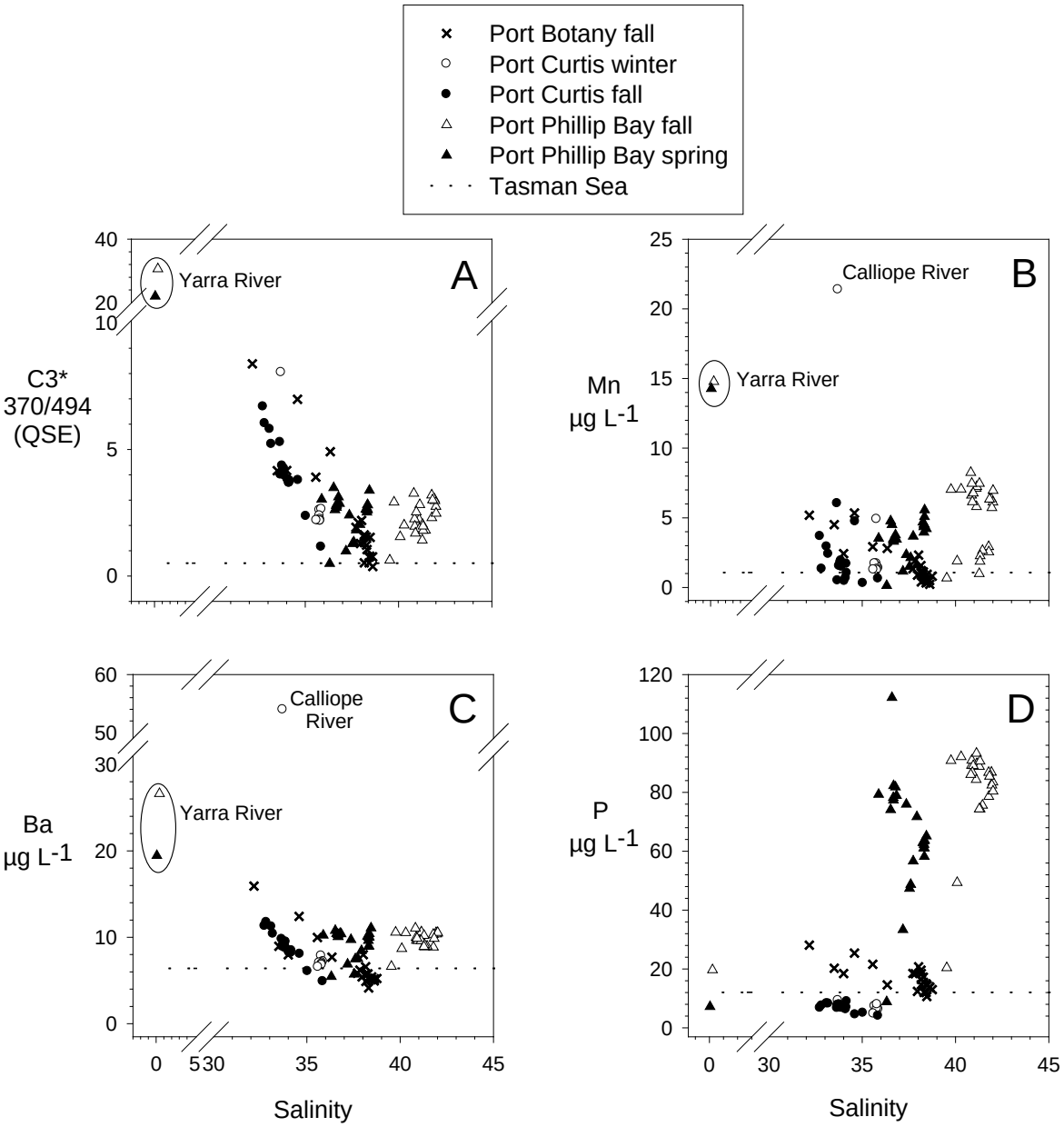
701 **Figure 2**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Port Curtis fall
Port Phillip Bay fall
Port Phillip Bay spring
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370/494
(QSE)
B
Calliope River
Yarra River
Mn
µg L-1
C
Calliope
River
Yarra River
Ba
µg L-1
D
P
µg L-1
Salinity
Salinity

Figure 3

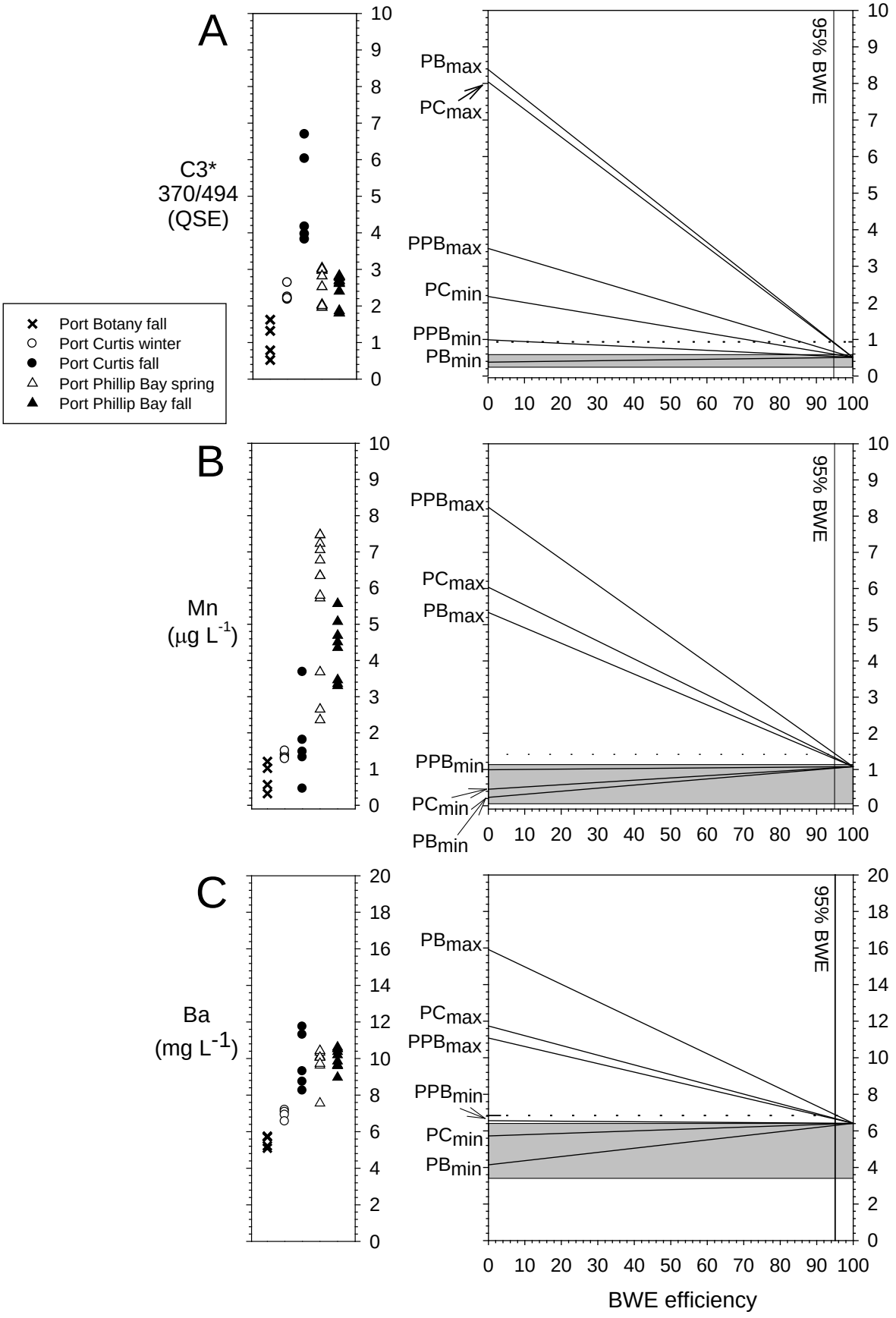
A
C3*
370/494
(QSE)
Port Botany fall
Port Curtis winter
Port Curtis fall
Port Phillip Bay spring
Port Phillip Bay fall
PBmax
PCmax
PPBmax
PCmin
PPBmin
PBmin
95% BWE
B
Mn
(μg L-1)
PPBmax
PCmax
PBmax
PPBmin
PCmin
PBmin
95% BWE
C
Ba
(mg L-1)
PBmax
PCmax
PPBmax
PPBmin
PCmin
PBmin
95% BWE
0 10 20 30 40 50 60 70 80 90 100
BWE efficiency

Figure 4

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