A preliminary ecotoxicological investigation of Bull Creek

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A preliminary ecotoxicological investigation of Bull Creek

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Summary

This study, *A preliminary ecotoxicological investigation of Bull Creek,* was conducted to determine whether contaminants known to be associated with the area are likely to be toxic to biota.

A previous investigation by the Water Science Branch, Department of Water, *A baseline study of contaminants in the sediments of the Swan and Canning estuaries* (Nice 2009), identified Bull Creek as an area that warranted further investigation. This was based on sediment concentrations of zinc, lead, mercury and selenium exceeding environmental guidelines (ANZECC & ARMCANZ 2000; Lemly 1996).

A preliminary ecotoxicological investigation of Bull Creek (this study) targeted two drain sites believed to be potential contaminant sources to Bull Creek, as well as downstream sites from these sources along a gradient through the creek. Sediment samples were collected from four sites within the creek and one site within the Swan River for toxicity analyses. Toxicity analyses were conducted on testing organisms native to the Swan Canning estuary system, which were exposed to field-collected sediment samples in the laboratory.

In summary, this study found that:

- sediments collected in the vicinity of the Bull Creek Main Drain and the Brentwood Main Drain were toxic to test organisms

- sediments collected in the vicinity of Brentwood Main Drain caused the highest degree of toxicity (although this may be due to the cumulative effect of both drains)

- the zone of impact (to organisms such as amphipods, copepods and mussels assessed here) is currently in the order of 300 m or less from the points of discharge to Bull Creek, since no significant toxicity was reported for sites located approximately 300 m or more downstream.

Given that toxicity was identified in Bull Creek, albeit in a localised area, there is justification for further examination of the issues: thus it is recommended that the next stage would investigate catchment disturbance. However, when compared with the extent and magnitude of toxicity and contaminants reported elsewhere in the Swan Canning estuary system such as Claisebrook (Nice & Fisher 2011) and other priority sites recently identified (Nice et al. 2009 & Nice 2009), this future work is considered a lower priority.

1   Introduction

1.1   Background

An assessment of contaminants in the sediments of the Swan and Canning estuaries (Nice 2009) identified Bull Creek as an area that warranted further investigation. This was based on sediment concentrations of zinc, lead, mercury and selenium exceeding environmental guidelines (ANZECC & ARMCANZ 2000; Lemly 1996). An ecotoxicological investigation was subsequently recommended to examine whether contaminants associated with the sediments did indeed have the potential to cause toxic affects to biota.

Two drains (Brentwood Main Drain and Bull Creek Main Drain) are known to discharge into Bull Creek approximately 100 m upstream from where the high levels of contaminants were recorded in the Nice (2009) study. As such, it was decided to assess sediments collected along a gradient away from the drains through the creek to determine the potential toxicity of the sediments and whether there was any pattern in toxicity appearing to originate from the drains.

1.2   Scope and objective

This report presents the results of the whole-sediment toxicity tests to provide an indication of the toxicity of the sediments in this area. It is a preliminary investigation because the budget was limited to ecotoxicological analyses only. However, some sediment chemistry information is available for this site from an earlier study (Nice 2009).

The objective of this investigation was:

- to assess the toxic potential of sediments collected from Bull Creek.

2   Methods

2.1   Site description

All sites in this study were estuarine sites located within Bull Creek (which feeds into the Canning Estuary) and Melville Water in the Swan Estuary (Figure 1 and Figure 2).

The distribution of sites included a gradient away from Bull Creek Main Drain and Brentwood Main Drain through Bull Creek (BC1–BC4) to the Canning Estuary and a reference site located approximately 6 km downstream in Melville Water in the Swan Estuary (BC5). Surficial sediment samples (top 2 cm according to Simpson et al. 2005) were collected from all sites. In addition, at one of the five sites (BC1), a sub-surface sample was also collected (10–20 cm depth).

This general area (BC1–BC4) was sampled in a previous study (Nice 2009), in which comparatively high levels of metals were identified, with some (zinc, mercury and lead) exceeding the Interim Sediment Quality Guidelines (ISQGs) (ANZECC & ARMCANZ 2000) and selenium exceeding the Moderate Hazard Concentration (Lemly 1996)[1].

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[1] Currently there is no ISQG for selenium.

Figure 1 Location of sites in the Swan Canning estuary

Figure 2 Detailed view of sampling sites within Bull Creek and potential contaminant sources

2.2   Field sampling procedure

Samples were collected with Perspex[TM] corers by scuba-assisted divers. Each sample comprised three litres of sediment collected from an area approximately 3 m x 3 m. Samples were preserved in food-standard zip-lock low-density polyethylene bags on ice for toxicity assessment by Ecotox Services Australasia (toxicity test methodology provided in Table 1 to Table 4). Surficial sediment samples comprised the top 2 cm (according to Simpson et al. 2005) and the sub-surface sample comprised the 10 to 20 cm portion of the core.

Temperature, salinity, pH and dissolved oxygen were measured in the water column at each site – between 5 and 20 cm above the sediment surface – before the sediment was disturbed (according to Simpson et al. 2005) by divers using a YSI Inc. (Yellow Springs Instrument) hand-held meter, model no: 6600. This supporting data is provided Appendix B.

2.3   Sediment toxicity test methods

A suite of four toxicity tests was conducted on each sample comprising different test organisms and life stages. Different organisms have varying sensitivities to contaminants due to their differing physiologies (Anderson et al. 2003; USEPA 2002). Further, for any one particular test organism, differing sensitivities to contaminants have been demonstrated from one life stage to the next (Nice et al. 2003; 2001). Each test was conducted in quadruplicate.

The test organisms were the mussel, *Mytilus edulis planulatus* (larvae)*; the* amphipod, *Grandidiella japonica* (adults)*;* the copepod, *Gladioferans imparipes* (adults); and the fish, *Pagrus auratus* (larvae)*.* All four test organisms selected were representative of those found in the Swan Estuary (SRCC 1955; Chubb et al. 1979; Trayler & McKernan 1997). Initially the black bream (*Acanthopagrus butcheri*) was the intended fish test organism because these have been recorded at the test site, but due to non-viable[2] stock cultures, the pink snapper (*Pagrus auratus*) was selected. This species is known to exist elsewhere in the estuary.

Toxicity is the degree to which a substance or combination of substances is able to damage an exposed organism. In this study, different endpoints were employed for different test organisms to represent toxic effects. For the mussel, developmental abnormalities and/or developmental delays were used as a measure of toxicity. Mortality was used as a measure of toxicity for the copepod and amphipod. Imbalance (larval fish unable to maintain an upright position in the water column) was used as a measure of toxicity for the pink snapper.

In the natural situation, the amphipod is a sediment-dweller. Therefore in this investigation, individuals were exposed to whole-sediment. The larvae of the mussel typically move vertically through the water column, but will make contact with the

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[2] There were high mortality rates in the stock cultures.

substrate intermittently due to their negative buoyancy. Hence in this investigation the test selected for the mussel incorporated a sediment-water interface, whereby sediment was present in the bottom of the test vials and overlain by clean seawater, into which the mussel larvae were introduced. The fish larvae and the copepods inhabit the water column. Therefore the tests selected for these organisms were sediment elutriate tests, in which sediments were agitated in clean seawater and the organisms subsequently exposed to the water (elutriate) only. This is considered representative of contaminants leaching from sediments that have been disturbed. Each of these methods was selected to provide the most ecologically relevant conditions. Summaries of the four test methods are provided in Table 1 to Table 4. A detailed description of each method is provided in Appendix A.

For the mussel, copepod and fish tests – in instances where toxicity was experienced with the 100% (i.e. undiluted) test solutions – subsequent dilution-series testing was performed to determine the degree of toxicity experienced. The concentrations of test solution were: 0% (filtered seawater control), 6.3%, 12.5%, 25%, 50% and 100%. It is not possible to perform dilution-series testing for the amphipod because this test is performed using whole-sediment and attempting to dilute whole-sediment with clean sediment would significantly affect the chemistry of the sample and lead to erroneous results.

Temperature, pH, salinity and dissolved oxygen concentrations of the test media were monitored to ensure no adverse conditions were contributing to the test results.

Table 1   Mussel (Mytilus edulis planulatus) test methodology

Test performed:	72-hour larval development test.
Test organism:	Mussel, *Mytilus edulis planulatus.*
Test protocol:	ESA Standard Operating Procedure 106 (ESA 2009a) based on APHA (1998) and USEPA (1996).
Preparation of test solution:	Sediments were prepared according to Puget Sound Estuary Program (PSEP) protocols (PSEP 1995). 18 g (wet weight) of sediment was weighed into 1 L glass jars. 900 mL of 0.45 μm filtered seawater was added to each jar. Jars were capped and shaken vigorously for 10 seconds and placed into a constant environment for approximately four hours to settle before the larvae were added. A filtered seawater control was tested concurrently with the samples.
Test organism life stage and exposure period:	Mussel embryos were exposed to test solutions for 72 hours.
Test endpoint:	Larval development to D-veliger stage*.
Test replicates:	Four
Source of test organism:	Farm-reared, Mercury Passage, Tasmania.

* D-veliger stage is a key developmental stage in bivalve molluscs. Abnormalities or delays in reaching this stage can result in subsequent inhibition of metamorphosis into viable adults (Nice 2000).

Table 2   Copepod (Gladioferans imparipes) test methodology

Test performed:	48-hour acute survival test.
Test organism:	Copepod, *Gladioferans imparipes.*
Test protocol:	Unpublished.
Preparation of test solution:	Sediment elutriates were prepared by combining sediment and filtered seawater in a 1:4 ratio on a volume-to-volume basis according to methods by USEPA (1991). 100 mL of sediment was placed into a 1 L glass beaker and combined with 400 mL of filtered seawater. The mixture was stirred vigorously for 30 minutes with a magnetic stirrer (manually shaken for sandy sediments or those containing large amounts of detritus). After mixing, the mixture was allowed to settle for one hour and the supernatant was collected. The test concentrations of each sample were prepared by serial dilution with filtered seawater. A filtered seawater control was tested concurrently with the samples.
Test organism life stage and exposure period:	Copepod adults were exposed to test solutions for 48 hours.
Test endpoint:	Survival.
Test replicates:	Four
Source of test organism:	Hatchery cultured, WA.

Table 3   Amphipod (Grandidiella japonica) test methodology

Test performed:	10-day whole-sediment survival test.
Test organism:	Amphipod, *Grandidiella japonica.*
Test protocol:	ESA Standard Operating Procedure 109 (ESA 2009b) based on Simpson et al. (2005).
Preparation of test sediments:	Sediments were prepared approximately 24 hours before test initiation by placing 40 g (wet weight) of whole-sediment in 250 mL glass beakers. Toxicity tests were conducted on the whole-sediments without additional dilutions. A clean sediment control was tested concurrently with the samples.
Test organism life stage and exposure period:	Amphipod adults were exposed to test sediments for 10 days.
Test endpoint:	Survival.
Test replicates:	Four
Source of test organism:	Lake Macquarie, NSW.

Table 4   Pink snapper (Pagrus auratus) test methodology

Test performed:	96-hour larval fish imbalance test.
Test organism:	Pink snapper, *Pagrus auratus.*
Test protocol:	ESA Standard Operating Procedure 117 (ESA 2009c) based on USEPA (2002).
Preparation of test solutions:	Sediment elutriates were prepared by combining sediment and filtered seawater in a 1:4 ratio on a volume-to-volume basis according to methods by USEPA (1991). 100 mL of sediment was placed into a 1 L glass beaker and combined with 400 mL of filtered seawater. The mixture was stirred vigorously for 30 minutes with a magnetic stirrer (manually shaken for sandy sediments or those containing large amounts of detritus). After mixing, the mixture was allowed to settle for one hour and the supernatant was collected. The test concentrations of each sample were prepared by serial dilution with filtered seawater. A filtered seawater control was tested concurrently with the samples.
Test organism life stage and exposure period:	Fish larvae were exposed to test solutions for 96 hours.
Test endpoint:	Survival (imbalance).
Test replicates:	Four
Source of test organism:	Hatchery-reared, Fremantle, WA.

2.4 Statistical analyses of toxicity data

Data were first tested for normality and homogeneity of variance using the Shapiro-Wilk's test and the Bartlett's test respectively. Parametric test data (normally distributed with equal variances) were subsequently compared with control data using the Bonferroni adjusted t-test. The results obtained using the Bonferroni t-test were confirmed by performing an independent t-test. Non-parametric test data were compared with control data using Steel's Many-One rank test.

Where high-level toxicity (defined below) was demonstrated and subsequent dilution-series testing was employed to compare a range of test concentrations with the controls, Dunnett's test was applied to parametric data; and Steel's Many-One rank test applied to non-parametric data.

The concentration of the samples affecting 10% and 50% of the test population (EC10/IC10 and EC50 respectively) was determined by the Maximum Likelihood Probit method (parametric data) or Trimmed Spearman Karber and Non-linear Interpolation methods (non-parametric data). The concentration causing no significant toxicity (no observable effect concentration – NOEC) and the lowest concentration causing significant toxicity (lowest observable effect concentration – LOEC) was determined by performing Dunnett's test (parametric data) and Steel's Many-One rank test (non-parametric data).

The statistical analyses were conducted using TOXCALC V5.0 software.

2.5   Categorising the level of toxicity

Sites were divided into three categories according to the degree of toxicity experienced in the samples collected from those sites. The categories were: *no toxicity, low-level toxicity* and *high-level toxicity* and are defined in Table 5.

Table 5   Toxicity categories

Level of toxicity	Criteria for copepod, mussel and fish tests	Criteria for amphipod test
No toxicity	No difference* in response between test and control organisms.	No difference* in response between test and control organisms.
Low-level toxicity	A difference* in response between test and control organisms observed with undiluted sediment elutriate; and no difference observed with subsequent dilution-series testing.	A difference* in response between test and control organisms with <50% of test organisms exhibiting the response.
High-level toxicity	A difference* in response between test and control organisms observed with undiluted sediment elutriate; and a difference* in response observed with dilution series testing in $\leq$50% sediment elutriate concentrations.	A difference* in response between test and control organisms with $\geq$50% of test organisms exhibiting the response.

* differences relate to a statistically significant effect ($p<0.05$)

3   Results

Toxicity was evident in the copepod and mussel tests, with the degree of toxicity experienced depending on the site (Table 6, Figure 3 and Figure 4). Toxicity was not evident in the amphipod tests. Data from larval fish tests have not been presented due to low confidence levels in the data[3].

Table 6   Summary of the toxicity experienced in each test for samples collected from each site.

	Toxicity test		
	Copepod	**Mussel**	**Amphipod**
Site			
BC1surface		X	
BC1sub-surface			
BC2surface	XX	X	
BC3surface			
BC4surface			
BC5surface (reference)			
Control			

Blank cells = no toxicity

X = low-level toxicity

XX = high-level toxicity

Note: the experimental design incorporated the collection of surficial sediment samples from five sites and one sub-surface sediment sample from one of the five sites. All sediment samples referred to in the following sections are surficial samples unless specifically stated otherwise.

Toxicity comparisons across sites

Mussel development was significantly affected by exposure to sediment from the two sites nearest the two drains (Bull Creek Main Drain and Brentwood Main Drain) (Figure 3). The surficial samples collected from these sites (BC1 and BC2) had 53% and 65% normally developed larvae respectively compared with 77% in the controls. However, subsequent dilution did not result in toxicity. Therefore the toxicity was classified as low-level.

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[3] Low confidence levels in the data were due to high mortality rates observed in reference organisms.

There was no significant difference in the number of normally developed mussel larvae from the sub-surface sample collected from site BC1 and the surficial samples collected from sites BC3, BC4 and BC5 when compared with the controls ($p > 0.05$) (Figure 3).

Copepod survival was affected by exposure to the surficial sample collected from site BC2 (near Brentwood Main Drain) where mean percentage survival was 65% compared with 100% in the controls (Figure 4). Toxicity was reported as high-level because there was also a significant reduction in survival in the 50% sediment elutriate concentration where mean percentage survival was 75% compared with 100% in the controls ($p < 0.05$). Refer to the dose-response plot for copepod survival at site BC2 (Figure 5), which shows decreasing copepod survival with increasing sediment elutriate concentration. There was no evidence of toxicity to copepods exposed to sediments collected from any of the remaining sites.

Refer to Table 7 for a summary of toxicity test data for the two sites where toxicity was experienced.
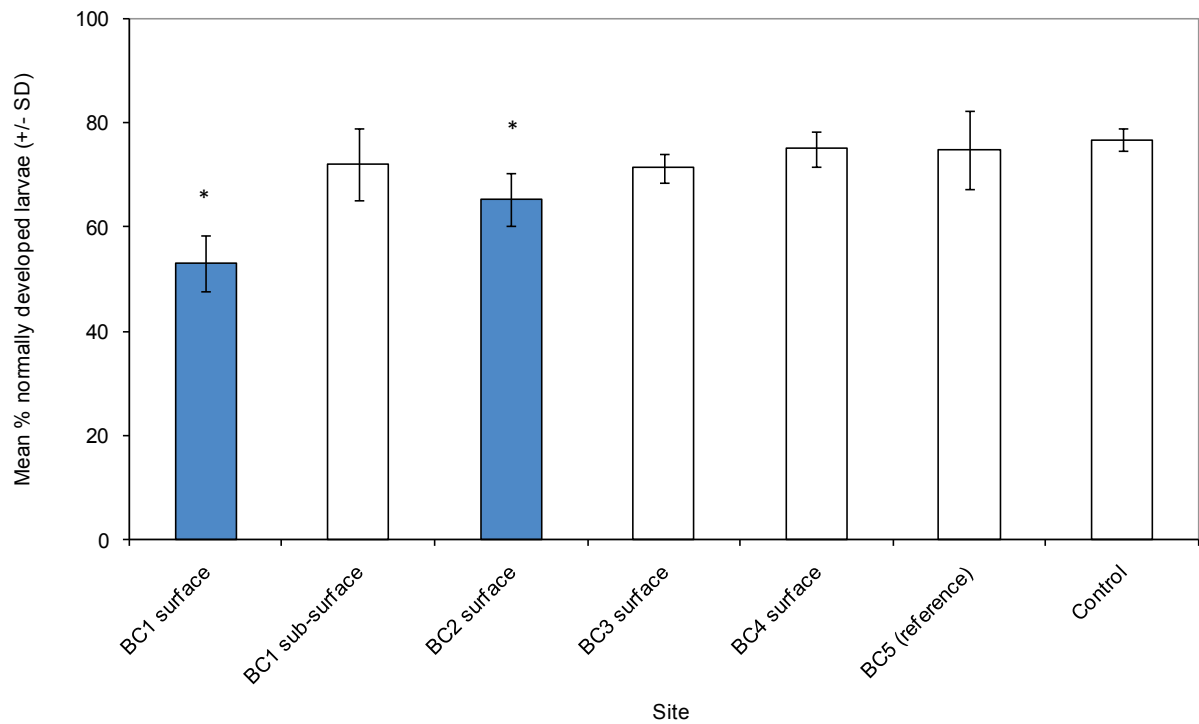
Figure 3 Mean percentage normally developed mussel larvae after 72-hour exposure to sediment elutriates.

*The asterisk * indicates significantly lower percentage of normally developed larvae compared with the control (Bonferroni adjusted t test, 1-tailed, p<0.05) Blue indicates low-level toxicity. White indicates no statistically significant difference in effect between field samples and control.*

Figure 4　Mean percentage copepod survival after 48-hour exposure to sediment elutriates.

*The asterisk * indicates significantly lower percentage survival compared with the control (Steel's Many-One rank test, 1-tailed, p<0.05). Red indicates high-level toxicity. White indicates no statistically significant effect between field samples and control.*
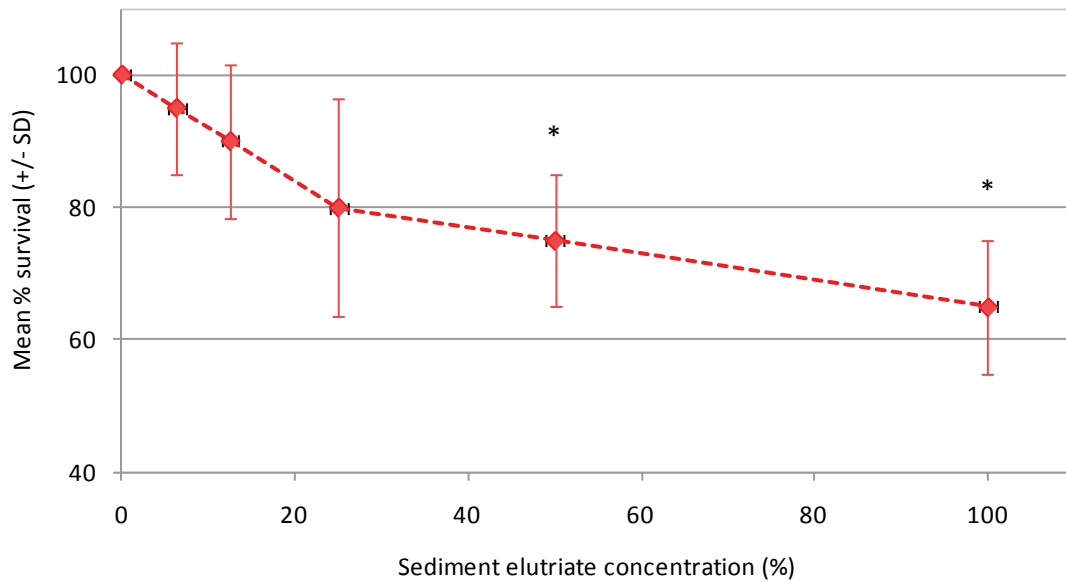
Figure 5 Dose-response plot for site BC2 where high-level toxicity was experienced in copepods.

The asterisk * represents significantly lower percentage of normal larvae compared with the control (Steel's Many-One rank test, 1-tailed, $p < 0.05$).

Toxicity test data

Table 7   Toxicity test data for sites where toxicity was exhibited

	Mussel			
Site	**72-hr EC/IC10 (%)**	**72-hr EC50 (%)**	**NOEC (%)**	**LOEC (%)**
BC1surface	60.3 (47.5–68.7)	>100	50	100
BC2surface	84.6 ^	>100	50	100

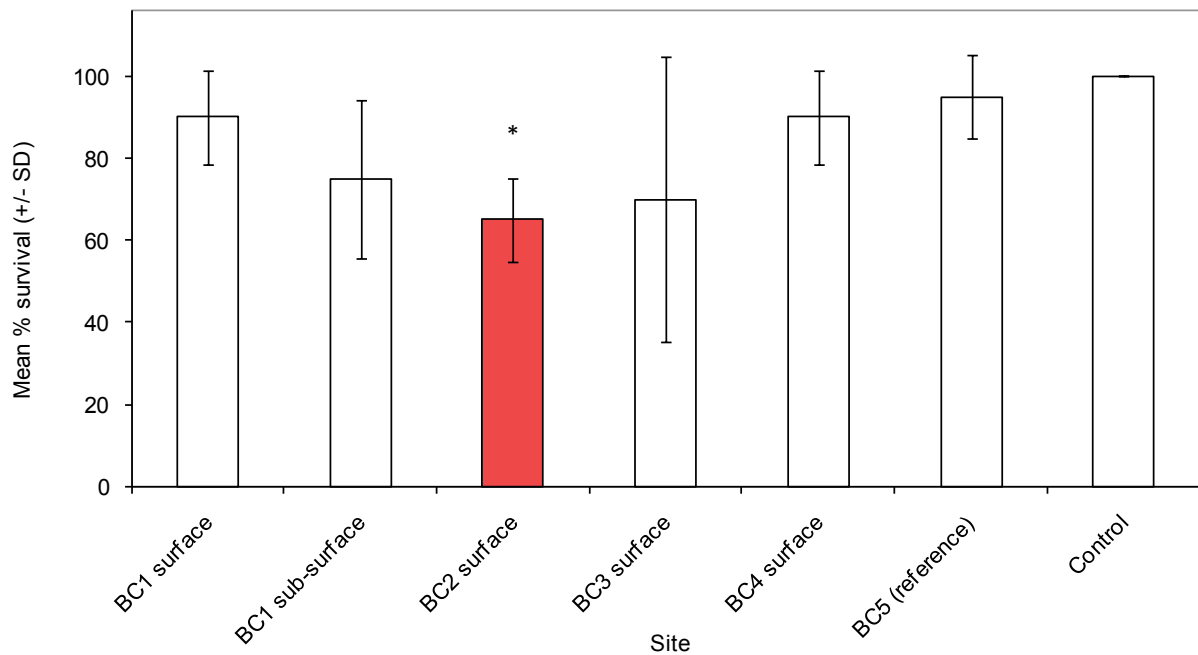|	Copepod			
Site	**48-hr EC/IC10 (%)**	**48-hr EC50 (%)**	**NOEC (%)**	**LOEC (%)**
BC2surface	NC	>100	25	50

EC/IC10 = concentration of sediment elutriate calculated to cause the effect in 10% of test organisms

EC50 = concentration of sediment elutriate which causes the effect in 50% of test organisms (median effect concentration)

NOEC = no observable effect concentration: the highest **tested** concentration at which organisms were unaffected compared with control organisms

LOEC = lowest observable effect concentration: the lowest **tested** concentration at which organisms were adversely affected compared with control organisms

Confidence limits shown in brackets

^ = 95% confidence limits not reliable

NC = not calculable

4   Discussion

All toxicity reported in this study was for organisms exposed to sediment collected from sites BC1 and BC2. These are the sites located closest to the two drains in the area – approximately 100 m downstream from the Bull Creek Main Drain and the Brentwood Main Drain respectively. Samples collected along a gradient from sites located further downstream from these drain sites did not induce toxic effects with the test organisms (based on the endpoints assessed in this study). Of the two sites where toxicity was reported, sediments collected from BC2 were considered to be the most toxic because they were found to result in low-level toxicity to mussel larvae and high-level toxicity to copepod adults. The sediments collected from BC1 were only found to result in low-level toxicity and only to mussel larvae. This is also reflected by sediments collected from site BC2 having the lowest NOEC (no observable effect concentration) and LOEC (lowest observable effect concentration) of the two sites.

It is likely that contaminants present in the discharge from Bull Creek Main Drain are responsible for the toxicity observed at BC1; and that contaminants discharged from both the Brentwood and Bull Creek main drains are responsible for the toxicity reported for site BC2, given the relative location of these drain outfalls to sites and the direction of flow. Earlier studies have shown the presence of contaminants in drain discharge further upstream in the Bull Creek subcatchment, including a range of metals, anionic surfactants, polycyclic aromatic hydrocarbons, petroleum hydrocarbons, microbial parameters such as faecal coliforms and enterococci (Nice et al. 2009), and a broad range of insecticides and herbicides (Foulsham et al. 2009). A third study (Nice 2009) reported metals (zinc, mercury, lead and selenium) present at concentrations exceeding environmental guidelines (ANZECC & ARMCANZ 2000; Lemly 1996) in the sediments of the receiving environment downstream from Bull Creek and Brentwood main drains (i.e. in the vicinity of sites BC1 and BC2). It is likely that any of these contaminants (or combinations of contaminants) reported in both the drains themselves or the downstream receiving environment are responsible for the toxicity observed.

Interestingly, toxicity was not observed with the amphipod test, most likely because contaminants known to affect these organisms such as zinc and copper (King et al. 2006) were unlikely to be present in high-enough concentrations to cause a measurable effect during the 10-day exposure period employed in this investigation. Toxicity was also not observed in the sub-surface sample collected at site BC1 for any of the organisms tested here, indicating that disturbance of the sediment at this site is unlikely to cause significant toxic effects. This may suggest that the contamination to the site is relatively recent; that is, only present in the top fraction of the sediment.

It can be concluded that although the likely main sources of toxic contaminants in Bull Creek are the two drains, their zone of impact (based on the invertebrate data

presented here) is currently in the order of 300 m or less from the points of discharge to Bull Creek, because no significant toxicity was reported for sites located approximately 300 m or more downstream.

Given that toxicity was identified in Bull Creek, further examination of the issues is justified: thus it is recommended that the next stage would investigate catchment disturbance. However, when compared with the extent and magnitude of toxicity and contaminants reported elsewhere in the Swan Canning estuary system such as Claisebrook Cove (Nice & Fisher 2011) and other priority sites recently identified (Nice et al. 2009 & Nice 2009), this future work is considered a lower priority.

Appendices

Appendix A   Toxicity testing methodology

The following method summaries have been provided by Ecotox Services Australasia:

Mussel test

The 72-hour larval development toxicity test using the larvae of the mussel *Mytilus edulis planulatus* was undertaken in accordance with ESA Standard Operating Procedure 106, which is based on methods described by USEPA (1995,1996) and APHA (1998), and adapted for use with *Mytilus edulis* by Krassoi (1995). Tests were performed in a constant temperature chamber of 20±1°C with a 16:8-hour light: dark photoperiod for the entire 72-hour exposure. Clean seawater was collected from the Sydney region and filtered to 0.45μm on return to the laboratory, and used for the maintenance and spawning procedures. Mussels used for the tests were obtained from mussel farms in Tasmania and spawned within six hours of arrival at the laboratory.

Sediments were prepared according to PSEP protocols (USEPA 1995). For each sediment sample, 18 grams of sediment was weighed out into 1 L glass jars, in quadruplicate. An additional replicate was also included for physical and chemical analysis. Nine hundred millilitres of 0.45 μm filtered seawater (FSW) was added to each of the glass jars. The jars were capped and shaken vigorously for 10 seconds, and then placed into a constant environmental chamber for approximately four hours to allow the sediment to settle. In addition, FSW control, consisting of seawater collected from the Sydney region (of 35.4‰), was also tested as a control treatment.

The temperature, pH, salinity and dissolved oxygen concentration of the physico-chemical replicate from each sample was measured. Salinity and conductivity were measured using a WTW LF330 salinity/conductivity meter with a WTW Tetracon 325 probe. The pH and temperature were measured using a WTW pH330 meter, with a WTW SenTix 41 electrode. Dissolved oxygen was measured using a WTW Oxi 330 Oximeter, with a WTW CellOx 325 probe. The pH and dissolved oxygen meters were calibrated each day prior to use, and the salinity/conductivity meter was calibrated on first use each week, with results recorded following each calibration.

Mussels were spawned by gonad stripping, and viable gametes selected on the basis of fertilisation success trials and visual examination of gamete maturity. The eggs were fertilised by adding spermatozoa to the egg suspension such that the final egg: sperm ratio was 1:100. The density of the egg suspension was determined using a Sedgwick-Rafter counting chamber to determine the volume required to achieve a final density of 100 eggs/mL in the test vessels. The test vessels were inoculated with 500±50 eggs within two hours of fertilisation. After 72 hours' exposure, the test was terminated and the pH, salinity and dissolved oxygen concentration of the physico-chemical replicate from each sample was measured, as detailed above. Ten millilitres of the solution was pipetted into vials and the contents preserved in formalin. One millilitre of the preserved test solution was drawn directly from the bottom of each test vessel and placed in a Sedgwick-Rafter counting chamber. The

first 100 oyster larvae were examined and the number of normal and abnormal D-veliger larvae was recorded. These data were used to calculate the percent survival (i.e. those larvae that have developed beyond fertilised eggs, including abnormal larvae, used as a QA measure), percentage normally developed larvae (i.e. the proportion of larvae counted that were normally developed to the D-veliger stage, used as a QA measure), and the percentage of normally developed surviving larvae (used for the assessment of overall toxicity).

Copepod test

The 48-hour acute copepod survival test was undertaken with the adult copepod *Gladioferans imparipes*. Tests were performed in a constant environmental chamber at 18±1°C with a 16:8-hour light: dark photoperiod for the entire 48-hour exposure. Clean seawater was collected from the Sydney region and filtered to 0.45μm on return to the laboratory. Copepods used for the tests were obtained from laboratory cultures and initially sourced from the Seahorse Sanctuary, WA.

Sediment elutriates were prepared by combining sediment and filtered seawater in a 1:4 ratio on a volume-to-volume basis, as outlined by the US EPA (1991). One hundred millilitres of sediment was placed into a 1 L glass beaker and combined with 400 mL of filtered seawater. The mixture was stirred vigorously for 30 minutes with a magnetic stirrer (or manually shaken for sandy sediments or those containing large amounts of detritus). At 10-minute intervals, the mixture was also stirred manually to ensure thorough mixing. After the 30-minute mixing period, the mixture was allowed to settle for one hour before the supernatant was carefully siphoned off without disturbing the sediment. The supernatant represented the 100% solution from which dilutions were prepared.

Toxicity tests were undertaken in 20 mL glass scintillation vials containing 18 mL of test solution. Five concentrations of the sediment elutriate sample were prepared and tested using four replicate vials. The test concentrations were 100, 50, 25, 12.5 and 6.3%. A 0.45 μm filtered seawater (FSW) control, consisting of seawater collected from the Sydney region (of 35.4‰), representing the diluent routinely used by the laboratory, was also tested as a control treatment.

The temperature, pH, salinity and dissolved oxygen concentration of a representative sample from each concentration/treatment was measured. Salinity and conductivity were measured using a WTW LF330 salinity/conductivity meter with a WTW Tetracon 325 probe. The pH and temperature were measured using a WTW pH330 meter, with a WTW SenTix 41 electrode. Dissolved oxygen was measured using a WTW Oxi 330 Oximeter, with a WTW CellOx 325 probe. The pH and dissolved oxygen meters were calibrated each day prior to use, and the salinity/conductivity meter was calibrated on first use each week, with results recorded following each calibration.

Adult copepods were removed from cultures and separated from nauplii by sieving through a 120–150 μm mesh. A concentrated stock of adult copepods was then used for transferring adult copepods into test vessels. Five healthy adult copepods were placed into each test vessel using a microscope prior to the addition of test solutions. Test solution was gently poured into corresponding test vessels immediately after the addition of five healthy

copepods. After 48 hours the test was terminated and the surviving amphipods were counted under the microscope. The pH, salinity and dissolved oxygen concentration of a representative sample from each concentration/treatment was measured, as detailed above.

Amphipod test

The 10-day acute survival toxicity test using the amphipod *Grandidiella japonica* was undertaken with ESA Standard Operating Procedure 109, which is based on methods described by Hyne et al. (2005) and Spadaro et al. (2008). Tests were performed in a constant environmental chamber at 20 ± 1°C with a 16:8-hour light: dark photoperiod for the entire 10-day exposure. Clean seawater was collected from the Sydney region and filtered to 0.45μm on return to the laboratory. Amphipods used for the tests were obtained from laboratory cultures.

Sediments were prepared approximately 24 hours prior to the initiation of toxicity tests by placing 40 g of homogenised sediment into 250 mL glass beakers. Toxicity tests with the whole-sediments, without additional dilutions, were run in quadruplicate. An additional replicate was used for physico-chemical analysis. The sediment was distributed along the bottom of the beaker by gently tapping the beakers against the palm of the hand. Overlying water consisting of filtered seawater (~35‰) was carefully added to each of the beakers to give a final approximate volume of 200 mL. The beakers were then covered with cling wrap and placed in an environmental chamber at 20±1°C overnight to equilibrate and allow suspended particles to settle out. On the day of testing, the overlying water from each of the test beakers was removed by gently siphoning with rubber tubing or a plastic syringe. Fresh overlying water was added gently by pouring down the sides of the beaker. A clean sediment control was tested concurrently with the samples.

The temperature, pH, salinity and dissolved oxygen concentration of the physico-chemical replicate from each sample was measured. Salinity and conductivity were measured using a WTW LF330 salinity/conductivity meter with a WTW Tetracon 325 probe. The pH and temperature were measured using a WTW pH330 meter, with a WTW SenTix 41 electrode. Dissolved oxygen was measured using a WTW Oxi 330 Oximeter, with a WTW CellOx 325 probe. The pH and dissolved oxygen meters were calibrated each day prior to use, and the salinity/conductivity meter was calibrated on first use each week, with results recorded following each calibration.

Amphipods were removed from culture trays and 10 of approximately 3 to 8 mm in length were placed into plastic weigh boats. Groups of 10 amphipods were then randomly placed into the overlying water of each test beaker. The test beakers were then covered and placed back into an environmental chamber where the overlying water was gently aerated for the duration of the test. At the termination of the test, the surviving amphipods were counted by wet sieving the contents of each beaker through a 180 μm stainless steel mesh. The pH, salinity and dissolved oxygen concentration of the physico-chemical replicate of each sample was also measured, as detailed above.

Fish test

The 96-hour toxicity tests using fish larvae were undertaken with the pink snapper, *Pagrus auratus*. Tests were undertaken in accordance with ESA Standard Operating Procedure 117, which is based on methods described by USEPA (1994), ISO 7346-1, and OECD Method 203. Research with vertebrates in the state of NSW is subject to the *Animal Research Act 1985*, and the toxicity test with larval fish was performed by ESA under the Animal Research Authority issued to ESA by the Director-General of NSW Department of Primary Industries (valid from 27 May 2008 to 27 May 2010) and Certificate of Approval from the Animal Care and Ethics Committee of the Director-General of the NSW Department of Primary Industries (valid from 16 May 2008 to 16 May 2010).

Larval fish of approximately 5 to 8 mm in length used for the tests were obtained from a hatchery in Fremantle, Western Australia. The larval fish were shipped overnight by express courier service in a foam box containing an ice brick and fish were contained within an air inflated bag containing approximately 4 L of seawater. The fish were transferred to an environmental chamber of 25°C on arrival, and provided gentle aeration using a Schego air pump. Clean seawater for holding the larval fish was collected from the Sydney region and filtered to 0.45μm on return to the laboratory, and used for holding fish. The seawater was acclimated to the appropriate temperature prior to use.

Sediment elutriates were prepared by combining sediment and filtered seawater in a 1:4 ratio on a volume-to-volume basis as outlined by the US EPA (1991). One hundred millilitres of sediment was placed into a 1 L glass beaker and combined with 400 mL of filtered seawater. The mixture was stirred vigorously for 30 minutes with a magnetic stirrer (or manually shaken for sandy sediments or those containing large amounts of detritus). At 10-minute intervals, the mixture was also stirred manually to ensure thorough mixing. After the 30-minute mixing period, the mixture was allowed to settle for one hour before the supernatant was carefully siphoned off without disturbing the sediment.

Toxicity tests were undertaken in 20 mL glass scintillation vials containing 18 mL of test solution. Five concentrations (100, 50, 25, 12.5 and 6.3%) of the sediment elutriate samples were prepared and tested using four replicate vials. A 0.45 μm filtered seawater (FSW) control, consisting of seawater collected from the Sydney region (of 35.4‰), representing the diluent routinely used by the laboratory, was also tested as a control treatment.

The temperature, pH, salinity and dissolved oxygen concentration of a representative sample from each concentration/treatment was measured. Salinity and conductivity were measured using a WTW LF330 salinity/conductivity meter with a WTW Tetracon 325 probe. The pH and temperature were measured using a WTW pH330 meter, with a WTW SenTix 41 electrode. Dissolved oxygen was measured using a WTW Oxi 330 Oximeter, with a WTW CellOx 325 probe. The pH and dissolved oxygen meters were calibrated each day prior to use, and the salinity/conductivity meter was calibrated on first use each week, with results recorded following each calibration.

Five fish were introduced into each of the test vials. The beakers were covered with cling-wrap film to minimise evaporation and placed in a constant temperature chamber of 20°C. The test vessels were monitored three times per day to examine fish for signs of distress or imbalance. Fish demonstrating such signs were removed and euthanased in accordance

with ESA SOP 117. Test vessels were also checked daily for dissolved oxygen concentration, with aeration to be provided should the dissolved oxygen concentration fall below 60% saturation, however this was not required. The beakers were examined every 24 hours and the number of surviving and apparently healthy larval fish recorded. The test was terminated after seven days, and the pH, salinity and dissolved oxygen concentration of a representative sample from each concentration/treatment was measured, as detailed above. At the termination of the test, the larval fish were euthanased by the addition of Aqui-S fish anaesthetic directly into each test vessel.

Appendix B   In-situ water quality data

Table 8 shows in-situ water quality data collected from all sediment chemistry and toxicity sites in the water column 5 to 20 cm above the sediment surface (according to Simpson et al. 2005).

Table 8   In-situ water quality data

Site code	Temperature (°C)	Salinity (ppt)	pH	Dissolved oxygen (%)	Dissolved oxygen (mg/L)
BC1	23.26	37.01	7.7	80	5.5
BC2	23.11	36.40	7.7	86	5.9
BC3	22.56	36.84	7.6	82	5.7
BC4	22.51	36.92	7.7	83	5.8
BC5	23.23	37.40	7.9	89	6.1

Shortened forms

AHPA　　　　　American Public Health Association

ANZECC　　　Australia and New Zealand Environment and Conservation Council

ARMCANZ　　Agriculture and Resource Management Council of Australia and New Zealand

DEWHA　　　Department of the Environment, Water, Heritage and the Arts (Australian Government)

EPA　　　　　Environmental Protection Authority (WA)

ESA　　　　　Ecotox Services Australasia

PSEP　　　　Puget Sound Estuary Program

SRRC　　　　Swan River Reference Committee

SRT　　　　　Swan River Trust

Glossary

Ecotoxicology	The integration of toxicology and ecology. Ecotoxicology aims to quantify the effects of stressors upon natural populations, communities, or ecosystems.
EC10	Concentration of sediment elutriate which causes the described effect in 10% of test organisms.
EC50	Concentration of sediment elutriate which causes the described effect in 50% of test organisms (median effect concentration).
High-level toxicity	Statistically significant effect (statistically significant difference from the control organisms; $p<0.05$); *and* when subsequent dilution-series testing was performed, the statistically significant effect was observed with $\leq 50\%$ sediment elutriate concentration. [Definition determined for this study].
IC10	Concentration of sediment elutriate calculated (by non-linear interpolation) to cause the described effect in 10% of test organisms.
ISQGs	Interim Sediment Quality Guidelines (Australian and New Zealand Environment and Conservation Council and Agriculture and Resource Management Council of Australia and New Zealand – ANZECC & ARMCANZ 2000). The *low ISQG* is the concentration below which the frequency of adverse biological effects is expected to be low. The *high ISQG* is the concentration above which adverse biological effects are expected to occur more frequently.
Low-level toxicity	Statistically significant effect (statistically significant difference from the control organisms; $p<0.05$) observed with undiluted sediment elutriate concentration but there was no such effect when subsequent dilution series testing was performed. [Definition determined for this study].
LOEC	Lowest observable effect concentration: the lowest tested concentration at which organisms are adversely affected compared with control organisms.
NOEC	No observable effect concentration: the highest tested concentration at which organisms are unaffected compared to control organisms.
No toxicity	No statistically significant effect (i.e. no statistically significant difference in response by the test organisms from the control organisms; $p>0.05$).
Toxicity	The degree to which a substance or combination of substances is able to damage an exposed organism. In this study, different

endpoints were employed for different test organisms to represent toxic effects:

Mussel 72-hour larval development test: developmental abnormalities or developmental delays were used as a measure of toxicity.

Copepod 48-hour survival test: mortality was used as a measure of toxicity.

Amphipod 10-day whole-sediment survival test: mortality was used as a measure of toxicity.

Fish 96-hour larval imbalance test: imbalance (fish unable to maintain an upright position in the water column) was used as a measure of toxicity.

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