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# Benthic Macroinvertebrate Assemblages in Remediated Wetlands Around Sydney, Australia

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# Abstract

To investigate potential high organisational level impacts of persistent organic pollution in the wetlands in the Sydney Olympic Park (SOP) remediated site, the benthic macroinvertebrate assemblages of seven wetlands within SOP and two off-site reference wetlands were examined. Sediment cores were collected, stained and preserved from each study site and the macroinvertebrates identified to the appropriate taxonomic level (Class, Order, Family, Subfamily). Data were analysed for taxon richness and macroinvertebrate abundance and multivariate techniques were used to identify chemical/physical characteristics of the sediment, which were important influences on the differences in the assemblage between study sites. Macroinvertebrate abundance was highly variable between

 study sites and taxon richness was low across all sites. Oligochaetes, nematodes, ostracods and chironomids were the most common taxa found and were the most important in influencing differences between the macroinvertebrate assemblages among the study sites. Sediment grain size and chemical characteristics of the sediments ( $\Sigma$ PAH,  $\Sigma$ PCB, TCDDeq and heavy metal concentrations) were important in separating the study sites based on taxon richness and abundance. Canonical correspondence analysis separated the macroinvertebrate assemblages at newly two created wetlands from those at other study sites including the urban reference sites. Increased sediment POP contamination (particularly as measured TCDDeq and  $\Sigma$ DDT concentrations) is a likely contributor in excluding pollution sensitive taxa and, therefore, alterations to benthic macroinvertebrate assemblages. Further, the influence of TOC suggests the significance of catchment inputs in contributing to changes in macroinvertebrate assemblage. The SOP remediation led to the establishment of wetlands with benthic communities representative of those expected in urban wetlands.

Keywords: Remediated site, macroinvertebrate, POPs, benthic community,

#### Introduction

Fine and coarse scale changes to a macroinvertebrate assemblage can indicate changes in the health of a wetland. Decreased overall taxon richness (or simply decreased richness in the sensitive taxa) may result from physico-chemical stress (e.g., Sandin and Johnson 2000; Hodkinson and Jackson 2005), chemical stress (Wallace et al. 1996; Hickey and Clements 1998; Sandin and Johnson 2000) or the introduction of exotic species (Stenroth and Nystrom 2003). Decreased macroinvertebrate abundance may be associated with seasonal effects (Boulton et al. 1992), site-specific physical characteristics (e.g., Quinn and Hickey 1990) or severe pollution (e.g., Burt et al. 1991; Hirst et al. 2002).

Exposure of benthic organisms to persistent organic pollutants (POPs) such as organochlorine pesticides (OCPs), polychlorinated biphenyls (PCB), polycyclic aromatic hydrocarbons (PAHs) and polychlorinated dibenzo-*p*-dioxins (PCDDs; e.g., 2,3,7,8-TCDD) can have important ecological consequences. Since POPs rapidly bind to fine sediment (Gustafsson et al. 1997) and are slow to exchange with surficial waters (Achman et al. 1996; Persson et al. 2005) sediments and pore water are likely sites for exposure of these contaminants to biota.

Bioconcentration in benthic organisms is usually high (Neely et al. 1974; Schrock et al. 1997; Thoman and Komlos 1999; Magnusson et al. 2006) and biomagnification, therefore, likely. The acute toxicity of POPs to benthic organisms can be high with targeted (e.g., organochlorine insecticides) and non-targeted effects (e.g., Mayer et al. 1977; Reynoldson 1987; Phipps et al. 1995; Boese et al. 1998) and exposure can cause changes to invertebrate diversity and abundance.

Assessment of benthic macroinvertebrate communities is a useful tool for the assessment of the ecological health of aquatic ecosystems linked to remediated sites and restoration projects. In particular, the rate of recruitment of macroinvertebrate taxa to a wetland in the post-remediation period has been used to assess continuing remediation success (e.g., Nelson and Roline 1996; LeFevre and Sharpe 2002; Simon et al. 2006).

Sydney Olympic Park (SOP) is a remediated site (425 Ha) situated in an urban residential and light commercial area of Sydney (Fig 1). Prior to remediation of the site, soils and sediments contained high concentrations of OCPs ( $\Sigma$ DDT: 0.7 mg / kg),  $\Sigma$ PAHs (430 mg / kg),  $\Sigma$ PCDD(F)s (316 mg / kg), and  $\Sigma$ PCB (14 mg / kg) (Laginestra et al. 2001) in many cases exceeding the Australian interim sediment quality guidelines (ISQG) trigger values (Ying et al. 2009). During the main remediation program (1992 to 1999) a number of wetlands was created on the site while some were remediated and others were left as remnant. Still others were remediated prior to the main program (Laginestra et al. 2001) (Table 1). Chemical and biochemical analysis have described the current POP contamination in some of the wetlands within SOP (Rawson et al. 2009; Ying et al. 2009). There were measurable concentrations of  $\Sigma$ PAHs,  $\Sigma$ PCBs, and TCDDeq in the wetlands of the Park and while these were generally toward the lower end of concentrations measured at remediated sites elsewhere in the world they still, in some cases, exceeded of the ISQG trigger value for these compounds (Ying et al. 2009). Metal (particularly lead and zinc) concentrations were also commonly above the ISQG trigger values (Ying et al. 2009).

The aim of this study was to investigate the effects of POP contamination on the long-term establishment of healthy benthic macroinvertebrate communities in created, remediated and remnant wetlands resulting from a very large remediation program (Sydney Olympic Park, Australia). It was hypothesised that wetlands with high concentrations of sediment POP

contamination would have benthic communities with lower taxon diversity and lower abundance relative to reference sites.

### Materials and Methods

#### Study Sites and Sample Collection

Seven wetlands were studied within SOP (Boundary Ck, Narawang 22, Northern Water Feature, Bicentennial Park, EWQCP, SWQCP, Wharf Pond) and two reference sites were chosen outside the Park (Upper Colo, a pristine site, and Macquarie University, an urban impacted site) (Table 1, Fig 2). Physico-chemical characteristics of these sites have been described elsewhere (Rawson et al. 2009) as have the sediment POP and metal concentrations (Ying et al. 2009) and the aqueous and sediment 2,3,7,8-TCDD equivalence (TCDDeq) (Rawson et al. 2009). Sediment cores (75 cm<sup>2</sup>) were collected from 5 random locations (over  $60m^2$ ) within each study site in February 2007. The top 15 cm of each core was excised, and preserved with borax buffered formalin containing 5 ml/L Rose Bengal stain. These were transferred on ice to the laboratory and stored at 4°C.

#### Sample Processing

Each sample was rinsed to remove as much formalin as possible and washed through 1 mm and 250 µm mesh sieves. The animals retained from each mesh size sub-samples and those removed after examination under a dissecting microscope were preserved in 70% ethanol. Preserved animals were sorted into coarse taxonomic groups before identification to lower levels. All arthropods were identified to Family level while other non-arthropod groups were identification to Family level for all arthropods was considered sufficient to detect broad differences in the benthic communities in this study. Further, with very low taxon richness at this level for most study sites, lower taxonomic identification of individuals was not considered advantageous. Taxon richness and overall macroinvertebrate abundance were calculated for each study site based on the average content of the sediment cores. Initially three cores for each site were examined and where variability in abundance was high (i.e., patchy distribution) additional cores were sorted and the macroinvertebrates identified as above.

#### **Data Analysis**

Differences between study sites based on (log-transformed) taxon richness and abundance (per m<sup>2</sup>) were examined using a single factor ANOVA (after confirming that the data fitted the assumption of homogeneity of variances). A similarity matrix (comparing all samples containing invertebrates) based on the Bray-Curtis similarity index (4<sup>th</sup> root transformed data) was constructed and a multivariate analysis of similarity (ANOSIM) used to investigate differences between macroinvertebrate assemblages noted under examination with a nonmetric multidimensional scaling ordination (PRIMER v6). A hierarchical clustering procedure was run to show within and between study site similarities and similarity profile permutation (SIMPROF) tests indicated the level of similarity required to detect significant differences between samples (PRIMER v6). A similarity percentages (SIMPER) routine was used to examine which species were important in driving the differences between macroinvertebrate assemblages which had been separated by the ordination SIMPROF and clustering routines. All multivariate analyses were conducted using the PRIMER statistical software (PRIMER v6). Canonical correspondence analysis (CCA) on the data was conducted to investigate the influences of selected environmental variables (pH, Total Organic Carbon (TOC), **DDT**, equivalence (TCDDeq) concentrations and sediment heavy metal concentrations from Ying et al. 2009) on individual taxa and benthic macroinvertebrate assemblages at the study sites (MSVP version 3.13p) except Upper Colo which was removed due to inherent differences between this study site and others in terms of the type of wetland (lotic, sandy substrate, geographic separation). To examine whether environmental factors influenced taxon richness or abundance a multiple regression was conducted including the potential predictors TOC, inorganic carbon, sediment grain size composition, sediment pH, sediment conductivity and concentrations of  $\Sigma$ PAHs,  $\Sigma$ PCBs, heavy metals,  $\Sigma$ DDT and TCDDeq in the sediment.

### Results

Thirty-two macroinvertebrate taxa were identified in the sediment cores from the study sites. Of these five were benthic infauna and six were benthic epifauna (Table 2). A further 18 taxa may spend some time in intimate contact with the benthos (e.g., benthic foraging, detrital feeding organisms) and were probably epifaunic at the time of collection (Table 2). Three taxa are generally not considered to be in intimate contact with the benthos but have been

included in the analysis as they were likely to have been foraging on the surface of the benthos at the time of collection.

There were significant (p<0.05) differences in both macroinvertebrate abundance and taxon richness between some of the study sites (Fig 3). This was apparent even over the small spatial scale of the study sites within Sydney Olympic Park (SOP). Macroinvertebrate abundance was variable both between and within study sites. The largest within-site variability was at EWQCP where abundance was between 2800 and 104 133 animals.m<sup>-2</sup> (a 37-fold difference). The lowest variability measured was at Upper Colo, the pristine reference (1.5-fold difference). The highest average abundance was at Macquarie University (121 366 animals.m<sup>-2</sup>) and the lowest was at Upper Colo (10 399 animals.m<sup>-2</sup>) (Fig 3). At Upper Colo, overall abundance was significantly (p<0.05) less than that at Macquarie University, Boundary Ck., Narawang 22 and the Wharf Pond and at SWQCP abundance was significantly (p<0.05) less than that at Macquarie University (Fig 3).

Macroinvertebrate taxon richness was low across all study sites ranging from an average of 4.5 taxa at Boundary Ck. to 12 taxa at Narawang 22. Taxon richness was significantly (p<0.05) higher at Narawang 22 than at Boundary Ck., Bicentennial Park, Macquarie University, Upper Colo and SWQCP. EWQCP, Northern Water Feature and Wharf Pond all had intermediate taxon richness (Fig 3).

The models for predicting of benthic macroinvertebrate abundance included positive coefficients for sediment grain sizes between 500 and 1000  $\mu$ m, pore water metal concentration, sediment  $\Sigma$ DDT concentration and negative relationships for sediment bound metals and  $\Sigma$ PAH concentration (r<sup>2</sup> = 1.000) (Table 3). A multiple regression model for predicting macroinvertebrate taxon richness in the study sites gave negative relationships (r<sup>2</sup> = 0.999) for sediment TCDDeq and  $\Sigma$ PCB concentrations and sediment grain sizes between 187 and 250  $\mu$ m (Table 3).

The nMDS ordination indicated that the macroinvertebrate assemblages at Narawang 22, Upper Colo and the Northern Water Feature are somewhat separated from the other study sites on the basis of a Bray-Curtis similarity index (Fig 4). However, there is considerable overlap between these groups as shown by the clustering procedure (Fig 5). Further, the similarity profile permutation (SIMPROF) tests show that above a similarity distance of 46.5% macroinvertebrate assemblages from individual cores were significantly (p<0.05) differentiated. This distinguished three groups of individual cores; Narawang 22 was separated from all study sites while Upper Colo was linked with one core each from the Northern Water Feature and SWQCP and all remaining cores constituted a third group. Analysis of similarity (Table 4) indicated that macroinvertebrate assemblages were significantly (p<0.05) different from each other but that the pattern was not obvious. Macroinvertebrate communities at Narawang 22 and Upper Colo were different from all other study sites. The macroinvertebrate community at Macquarie University was significantly (p<0.05) different from that at Narawang 22, Upper Colo and SWQCP. SIMPER analysis of the contribution of individual taxa to the dissimilarity between macroinvertebrate assemblages revealed the importance of four common taxa and a number of rare taxa (Table 5). In particular, the lack of common taxa (Oligochaeta, Chironominae, Ostracoda, Nematoda) separated the assemblages at Upper Colo from those at other study sites and the presence of rare taxa (including Baetidae, Caenidae, Leptophlebidae, Cladocera and Ceratopogonidae) at Narawang 22 separated the assemblages at this wetland from that of the other wetlands (Table 5). Table 5 illustrates the differences between the 3 groups of sites identified by the SIMPROF routines in Figure 5 with Boundary Ck representing the sites grouped together (other pairwise comparisons have been removed for clarity).

The CCA separated the macroinvertebrate assemblages at Narawang 22 and Northern Water Feature from those at other study sites along an axis which was correlated with decreasing levels of contamination, in particular concentrations of  $\Sigma$ DDT and TCDDeq (Fig 6). Decreasing TOC was also important in describing the separation of these sites. The separation of the sites other than Narawang 22 and Northern Water Feature was along a second axis which was less correlated with contamination. The taxon centroids indicated that Narawang 22 and Northern Water Feature had higher numbers of rare taxa while the other wetlands lacked these taxa.

Multiple regression analysis indicated that the abundance of Chironominae was negatively affected by sediment  $\Sigma$ PAH concentrations but positively affected by sediment grain sizes between 250 and 500 µm (Table 3). Differences in nematode abundance were negatively affected by sediment TCDDeq concentrations and oligochaete abundance was positively

affected by sediment TCDDeq concentrations and negatively affected by sediment grain sizes between  $1000\mu m$  and  $5000\mu m$ . There were no significant predictors for the abundance of ostracods (Table 3).

## Discussion

Urban wetlands generally have reduced benthic macroinvertebrate taxon richness (Hall et al. 2001; Shutes 1984) represented by taxa that are tolerant to urban contaminants (Whiting and Clifford 1983). Compared to similar pristine wetlands, taxa commonly not found in urban wetlands include odonates, trichopterans, ephemeropterans and plecopterans, while oligochaetes, nematodes and chironomids often dominate urban benthic communities (Lenat and Crawford 1994; Hall et al. 2001). The common taxa found in this study were those expected for degraded urban habitats with all wetlands having abundant tolerant taxa. However, a few wetlands contained pollution sensitive taxa such as trichopterans and ephemeropterans. Only Narawang 22 and Northern Water Feature within SOP contained odonates and ephemeropterans but in very low numbers while trichopterans were moderately abundant only at Narawang 22. While these are not strictly benthic dwellers they can be benthic foragers and are, therefore, intimately associated with the benthos.

The times required for macroinvertebrates to recruit to created and newly remediated wetlands depend on a number of factors including organism life-history (e.g., generation time and dispersal strength) and the distance to a refuge or a number of refugia (Niemi et al. 1990). In lotic systems drift is an important source for recruitment (Nelson and Roline 1996) and increases in taxon richness after remediation can occur quickly if there are upstream seeding sites (Simon et al. 2006). The two urban lotic sites included in the current study (Macquarie University and Boundary Ck) have highly degraded upstream reaches located in highly urbanised land. It is unlikely that taxon rich regions exist upstream of either wetland. This will contribute to decreased taxon richness at these wetlands.

In lentic sites, only highly aerially dispersive organisms (dipterans, odonates and ephemeropterans) are likely to recolonise these wetlands quickly. SOP is located in the highly urbanised Sydney metropolitan area and there are few nearby undisturbed wetlands. The wetland with the highest taxon richness (Narawang 22) is located adjacent to the Newington Nature Reserve, which may contain refugia for rare taxa. In their meta-analysis of post-

disturbance recovery times Niemi et al. (1990) broadly described that (in lotic systems) dipterans were the first insect colonisers, followed by ephemeropterans, then trichopterans and finally plecopterans. The lack of the latter order in these wetlands may indicate slow recruitment to newly remediated wetlands or that conditions were unsuitable for its survival. The time taken for recovery to pre-disturbance taxon richness in lotic wetlands is generally less than 12 months (Simon et al. 2006) but where the disturbance is extreme this can be extended significantly. In the case of the SOP wetlands, given the high degree of disturbance it is possible that the recovery process is incomplete but given the time interval (8 years) this is considered unlikely.

Within the study sites, assemblage patchiness was high, particularly in terms of abundance (37-fold difference between cores at EWQCP). It is unclear whether the patchiness recorded in this study was due to habitat patchiness (often high: Downes et al. 1993; Heino et al. 2004), or chemical pollutant patchiness (also often high: Johnson and Larsen 1985; Swartz et al. 1989; Feng et al. 1998; Koh et al. 2004). Correlation between contamination and benthic community measures in a patchy environment has been recorded over very small spatial scales (<500m) (Stark et al. 2005) and thus the effects of contaminant heterogeneity cannot be ruled out here.

In a study of post-remediation recolonisation, den Besten and van den Brink (2005) found that that differences between the sites were likely due to differences in sediment characteristics. In the current study, sediment grain size was important in predicting chironomid and oligochaete abundances (and total macroinvertebrate abundance). Benthic habitat is dependent on interstitial pore size and, therefore, sediment grain size. Most of the wetlands in the current study had sediment which was dominated by fine sand to silt, (62.5 - 250  $\mu$ m) the exception being Upper Colo which had a much larger proportion of medium sand (>250  $\mu$ m) (Rawson et al., 2009). The proportion of fine sediment is generally positively correlated with total organic carbon therefore a site (such as Upper Colo) with a lower proportion of fine sediment will likely have lower total organic carbon content. This will restrict the prevalence of macroinvertebrates using sediment TOC as a food source and hence impact the entire macroinvertebrate assemblage.

The different assemblage at the pristine reference site Upper Colo (low abundance of common taxa) is therefore likely due to differences in sediment type but also to its

geographic separation from the other study sites (about 100 km) and its different wetland characteristics (a lotic system) with concomitant sediment differences (dominated by sand as opposed to silt). At Narawang 22 the different assemblage (increased abundance of both common and rare taxa) appears indicative of the reduced concentrations of organic contaminants at this site (Rawson et al. 2009; Ying et al. 2009), suggesting that these rare taxa are sensitive to this type of pollution.

Canonical Correspondence Analysis separated the macroinvertebrate assemblages at Narawang 22 and Northern Water Feature (the sites with the highest taxon richness) wetlands along an axis, representing a gradient of decreasing sediment TCDDeq,  $\Sigma$ DDT contamination and TOC. The influence of increasing TCDDeq and concentrations of the pesticide DDT and its metabolites ( $\Sigma DDT$ ) was to reduce the occurrence of rare taxa at the study sites. While the acute toxicity of TCDD and other aryl hydrocarbon receptor (AhR) ligands to benthic invertebrates is not particularly high (West et al. 1997), there is evidence to suggest they can cause significant chronic effects in even the most pollution tolerant taxa (Lotufo 1998b; Hwang et al. 2004) and may reduce taxon richness in the long-term by excluding sensitive taxa. Many studies have shown the tendency of TCDD and other AhR ligands to bioaccumulate in benthic organisms (e.g., West et al. 1997; Froese et al. 1998; Lotufo 1998a; Timmermann and Andersen 2003). These two processes (reduction of taxon richness and bioaccumulation of toxicants) are likely to have negative impacts on the health of vertebrate consumers (e.g., fish and birds) and must be considered together. TOC is usually high in wetlands affected by urban catchments and catchment land-use can be an important predictor of sediment contaminant load (Hoffman et al. 1984). The strength of the influence of TOC on the macroinvertebrate assemblages in the study sites indicates that catchment input is important in restricting the occurrence of some taxa. These results are of importance in the context of remediation since it appears that reductions in contamination and TOC result in the recruitment of more rare taxa. Variation between the macroinvertebrate assemblages at wetlands other than Northern Water Feature and Narawang 22 was mainly along an axis which poorly correlates with the contaminant variables included in the analysis. This suggests the importance of other factors (e.g., physico-chemical characteristics, habitat variety) in influencing the macroinvertebrate assemblages in these wetlands. The cores from the urban reference site, Macquarie Uni, were spread throughout the analysis (not separated from the

remediated sites) demonstrating that none of the remediated sites were different from the expected state of an urban wetland.

Pratt et al. (1981) described the disruption to macroinvertebrate communities due to runoff from urban catchments and Lenat and Crawford (1994) showed that the taxon richness within the orders Ephemeroptera, Plecoptera and Trichoptera was reduced in urban wetlands subject to urban runoff. Taxa within these orders (the EPT index) are considered relatively sensitive to a range of stressors (e.g., changes in water quality) and relatively insensitive to natural disturbances (e.g., changes in flow regime) leading to their wide use as an indicator of wetland health. In the current study no Plecoptera were recorded. Only three families of Ephemeroptera and five families of Trichoptera were found and, in general, these were at the wetlands (Narawang 22 and Northern Water Feature), which were least subject to urban catchment inputs.

The SOP wetlands were subject to a variety of remediation histories. Some were remnant (Wharf Pond), others were remediated either pre-1991 (Boundary Ck.) or created post -1991 (e.g., EWQCP) (Laginestra et al. 2001). There was no clear trend between remediation history and macroinvertebrate community. Nor was there a trend between contamination history and macroinvertebrate assemblage. The Northern Water Feature is situated on land previously contaminated with PCBs and dioxins (Laginestra et al. 2001) while SWQCP is situated on land that did not require remediation. Yet these sites are separated on the basis of the presence of rare taxa at the Northern Water Feature. It is unlikely that differences between the macroinvertebrate assemblages are the result of incomplete remediation or variation in remediation efficacy.

Differences in habitat variety were observed at the study sites. At Boundary Ck there is a stand of emergent macrophytes (*Phragmites australis*) with no riparian vegetation or submerged macrophytes. SWQCP and EWQCP are surrounded by a riparian zone (*Casuarina* spp.) and a significant littoral region with emergent macrophytes (*Baumea articulata*, *P. australis*) while Narawang 22 has a benthic cover of submerged macrophytes, emergent macrophytes (*B. articulata*) in the littoral zone and some riparian vegetation. While sampling at each study site attempted to cover a representative portion of the wetland to account for small-scale patchiness, the absence of healthy riparian, littoral and submerged vegetation at many of the study sites may contribute to a reduction of variety in the benthic habitat. This

may influence the macroinvertebrate assemblages present at the study sites particularly in terms of taxon richness.

The highest taxon richness was in the created wetland Narawang 22 indicating that sufficient time has passed to allow the establishment of a healthy benthic invertebrate community and to allow effective monitoring across the wetlands of the site. As Narawang 22 is primary wading bird and frog habitat, it is essential that a healthy invertebrate population is maintained. This area is regularly subject to inundation as a result of stormwater amelioration action (Laginestra et al. 2001) allowing some recruitment from upstream. However, it is also regularly drained in an attempt to control mosquitofish populations in the wetland. The results suggest that inundation is sufficient to allow recruitment and drainage does not cause long-term depauperation of the benthic community. This wetland should be designated as a reference site to benchmark taxon richness in future monitoring of macroinvertebrate communities in SOP wetlands.

# Conclusions

While there was variation in the current POP concentrations between the study sites, there was no observable correlation with diversity or abundance. The benthic macroinvertebrate communities inhabiting the wetlands of Sydney Olympic Park (SOP) were consistent with those expected in urban wetlands (low taxon diversity and an abundance of tolerant taxa) as represented by the urban reference site Macquarie Uni. This indicates the success of the remediation in returning these highly contaminated wetlands to a condition expected for an urban wetland in Sydney. Only one wetland (Narawang 22) had noticeably high taxon richness and this study site was adjacent to remnant bushland. On the other hand, wetlands without nearby recruitment sources (those with degraded upstream catchments in urban surrounds) are likely to maintain depauperate invertebrate communities. While it is possible that the SOP wetlands are still undergoing the establishment of a healthy benthic community following their remediation or creation, this is considered unlikely as eight years has passed since the remediation. There may also be an influence of differences in habitat diversity between the wetlands, which could affect taxon richness. However, given the strength of the canonical correspondence analysis (CCA) relationship, increased sediment POP contamination (particularly as measured TCDDeq and  $\Sigma$ DDT concentrations) is a likely contributor in excluding pollution sensitive taxa and, therefore, alterations to benthic macroinvertebrate assemblages. Further, the influence of TOC suggests the significance of catchment inputs in contributing to changes in macroinvertebrate assemblage.

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Fig 1 Location of Sydney Olympic Park (shaded area) in the Sydney metropolitan area. The solid circle shows the location of the Sydney central business district.



**Fig 2** Map of benthic invertebrate sampling sites in Sydney Olympic Park. 1. Wharf Pond, 2. Narawang 22, 3. EWQCP, 4. Northern Water Feature, 5. Bicentennial Park, 6. SWQCP, 7. Boundary Ck.



Fig 3 Mean ( $\pm$  SEM) Species richness (top) and abundance (bottom) of benthic macroinvertebrates (m<sup>-2</sup>) at Sydney Olympic Park and key reference sites. Letters represent significantly different homogeneous subsets (columns with the same letter are not significantly different) at  $\alpha = 0.05$ .



Fig 4 Non-metric multi dimensional scaling (nMDS) ordination plot of SOP and reference sites using all benthic macroinvertebrate data. Contours show significantly (p<0.05) similar groups as defined by similarity profile permutations (SIMPROF).



Fig 5 Dendogram of similar samples based on benthic macroinvertebrate assemblage. Fourth-root transformed data were analysed by Bray-Curtis similarity. The dashed vertical line represents the Bray-Curtis similarity value above which divergence is not significant as determined by similarity profile permutation tests (SIMPROF) at  $\alpha = 0.05$ .



**Fig 6** Canonical correspondence analysis ordination for macroinvertebrate assemblages at SOP and reference study sites. Vectors representing environmental variables are scaled x3. TOC = Total Organic Carbon. PCB, DDT and PAH values are summed analyte concentrations. + denotes centroids for major taxa influencing the significant dissimilarity between Narawang 22 and other wetlands. Nem. = Nematoda, Ost. = Ostracoda, Olig. = Oligochaeta, Chir. = Chironominae, V. = a group of rare taxa including Baetidae, Caenidae, Culicidae, Odontoceridae, Orthocladinae.

L [1 11]	111-11-11-		
weuand	w euana 1 ype	Catchment Area (km <sup>2</sup> )	Description / Current Languse
1. Wharf Pond	Remnant Pond	~5~	Bounded by Parramatta River (estuarine) and park. It is a protected remnant
			pond providing habitat. Fed by a small creek originating at a nearby prison
			complex then running through parkland.
2. Narawang	Remediated/	$\sim 1.5$	Situated between saline creek, containment mound, remnant woodland, road &
Wetlands	<b>Created Pond</b>		residential village. Used as passive recreation, irrigation storage, floodplain,
			habitat, green corridor.
3. EWQCP	Created Water	*	Stormwater collection pond buffered by low vegetated area and habitat ponds.
	Treatment Pond		Provides 1 <sup>st</sup> flush before water is recycled to other ponds for re-use and
			recycling.
4. Northern Water	Remediated/	~2~	Consists of several freshwater ponds over 7 ha. Bounded by road and estuarine
Feature	Created Water		creek, containment mounds & parkland, it is part of a green corridor and
	Feature		provides habitat and stormwater control for re-use and irrigation.
5. Bicentennial Pk.	Remnant	$\sim 0.5^*$	On the eastern edge of SOP, Bicentennial Park is bounded by roads,
	Mangrove		Homebush Bay (estuarine) and commercial precinct. Mangrove section
			provides recreational, educational and conservation value. Inflow from Powells
			and Boundary creeks and tidal input from Homebush Bay.
6. SWQCP	Created Water	∽ <mark>1</mark> *	Oval, steep sided pond surrounded by commercial precinct, road and rail line.
	Treatment Pond		Provides stormwater 1 <sup>st</sup> flush before release to Bicentennial Park.
7. Boundary Ck	Remediated	$\sim 2.1$	Flows from heavily urbanized areas, under major freeway then runs south of
	Urban Creek		Sydney Olympic Park into a freshwater lake at Bicentennial Park. It was
			relocated and lined with pools and riffles installed to improve aesthetics and
			provide water quality service.
R1. Upper Colo	Pristine river	~3277.2	National park, recreation, light agriculture
R2. Macquarie Uni.	Created Pond	~2.8	University, open space, standing pond

Table 1 Description of the study sites in Sydney Olympic Park and reference sites. \* denotes sites which receive inputs beyond this immediate catchment area

Table 2 Macroinvertebrate taxa collected at SOP and reference sites. Average density $(m^{-2})$ shown: * < 1000, ** < 10000, ** > 100000. BC = Boundary Ck, EWQ =
EWQCP, NWF = Northern Water Feature, N22 = Narawang 22, BP = Bicentennial Pk., WP = Wharf Pond, MCU = Macquarie University, UPC = Upper Colo, SWQ =
SWQCP, H = Habitat Classification.

Phylum	Class	Order	Family	BC.	EWQ	NWF	N22	BP	WP	MCU.	UC	<b>D</b> MS	$\mathrm{H}^{\mathrm{a}}$
Nematoda				* *	* * *	* *	* * *	* *	*	* * *	*	×	A
ni	Temnocephalidea										*		С
Platynelmintnes	Turbellaria				*	*		*	*	*			С
Mallucoo	Continon od o	Dulmonoto	Lymnaeidae		*		*		*	*			С
Mollusca	Gastrapoua	ruimonata	Physidae				*		*			*	С
A multiple	Hirudinea	Rhychobdellida	Glossiphoniidae	*		*				*	*		С
Ашециа	Oligochaeta			***	***	***	**	**	***	* * *	*	* *	A
<u> 4 14 1 10 10 10 10 10 10 10 10 10 10 10 10 1</u>	Maxilliopoda	Cyclopoida			*	*	**	*	**	*			A
Arunropoua	Branchiopoda	Cladocera			*	*	*		*		*		в
	Ostracoda			* *	* * *	*	* *	* *	* *	* * *	*	* *	В
	Malacostraca	Isopoda					*						c
	Arachnida	Acarina					*						D
	Tuccota	To be an an and a set	Baetidae				**						С
	Шзеста	ърпешегориега	Caenidae				*						в
			Leptophlebidae			*	*						в
		Colcontore	Ptilodactylidae			:			*				A
			Hydrophilidae						*				C

Phylum	Class	Order	Family	BC	EWQ	NWF	N22	BP	WP	MCU	UPC	SWQ	$\mathrm{H}^{\mathrm{a}}$
Arthropoda	Insecta	Diptera	Chironominae <sup>2</sup>	* *	* *	* * *	***	* *	*	* * *	* *	*	В
			Orthocladiinae <sup>2</sup>				*						C
			Tanypodinae <sup>2</sup>		*	*	*	*	*		*	*	B
			Ceratopogonidae		*		*	*			*	*	A
			Culicidae				*						C
			Syrphidae			*							C
		Odonata	Corduliidae			*				*			C
			Coenagrionidae			*							C
		Tricoptera	Hydroptilidae				*					*	C
			Leptoceridae		*		* *				*		C
			Odontoceridae				*						C
			Unknown 1			*							C
			Unknown 2		*								C
		II. and the second	Notonectidae			*		*					D
		nempuera	Corixidae						*				D

Table 2 (cont.)

<sup>1</sup>Suborder, <sup>2</sup>Subfamily of Chironomidae. <sup>a</sup>A = Benthic infauna, B = may be either benthic infauna or epifauna at different life-stages, C = may spend time as epifauna, D = Rarely in direct contact with sediment (from information in Williams 1980 and Gooderham and Tsyrlin 2002.

**Table 3** Results of multiple regressions on macroinvertebrate community data using physical and chemical characteristics of the sediments as predictors. Individual taxa are those that regularly influenced the pairwise differences between the sites (SIMPER). The direction of the relationships is denoted as + (positive relationship) and – (negative relationship).

	F	p-value	Predictors	$r^2$
			Grain size <1000µm, >500µm (+) Pore Water Metals (+)	
Abundance	3551.5	0.000	Sediment Metals (-)	1.000
			<b>ΣDDT</b> (+)	
			ΣΡΑΗ (-)	
T			Sediment TCDDeq (-)	
Taxon Richness	948.63	0.000	ΣΡСВ (-)	0.999
			Grain size <250µm, > 187.5µm (-)	
Chironomidae	24.45	24.45 0.03 Grain size <250μm, >187.5 ΣΡΑΗ (-)		0.952
Nematoda	58.931	0.000	Sediment TCDDeq (-)	0.959
Oliza aka ata	27.255	0.001	Sediment TCDDeq (+)	0.027
Ongocnaeta	37.233	0.001	Grain size <1000µm, >500µm (-)	0.93/
Ostracoda			No significant Predictors	

	BC	EWQ	NWF	N22	BP	WP	MCU	UPC	SWQ
Boundary Ck (BC)		0.03	0.02	0.03	0.02	0.03	0.06	0.03	0.03
EWQCP (EWQ)			0.03	0.03	0.11	0.09	0.06	0.03	0.20
Nth Water Feat (NWF)				0.02	0.01	0.02	0.06	0.01	0.02
Narawang 22 (N22)					0.03	0.03	0.03	0.03	0.03
Bicent. Pk (BP)						0.03	0.06	0.03	0.11
Wharf Pond (WP)							0.06	0.03	0.03
Macquarie Uni (MCU)								0.03	0.03
Upper Colo (UPC)									0.10
SWQCP (SWQ)									

**Table 4** Matrix of pairwise comparisons of macroinvertebrate assemblages at Sydney Olympic Park (SOP) andreference study sites using ANOSIM. Significantly (p < 0.05) different values in bold.

**Table 5** Relative contribution of the main taxa influencing pairwise dissimilarity between study sites. All pairs of study sites represented are significantly (p<0.05) different from each other. Contribution to dissimilarity is represented categorically; + signifies greater abundance of taxa in study site (a), - signifies greater abundance of taxa in study site (b). 1 symbol > 5%, 2 symbols >6%, 3 symbols >7%, 4 symbols >8%, 5 symbols >9%, 6 symbols >10%. Boundary Ck is included as a representative of the main group of study sites separated by similarity profile permutations (SIMPROF). Other pairwise comparisons have been omitted for clarity.

Study Site (a)	Narawang 22 $\rightarrow$	Upper Colo $\rightarrow$	Narawang 22 $\rightarrow$
Study Site (b)	Upper Colo	Boundary Ck	Boundary Ck
Average	65 72	46.0	56.00
Dissimilarity (%)	03.72	40.9	30.88
Chironominae	+ + + + +		+ + + +
Ostracoda	+ + + +		++
Oligochaeta	+ + +		
Nematoda	+ + + + +		+
Leptophlebidae	++		++
Cladocera			+
Caenidae	+		++
Baetidae	++		++
Copepoda	++		+ + +
Tanypodonidae		+ + + + + +	
Ceratapogonidae		+	++
Hirudinea			