Ulva australis as a tool for monitoring zinc in the Derwent Estuary and implications for environmental assessment.

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Abstract

This study investigated temporal and spatial patterns of zinc content in *Ulva australis*. Samples were collected from the Derwent Estuary, Tasmania, Australia, between 2013-2015, at locations where zinc levels were elevated in both sediments and seawater historically. Zinc content was high (~321 $mg \cdot kg^{-1}$) in *U. australis* at all sampling times, with levels consistent with the spatial distribution of metal within the system. Zinc in *Ulva* varied seasonally (5 – 321 $mg \cdot kg^{-1}$) and was highest in the middle-upper estuary, where seawater concentrations were highest, attributed to a nearby zinc smelter. The results suggest that spatial variation of zinc content in *Ulva* reflects the variability in the seawater, which in turn indicates that *U. australis* could be used for monitoring the effects of zinc in estuarine systems more broadly, and that *U. australis* could be a useful addition to existing management strategies in the Derwent and elsewhere.

Keywords: Biological indicators, Contamination, Macroalgae, Seasonal variability, Monitoring.

1. Introduction

Metal pollution in aquatic environments is a worldwide concern. Contamination of estuaries and coastal zones is increasing (Kennish 2002, Lionetto *et al*. 2012). Biological monitoring or biomonitoring is one way to obtain information on the potential effects of metals pollutants (Campbell 2002, Zhou *et al*. 2008). However, reliable indicators are needed to provide an understanding of the ecological effects (Rainbow 1995, Conti *et al*. 2002, Zhou *et al*. 2008). Measuring contaminant levels in seawater and sediments can provide a very accurate result (Campbell 2002), but does not provide a complete a complete picture of exposure or potential impacts from the contaminants. Measurement of levels in biota (flora and/or fauna) provides an better understanding of biological uptake over time, and as such may be a better indicator of overall ecosystem health (Holt and Miller 2011). Seaweeds have been recommended by a number of authors as potentially valuable bioindicators (Rainbow and Phillips 1993, Rainbow 1995). Many species have a high tolerance to metal pollution and being static will reflect local conditions (Zhou *et al*. 2008). However, there can be temporal differences associated with plant physiology (e.g. growth rate) (Malea and Haritonidis 1999b), environmental conditions (Malea *et al*. 1995, Brown *et al*. 1999) and even in pollutant inputs. The Derwent Estuary in Tasmania, Australia, is a highly metal polluted system (Bloom and Ayling 1977) with zinc (Zn) being a significant contaminant in the middle estuary (Coughanowr *et al*. 2015). Monitoring and research data collected over the past 15 years has shown that arsenic (As), cadmium (Cd) copper (Cu), lead (Pb) and Zn are all present at elevated concentrations in sediments (Bloom and Ayling 1977, Wood *et al*. 1992, Coughanowr *et al*. 2015), shellfish (Eustace 1974, Bloom and Ayling 1977) and fish (Eustace 1974, Langolis *et al*. 1987, Verdouw *et al*. 2010) in the Derwent. However, there is no information on the content of heavy metals in seaweeds in the Derwent. This study used *Ulva australis*, to characterise the spatial and temporal changes in zinc content within the estuary and examine the effectiveness of this species as a tool for monitoring.

2. Methods

2.1 Study area

Eight study sites were selected to reflect different metal loads and sources of pollution, i.e. levels of industrial discharge, sewage treatment levels, and heavy metal concentrations (Fig. 1). The estuary was divided into four regions where *U. australis* is located (upper, middle-upper, middle-lower and lower estuary).

2.2 Field collection

U. australis samples (n = 3) were collected from all study sites every three months from October (Spring) 2013 to October (Spring) 2015. Approximately 50 g of *U. australis* was collected from intertidal areas at low tide. Metal analysis was undertaken as per methods described by Farias *et al*. (2017). The Derwent Estuary Program (DEP) provided monthly data for surface water (0.1 m depth) zinc concentration ($\mu g \cdot L^{-1}$) for the middle estuary study sites (PWB: Princes of Wales Bay, SB: Shag Bay, KB:

Kangaroo Bay, TRA: Tranmere, LSB: Lower Sandy Bay). All water and seaweed samples were analysed at Analytical Services Tasmania (AST).

2.3 *Statistical analysis*

Inter-annual differences were assessed between spring 2013 and spring 2015, using a general linear mixed model (GLMM) with area as random effect, year as factor, and gamma distribution. Where this showed a significant interaction, pairwise contrasts were undertaken using a Least-Square Means (lsmeans) package (Lenth and Hervé 2015), with Bonferroni adjustment. Seasonal variability was also analysed using GLMM, with area as random effect, year as factor, season as fixed effect, a gamma distribution and restricted suite of post-hoc multiple contrasts using the Least-Square Means (lsmeans) package (Lenth and Hervé 2015) and Bonferroni adjustment. Seasonal Zn variability in seawater was assessed using a GLMM with a gamma distribution. All GLMM analyses were performed in R with significance set at alpha = 0.05 (R Core Team 2013).

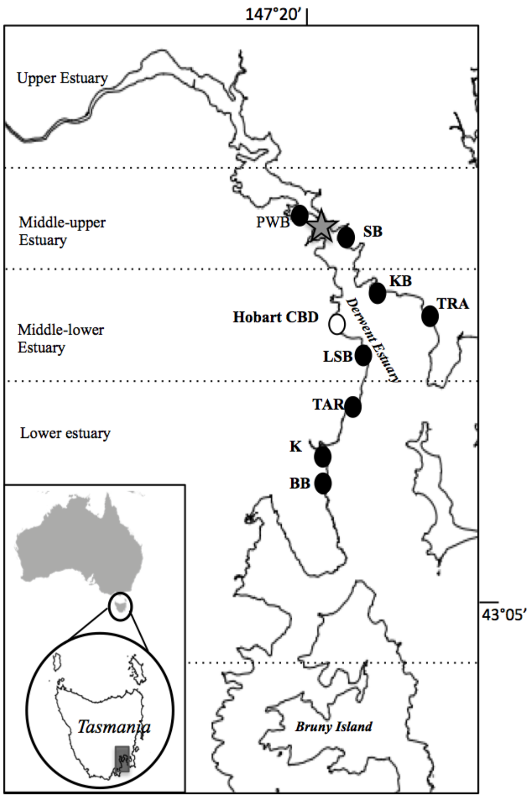

Figure 1. Study area and sampling sites in the Derwent Estuary, Tasmania, Australia. Grey star indicates Zinc smelter (historic source of metal contamination). Study sites were PWB: Princes of Wales Bay, SB: Shag Bay, KB: Kangaroo Bay, TRA: Tranmere, LSB: Lower Sandy Bay, TAR: Taroona, K: Kingston Beach and BB: Blackmans Bay.

3. Results

3.1 Inter-annual variability

Total Zn in *U. australis* content differed markedly between years; in 2013, mean Zn content was 320.7 ± 18.3 mg·kg^{-1}, whereas in spring 2015 levels were considerably lower (Zn = 76.5 ± 1.4 mg·kg^{-1}) (Fig. 2).

3.2 Seasonal variability

Zn content varied seasonally ($p < 0.05$, F = 8.076, df = 3) and spatially ($p < 0.05$, F = 24.3439, df = 2). Seasonal changes were most pronounced in the middle-upper estuary (Table 1). Metal levels were relatively consistent over time, except in winter 2015, when Zn levels increased in the middle-upper estuary. The highest Zn levels occurred in the middle-upper estuary in spring 2013, and there was a clear spatial gradient in every season. There was a strong interaction between season and region ($p < 0.05$, F = 4.0979, df = 6). Post-hoc analysis suggested that this was due to plants from the middle-upper estuary having a much higher Zn content (244.3 ± 108.0 mg·kg^{-1}) compared to the middle-lower estuary (42 ± 27.2 mg·kg^{-1}) and the lower estuary (15.5 ± 7.3 mg·kg^{-1}) in all seasons ($p < 0.05$, df = 5). This suggests that seasonal patterns are region-specific. Zn content was significantly higher in the middle-upper estuary in summer and spring, than in autumn or winter (Table 1). However, in autumn and winter, there were still clear spatial differences in Zn content in *U. australis* between the middle-upper and the lower estuary ($p < 0.05$, df = 5). There was no significant difference in Zn levels between the middle-lower and the lower estuary ($p > 0.05$) in any season.

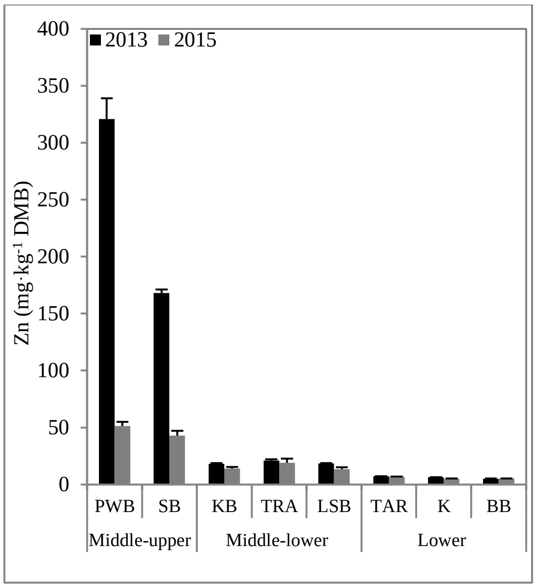

Figure 2. Inter-annual zinc (Zn) content (mg·kg^{-1} DMB) in *Ulva australis* from sites in the Derwent Estuary in Spring 2013 and Spring 2015. Metal levels are expressed as mean (± SE). PWB: Princes of Wales Bay, SB: Shag Bay, KB: Kangaroo Bay, TRA: Tranmere, LSB: Lower Sandy Bay, TAR: Taroona, K: Kingston Beach and BB: Blackmans Bay. Note different scales on y-axis.

3.3 Metal content in seawater

There was significant spatial variability in Zn concentration in the surface waters ($p < 0.05$, F = 45.623, df = 1), with the middle-upper region of the estuary markedly higher than the middle-lower estuary (Table 1). This spatial difference was consistent over time, with no significant seasonal variability.

Table 1. Zinc (Zn) concentration in surface water (μg·L^{-1}) and *Ulva australis* (mg·kg^{-1} DMB) collected from spring 2013, summer, autumn, winter and spring 2014 and 2015, from the three different regions defined in the current study. Metal levels expressed as mean (± SE), at Middle-upper estuary (n = 2), Middle-lower estuary (n = 3), and Lower estuary (n = 3).

		Middle-upper		Middle-lower		Lower
Year	Season	Water	*Ulva*	Water	*Ulva*	*Ulva*
2013	Spring	16.5 ± 1.5	244 ± 76.3	9.7 ± 0.3	19.3 ± 0.9	4.2 ± 1.3
2014	Summer	23 ± 1	156 ± 128.1	9 ± 0.3	24.8 ± 3.1	6.6 ± 1.1
	Autumn	26.8 ± 4.2	67.4 ± 41.6	9.4 ± 0.8	42 ± 15.7	15.5 ± 3.3
	Winter	28.8 ± 9.5	98 ± 39.1	12.6 ± 0.9	30.1 ± 1.2	8.9 ± 3.2
	Spring	20.5 ± 2.8	106 ± 35	13.2 ± 0.9	10.3 ± 1.3	4.2 ± 1.1
2015	Summer	22.5 ± 2.5	83.3 ± 55.8	14.9 ± 0.7	19.2 ± 3	6.5 ± 1.1
	Autumn	23.3 ± 2	50.6 ± 28.5	12 ± 0.8	17.2 ± 6.6	7.3 ± 1.1
	Winter	17.1 ± 1.8	73.2 ± 2.7	15.1 ± 0.2	35.2 ± 2.6	9.7 ± 3
	Spring	25.7 ± 0.7	47.2 ± 4.2	14.2 ± 0.4	15.5 ± 1.8	4.3 ± 0.9

4. Discussion

U. australis appears to be a good biological monitor for zinc in the Derwent estuary, broadly reflecting environmental conditions and providing a better understanding of the biological contamination. *Ulva australis* accumulated Zn showing a clear spatial gradient. The highest concentrations in algae were found in areas were the environmental loading was also high, i.e. middle-upper estuary (PWB and SB), closest to the Nyrstar Zn smelter. The distribution of metal contamination in *Ulva* relates well to the patterns of contamination described by the DEP monitoring program (Coughanowr *et al*. 2015)Metal content in algae from the lower estuary was consistently low, and comparable to levels reported for unpolluted systems worldwide, suggesting that this region could act as a reference for the rest of the system. There were clear inter-annual and seasonal differences in zinc levels in *Ulva* throughout the estuary. This likely reflects a combination of both the scale of Zn contamination in the Derwent and the significant remediation efforts to capture industrial inputs over the last few years (Coughanowr *et al*. 2015). Zn is the predominant contaminant in the Derwent, and has been established as a suitable proxy for most other heavy metals contaminants (Cu, As and Pb) (Coughanowr *et al*. 2015). Zinc has been consistently detected at high concentrations in the water column, sediments, fish and shellfish (Coughanowr *et al*. 2015). Consequently, evaluating of Zn levels should provide a good indication of the broader metal contamination in this system. The temporal differences observed could be due to a number of factors. In a biomonitoring context, it is the differences in the metal loading/ inputs that are important, but temporal differences can also arise as a result of changes in the alga's ability to accumulate metals, that due to physiological status or its environment, both of which could be affected by the timing of sampling. In the current study, Zn levels in *U. australis* were highest in the most polluted (middle-upper) region of the estuary. In this area the contamination levels are very high and would likely obscure any subtle seasonal or temporal influences. Zn content in *U. australis* was greater in spring and summer than other times of the year, which is perhaps not surprising as this is the time when growth/ metabolic rate was highest (in summer) and/ or when the plants were reproductive (in spring). Temporal variability in metal contamination in algae can be related to biological factors such as the growth strategy (annual/ perennial) or to the morphology of the particular species (Stengel *et al*. 2004). For *Ulva*, many studies have shown species specific differences: *Ulva linza* has been shown to take up metals to a greater extent in spring and summer (Haritonidis and Nikolaidis 1990), *U. lactuca* was found to have the highest Zn content in winter (Brown *et al*. 1999), *U. rigida* and *U. intestinalis* uptake was greatest in autumn/winter (Villares *et al*. (2002) and Haritonidis and Malea (1999) observed maximum metal content in *U. rigida* in autumn. These differences are likely due to changes in plant metabolism, which in turn will be affected by a range of environmental variables such as light and temperature. Previous research has shown that salinity can influence metal uptake in a range of *Ulva* species (Ho 1990, Malea and Haritonidis 1999a), with levels generally increasing as salinity declines (Villares *et al*. 2002, Mamboya *et al*. 2009). There is a reasonably strong salinity gradient in the Derwent estuary, with salinity dropping 10 ppt in surface waters through the middle estuary, the area most contaminated with Zn and where the highest levels were detected in *U. australis*. The levels observed in *U. australis* at these sites were clearly elevated, with biological implications for both the plants themselves and the food web. Should the salinity in this region decline (i.e. due to catchment management practices) this could affect the biomonitoring potential of *Ulva* in two important ways: i) directly - resulting in higher metal levels in the plants and associated increase in biological risk, and ii) indirectly - affecting the temporal and spatial comparability of results in a biomonitoring context. Consequently, it is important to consider salinity when using *Ulva* in a biomonitoring program. Using a biomonitoring species such as *Ulva* can provide an improved understanding of Zn contamination throughout the estuary over a biologically meaningful timeframe (the growing period of the plant), whilst samples from the water column will only ever provide a snapshot of the concentration at the time of sampling. The results showed that although *U. australis* broadly reflected a similar contamination gradient and pattern to that observed in the water column/ sediment sampling, reflecting the different matrices. Monitoring *U. australis* could provide important information on changes in a bioavailable contamination source over time, something which has been missing in monitoring to date and which could provide an important new approach to evaluate the effect of remediation/ deterioration activities. Finally, *U. australis* has the added advantage of being both locally abundant and ubiquitous throughout the estuary. Previous studies have suggested that local species are generally the most suitable for biomonitoring (Phillips and Rainbow 1994, Rainbow 2006), and that cosmopolitan, widely distributed (O'Leary and Breen 1997) or abundant (Amado Filho *et al*. 1999) species are to be preferred. Currently seaweeds are not included in the Derwent Estuary monitoring program. Adopting *U. australis* as an indicator in this program, in conjunction with seawater and sediment monitoring, would provide another line of evidence with which to assess changes (improvement/ deterioration) in the "biological" condition of this metal-impacted system.

5. Conclusion

U. australis can play a valuable role as biomonitoring species, and can help improve our understanding of the potential risks and changes (improvements and deteriorations) in metal contaminated systems.

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