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# The challenge of choosing environmental indicators of anthropogenic impacts in estuaries

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ABSTRACT

Ecological assessments over large spatial scales require that anthropogenic impacts be distinguishable above natural variation, and that monitoring tools are implemented to maximise impact detection and minimise cost. For three heavily modified and four relatively 'pristine' estuaries (disturbance category), chemical indicators (metals and PAHs) of anthropogenic stress were measured in benthic sediments, suspended sediments and deployed oysters, together with other environmental variables. These were compared with infaunal and hard-substrate invertebrate communities. Univariate analyses were useful for comparing contaminant loads between different monitoring tools and identified the strongest relationships between benthic and suspended sediments. However, multivariate analyses were necessary to distinguish ecological response to anthropogenic stressors from environmental "noise" over a large spatial scale and to identify sites that were being impacted by contaminants. These analyses provide evidence that suspended sediments are a useful alternative monitoring tool to detect potential anthropogenic impacts on benthic (infaunal and hard-substrate) communities.



## 1. Introduction

Estuaries are a major focus of anthropogenic activities and are subject to high levels of disturbance from multiple stressors. Chemical contaminants from urban and industrial activities are released into estuaries and accumulate in benthic sediments (Birch, 2000), that are resuspended by physical disturbance from shipping, dredging and storms (Eggleton and Thomas, 2004; Hedge et al., 2009). This creates the potential for organisms living both within the sediments and the water-column to be exposed to toxic chemicals. Comprehensive legislation exists to protect water quality, but we currently lack the appropriate monitoring tools to identify impacts (Borja et al., 2008). To assess the ecological health of benthic communities, monitoring tools are selected that provide a cost-effective method to distinguish meaningful anthropogenic impacts.

Past management efforts have largely focused on monitoring water quality alone and have therefore been criticised for lacking ecological relevance (Scanes et al., 2007). Recent efforts have aimed at developing integrative assessment tools and substantial advances have been made in a number of areas (Borja et al., 2008; Hill et al., 2011). However, fewer studies have combined multiple tools and measures with the aim to identify redundant variables and streamline large-scale impact assessments. To develop targeted sampling programs for large-scale impact assessments, it is important to compare the efficacy of different monitoring tools, and their ecological relevance, and prioritize sampling of the variables used in the assessment (Luoma et al., 2010).

Where biological monitoring tools have been identified and investigated, these are often limited to a single group of biomonitors (e.g. bivalves (Rainbow, 1995)) or a single community (e.g. benthic infauna and fish (reviewed by Diaz et al., 2004)). Such studies do not allow for comparative analyses of the sensitivity of different biological communities to anthropogenic impacts. Where chemical monitoring tools are implemented in impact assessments they often target a particular type of contaminant (e.g. metal (Birch, 2000) or organic (Martínez-Lladó, 2007) contaminants) or a particular exposure media (e.g. benthic sediments or water samples (ANZECC/ARMCANZ, 2000)). For integrated assessments a range of monitoring tools is usually necessary to ensure ecological health is assessed in relation to multiple exposure sources (waters and sediments) for different

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contaminants, and to allow comparison and improve future sampling designs (Borja et al., 2009). Studies that target a particular type of contaminant cannot distinguish the comparative importance of different anthropogenic stressors under field conditions, where environmental variables (e.g. temperature, salinity and pH) differ greatly over large spatial scales. For management and remediation purposes, it is important to determine if contaminant effects are stronger than variations in natural stressors (Burton and Johnston, 2010; Chariton et al., 2010).

Biomonitoring tools are increasingly used in integrated assessments of contaminant hazards and other stressors, and some argue that the tissue concentrations from biomonitors are a more accurate representation of potential exposure than chemical measurements of the exposure media (Rainbow, 1995; Romeo et al., 2003). However, while guidelines exist to manage sediment and water contaminants and identify levels that are likely to cause an ecological impact, these guidelines cannot yet be applied to measures derived from biomonitoring tools (but see Birch and Hogg, 2011).

For three heavily modified and four relatively 'pristine' estuaries, chemical indicators (metals and PAHs) of anthropogenic stress were measured in benthic sediments, suspended sediments and deployed oysters, together with other environmental variables. In this study we discuss the deployed oysters as a monitoring tool (similar to the sediments) to estimate exposure risk to other water-column organisms. Chemical indicators from all three monitoring tools and other environmental variables were compared with infaunal and hard-substrate invertebrate communities. We investigated the potential for each monitoring tool to explain ecological variation in the communities sampled. Suspended sediment contamination was expected to represent the strongest link between benthic sediment contamination and the fraction of contaminants in the water-column available for uptake by organisms. We discuss whether monitoring suspended sediments could provide a useful alternative, and possibly replacement, monitoring tool for benthic sediments and transplanted oysters. The study identifies anthropogenic impacts from environmental "noise" over a large spatial scale and highlights how integration of information from these monitoring tools could be used to prioritise what variables to measure and where to measure these variables in future sampling programs.

## 2. Materials and methods

### 2.1. Sampling design

We investigated variation in contamination within three heavily modified and four relatively unmodified estuaries along the coast of New South Wales, Australia (Fig. 1). Port Kembla, Port Jackson and Botany Bay are highly urbanised estuaries with histories of industrialisation. The southern arm of Port Hacking, The Clyde, Wagonga Inlet and Jervis Bay are far less modified by urbanisation and have no history of major industry (although the catchments have a history of mining activities). In addition The Clyde, Wagonga Inlet and Jervis Bay are part of the NSW marine park system. Estuaries were divided into inner and outer zones based on qualitative observations of physical conditions (Fig. S1). Outer zones were characterised by greater wave exposure, sandier sediments, more oceanic flushing and tidal influence than inner zones. Seven sites (~1.5 km apart) were sampled in each zone (Fig. S1).

At each site benthic sediments were collected between February and March 2010. Plastic ware used in sediment collection was previously soaked in 5% $HNO_3$ for a minimum of 24 h and then rinsed in Milli-Q™ water. Four sediment grabs were collected at each site from 5 m depth using a Van Veen grab to target surficial sediments. Grab sediments were homogenised in a clean tray and sub-samples were taken for analyses of particulate metals and PAHs, total organic carbon (TOC) and grain size. These samples were kept in the dark on ice for transport and then stored frozen at −20 °C until time of analysis. For sediment porewater analyses, a 50 mL centrifuge tube was filled with sediment and stored on ice until porewaters were extracted (within 24 h of collection). Analyses of metals, TOC and grain size were made on one sample from each site, with a replicate grab analysed for approximately 20% of sites. Analyses of PAHs and porewater metals and ammonia were made on one sample from each of the inner zone sites only because outer zone sites were primarily sandy and concentrations were expected to be negligible (Simpson et al., 2005).

Sediment sub-samples (500 mL) were also collected from two homogenised replicate grabs at inner zone sites to assess the infaunal assemblages. Infaunal samples were sieved and half the volume (250 mL) was sorted for comparison with benthic sediment contaminants. Samples were stained with Rose Bengal and preserved in 7% formaldehyde, then passed through 2-mm (to remove large debris) and 500-μm sieves (to collect organisms). Organisms were sorted under a dissecting microscope and identified in most cases to family or order.

Settlement plates (to census hard-substrate invertebrates), sediment traps (to collect suspended particles) and oysters (as biomonitors) were also deployed at all sites for 3 months between November 2009 and March 2010. Settlement plates, sediment traps and oyster bags were attached to backing panels (60 × 60 cm, Perspex) that were anchored to the seafloor at 5 m depth and held upright in the water-column by a float. Settlement plates were 11 × 11 × 0.5 cm black Perspex roughly sanded with a central hole for attachment to the backing panels. Settlement plate assemblages were preserved in 7% formalin. Percent covers of different species were censused under a dissecting microscope. Sediment traps were Perspex cylindrical pipes capped at one end with an internal aperture of 50 mm diameter and a height of 250 mm (Larsson et al., 1986). During collection, the trap was capped at the other end and kept cool on ice for transport before being frozen. Oyster bags were square black polyethylene mesh (25 × 25 cm with 15 mm aperture) containing 15 oysters (2 year old *Saccostrea glomerata*, of ~7 cm length sourced from an oyster farm located in Port Stephens, NSW). Upon collection, three oysters were randomly selected from each bag and allowed to depurate for 12 h in clean containers of site water before being frozen. Sediment traps and oyster bags were recovered from at least 3 of the 7 sites within each zone of each estuary, but in most cases all were recovered (Table S1). Mortality in oyster bags (e.g. from crab predation) reduced oyster numbers considerably and only three live individuals remained at some sites. Therefore each site collection consisted of two replicate settlement plates, sediments from a single trap, and a homogenised composite of three oysters.

Sediment grain size analyses were made on benthic sediments by wet sieving through stainless steel sieves; gravel (2 mm), sand (2 mm–63 μm), and fines (<63 μm). Samples were then oven-dried (24 h at 60 °C) and weighed to determine the percentage contribution of each fraction.

Inorganic carbon in benthic sediments was removed by acidification with 2 mL of 1 M HCl overnight (Hedges and Stern, 1984), and TOC was analysed using a Leco CN2000 analyser (Leco Corporation, USA) at a combustion temperature of 1050 °C.

### 2.2. Porewater extraction and analyses

Porewaters were extracted from the sediments by centrifugation at 800 g for 5 min and then filtered (0.45 μm, Sartorius Minisart) immediately to minimise exposure to air. Samples were taken for metal analyses (acidified with 2% $HNO_3$ (v/v) (Tracepure, Merck)) and ammonia (stored frozen until analyses). Dissolved ammonia was analysed colorimetrically using a Merck Spectroquant Kit (14,752) and metals by inductively coupled plasma-atomic emission spectrometry (ICP-AES, Varian 730-ES, Varian Australia).

### 2.3. Metal analyses

Sediments for metal analyses were oven-dried at 50 °C and oyster tissue was freeze-dried for 48 h before being homogenised to a fine powder in a ball mill (Retsch, GmbH-301mm, Germany). A 0.5 g subsample of dry suspended and benthic sediments from each replicate was digested according to Method 3051A (USEPA, 2007) and a 0.3 g subsample of freeze-dried oyster tissue from each site was digested following Hardiman and Pearson (1995). Approximately 0.5 g of sediment was digested in 9 mL $HNO_3$ and 3 mL HCl and 0.3 g of oyster tissue digested in 5 mL $HNO_3$, 2 mL $H_2O_2$, and 3 mL Milli-Q™ water, for 20 min at 200 °C in a 1000 W microwave (Milestone, Ethos-1 Advanced MW Digestion System, Italy). Microwave digestion vessels were soaked for at least 12 h in 2% $HNO_3$ and rinsed with Milli-Q™ water in between sample batches. Following digestion, samples were diluted to 30 mL with Milli-Q™ water and the metal concentrations analysed using ICP-AES (Perkin Elmer, Optima7300DV, USA). Sediment samples were diluted an additional five times before analysis. The instrument was calibrated with matrix-matched standards and analysis of certified reference materials (CRM) (sediment LGC6137 and oyster tissue 1566b, Graham B. Jackson Pty Ltd, Australia) indicated adequate recoveries (within +/−15%) for all metals used in further analyses (full details of CRMs and limits of reporting are provided in Table S2).

### 2.4. Polycyclic aromatic hydrocarbon (PAH) analyses

For both sediments and oyster tissues the PAHs analysed were naphthalene (Nap), acenaphthylene (Acel), acenaphthene (Ace), fluorene (Flu), phenanthrene (Phe), anthracene (Anth), fluoranthene (FluA), pyrene (Pyr), benzo(a)anthracene (BaA), chrysene (Chry), benzo(a)pyrene (BaP), benzo(b & k)fluoranthene (BbkF), indeno(1,2,3-cd)pyrene (Ind), dibenzo(a,h)anthracene (DahA) and benzo(g,h,i)perylene (BghiP).