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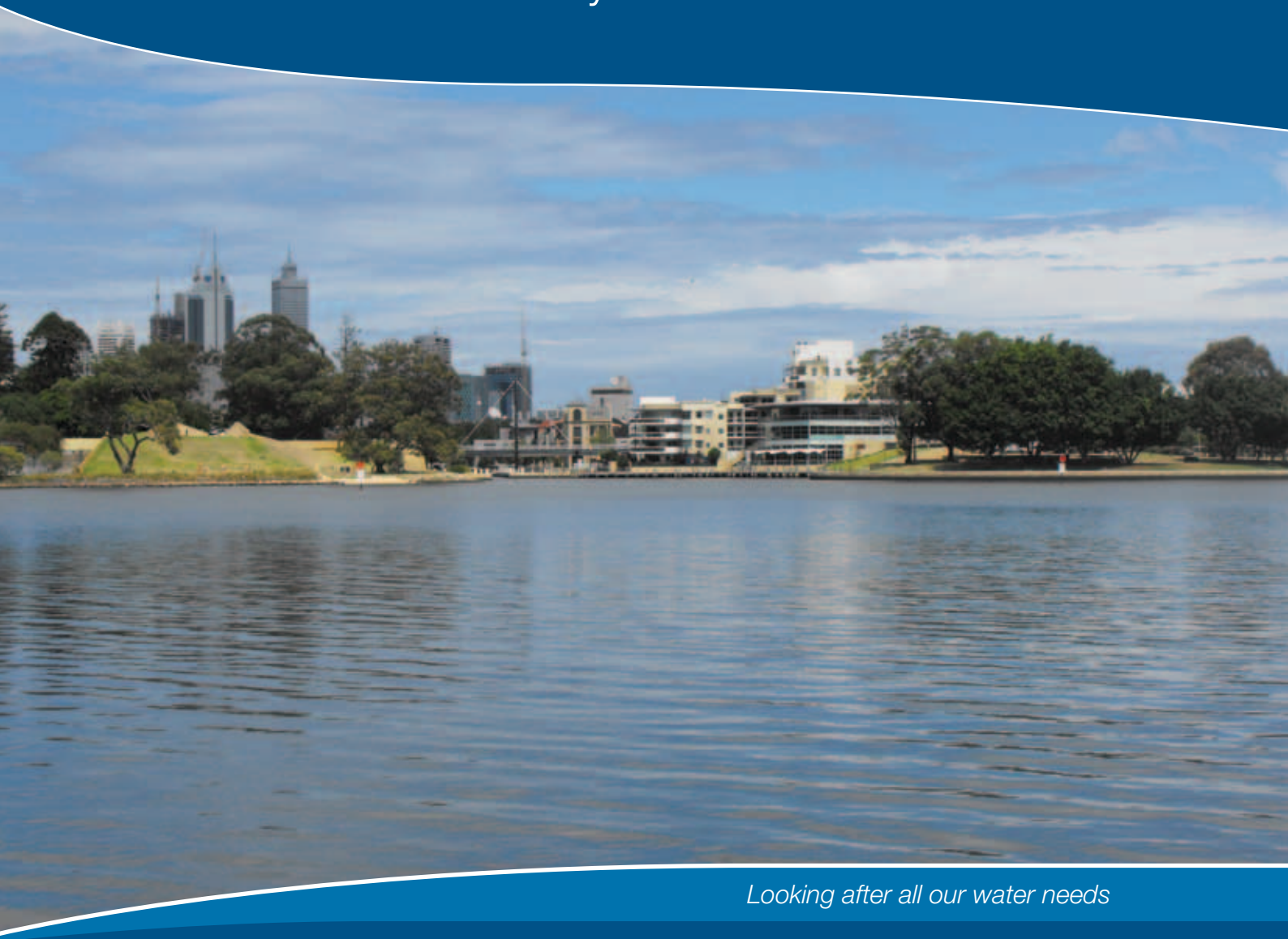
Department of **Water**

Ecotoxicological and bioaccumulation investigations of the Swan Estuary in the vicinity of Claisebrook



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Report no. WST 28
August 2011

Ecotoxicological and bioaccumulation investigations of the Swan Estuary in the vicinity of Claisebrook

Department of Water

Water Science Technical Series

Report no. 28

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August 2011

ISSN 1836-2869 (print)
ISSN 1836-2877 (online)

ISBN 978-1-921736-48-3 (print)
ISBN 978-1-921736-49-0 (online)

Acknowledgements

This project was funded by the Government of Western Australia through the Swan River Trust and the Department of Water.

Citation details

The recommended citation for this publication is:

Nice, HE & Fisher, SJ 2011, *Ecotoxicological and bioaccumulation investigations of the Swan Estuary in the vicinity of Claisebrook,* Water Science Technical Series, Report no. 28, Department of Water, Western Australia.

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Disclaimer

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Summary

This study, *Ecotoxicological and bioaccumulation investigations of the Swan Estuary in the vicinity of Claisebrook,* was conducted to determine whether contaminants known to be associated with the area are likely to be *a)* toxic to and *b)* bioaccumulating in biota. It is the intention that the Swan River Trust use the information contained in this report to develop options for management of the Swan Estuary in this area.

A previous investigation by the Department of Water's Water Science Branch, *A baseline study of contaminants in the sediments of the Swan and Canning estuaries* (Nice 2009), recommended that this study be undertaken. In the previous assessment, Claisebrook Cove was identified as an area that warranted further investigation based on the concentrations of particular organochlorine (OC) pesticides and metals exceeding environmental guidelines. Additionally, out of 20 sites investigated, the Claisebrook site had consistently the highest concentrations of all polycyclic aromatic hydrocarbons (PAHs), all OC pesticides (except one) and among the highest for the metals targeted by the study. As such, it was recommended that a comprehensive investigation incorporating whole-sediment toxicity tests and in-situ bioaccumulation studies be conducted.

The Swan Estuary in the vicinity of Claisebrook, including Claisebrook Cove, receives drainage from a catchment impacted by a variety of land uses typical of an inner city area. *Ecotoxicological and bioaccumulation investigations of the Swan Estuary in the vicinity of Claisebrook* (this study) targeted the potential sources of contamination at the site including the Claisebrook Drain and Claisebrook Diversion Drain; and downstream sites from these source(s) incorporating a gradient study design through the cove (parallel to a barrier wall separating the cove from an historic contaminated site) and beyond into the Swan Estuary.

Sediment samples were collected from four sites within Claisebrook Cove and eight sites within the Swan Estuary for both toxicity and chemical analyses. Toxicity analyses were conducted on four testing organisms native to the estuary: the blue mussel (*Mytilus edulis planulatus*), the amphipod (*Grandidiella japonica*), the copepod (*Gladioferans imparipes*), and the pink snapper (*Pagrus auratus*). These test organisms were exposed in the laboratory to field-collected sediment samples. The chemical analyses targeted contaminant groups previously demonstrated to be present at levels of concern in the Claisebrook area (Nice 2009): the PAHs, OC pesticides and metals.

In addition, naturally growing mussels were collected from three sites within Claisebrook Cove and three sites in the adjacent Swan Estuary for whole-tissue analyses for the same contaminant groups.

In summary, this study found that:

- Samples collected in the vicinity of Claisebrook Drain and Claisebrook Diversion Drain were considered to cause the highest degree of toxicity in the testing organisms compared with other samples.

- The evidence suggests that different contaminants (or combinations thereof) are responsible for the toxicity observed for samples collected from these two sites.
- In addition to the two drains, it is likely there are other sources of toxic contaminants to this area of the Swan Estuary.
- The toxicity experienced by test organisms in this study is not completely explained by the three contaminant groups targeted by the analyses (PAHs, OC pesticides and metals). Other contaminants are therefore likely to be contributing to the observed toxicity.
- Chemical analyses of the sediments showed the presence of a range of PAHs in concentrations that warrant further investigation at several sites within the adjacent Swan Estuary; and it was evident from the spatial distribution of PAH contamination that a source other than the two drains may be at least partly responsible.
- The metals and OC pesticides recorded at concentrations of concern (lead, zinc and p,p'-DDE in particular) were fairly evenly distributed throughout the study area and were not attributable to any one source, although Claisebrook Drain (discharging periodically within Claisebrook Cove) did appear to be a potential source of these contaminants (and also dieldrin and *trans*-chlordane), along with diffuse sources in the area such as runoff and the likely historic signature of these contaminants within the sediments. These contaminants were considered to be contributing to some of the toxicity experienced by the testing organisms in this study.
- The metals and two OC pesticides (dieldrin and p,p'-DDE) were also found to be accumulating in the mussels collected from the study area. While the concentrations of these did not exceed maximum levels or extraneous residue limits for any of the contaminants for which these are specified in the Australia New Zealand Food Standards Code (FSANZ 2009), the resulting ecological effects of this level of bioaccumulation remain unknown since environmental guidelines for bioaccumulation are not yet available.

Overall, the spatial distribution of the sites where toxicity was experienced, and the concentrations of potentially toxic contaminants in the sediments reported in this study, warrant further targeted investigation to establish sources of the contamination and the extent of the likely impact.

1 Introduction

1.1 Background

An assessment of contaminants in the sediments of the Swan and Canning estuaries (Nice 2009) identified the estuary in the vicinity of Claisebrook as an area that warranted further investigation. This was based on the concentrations of the organochlorine (OC) pesticides, dieldrin and p,p'-DDE, and the metals, zinc, lead and copper exceeding environmental guidelines. Additionally, out of 20 sites investigated, the Claisebrook site had the highest concentrations of all polycyclic aromatic hydrocarbons (PAHs), all OC pesticides (except one) and among the highest for the metals targeted by the study.

Two other sites in the vicinity of Claisebrook were also categorised as high priority areas in the Nice 2009 study: Maylands, located in the estuary approximately 600 m to the north of Claisebrook Cove, adjacent to the former East Perth Power Station; and Burswood, located in the estuary approximately 1.2 km south east of Claisebrook Cove near a disused landfill site and golf course. While contaminants measured at both of these neighbouring sites exceeded environmental guidelines, the Claisebrook site contained markedly higher concentrations of all PAHs than either of these, and was the only site where the environmental guideline for copper was exceeded.

The land immediately to the north of Claisebrook Cove, is the site of the former East Perth Gasworks. The gasworks operated between 1922 and 1971 generating gas from coal. After the gasworks were decommissioned, the site became a State Energy Commission of Western Australia (SECWA) services depot. In 1989 SECWA commenced an assessment of contamination associated with the site. It was acknowledged in 1992 that the site (in addition to the adjacent Claisebrook Drain and Swan Estuary) was extensively contaminated by coal tar and its derivatives – including a broad range of carcinogenic and toxic compounds such as PAHs – and was regarded as a seriously contaminated industrial site (EPA 1992). The contaminated zone extended from approximately 50 m north to 250 m south of the gasworks site, over a distance of approximately 600 m including the western half of the Swan Estuary extending to at least 0.5 m sediment depth in the centre of the contaminated zone (Bowman Bishaw Gorham 1992). The East Perth Redevelopment Authority has subsequently redeveloped the site, whereupon an artificial canal-type waterway has been created at the outlet of the Claisebrook Drain. The waterway is surrounded by both domestic (1,450 homes) and retail properties (EPRA 2009). In accordance with the Conditions of Approval for the redevelopment, extensive remediation was conducted including the replacement of 30 000 m^3 of PAH-contaminated sediment from the Swan River (to a depth of 1 m below the riverbed level) with 12 200 m^3 of clean fill in September 1994 (Tingay & Associates 1994, cited by Trayler & McKernan 1997). A barrier wall was constructed along the southern boundary of the gasworks site prior to dewatering for canal construction.

In the 2009 baseline study of contaminants in the sediments of the Swan and Canning estuaries (Nice 2009), the Claisebrook site was located in the remediated

zone, yet surficial sediments exhibited comparatively high levels of a range of contaminants (including PAHs) – indicating a recent or ongoing source of pollution to the area.

As such, a comprehensive investigation at Claisebrook Cove and the adjacent estuary was recommended (Nice 2009), following the multiple-lines-of-evidence approach proposed by Chapman et al. (1997) and incorporating whole-sediment toxicity tests, in-situ bioaccumulation studies, an assessment of sediment chemistry and an assessment of fish health. It was recommended that these studies target the potential sources of contamination at the site including the Claisebrook Drain and the Claisebrook Diversion Drain; and downstream sites from these potential source(s) incorporating a gradient study design through the cove (parallel to the barrier wall) and beyond into the Swan Estuary.

1.2 Scope

This report presents the results of the whole-sediment toxicity tests, the in-situ bioaccumulation studies and the assessment of sediment chemistry conducted by the Water Science Branch, Department of Water. The assessment of the health of fish collected from Claisebrook Cove measured physiological responses to indicate deleterious effects of contaminants in the cove and is presented in a separate report (Rawson et al. in prep). The latter study was conducted as a collaborative research project between Curtin University and the Water Science Branch, Department of Water through the Swan Canning Research and Innovation Program funded by the Swan River Trust.

1.3 Objectives

- To assess the toxic potential of sediments collected from Claisebrook Cove and the adjacent Swan Estuary.
- To determine whether PAHs, OC pesticides and metals (known to be present in the sediments) are accumulating in mussels inhabiting Claisebrook Cove and the adjacent Swan Estuary.

    To determine the concentrations of these chemicals (if present) in the mussels and the likely sources where possible.

2 Methods

2.1 Site description

All sites in this study were estuarine sites located within the Swan Estuary including Claisebrook Cove (Figure 1 and Figure 2). Surficial sediment samples (top 2 cm according to Simpson et al. 2005) were collected from 12 sites. At three of the 12 sites, a sub-surface sample was also collected (10–20 cm depth). The distribution of sites included a gradient away from Claisebrook Drain through Claisebrook Cove (CBC01–CBC04) to the Swan Estuary (CBC07); a site adjacent to Claisebrook Diversion Drain in the Swan Estuary (CBC08); and sites upstream and downstream of Claisebrook Cove, adjacent to the shoreline on the western side of the Swan Estuary, CBC05 and CBC06 being adjacent to the former East Perth Gasworks site and CBC09 further downstream. In addition, two comparison sites were located further from the cove in the main channel of the Swan Estuary (CBC10 and CBC11) and a reference site unaffected by potential contaminants from the gasworks or drains (CBC18) was located approximately 6 km downstream in Melville Waters. Note: CBC07, in the Swan Estuary adjacent to the mouth of Claisebrook Cove, was the same site sampled in a previous study (Nice 2009) where comparatively high levels of PAHs were identified and some metal and OC pesticide concentrations exceeded Interim Sediment Quality Guidelines (ISQGs) (ANZECC & ARMCANZ 2000).

Mussels (*Xenostrobus* sp.) were collected from six sites (CBC12–CBC17). Sites CBC12, CBC13 and CBC14 (within Claisebrook Cove) followed a gradient away from Claisebrook Drain. Sites CBC15 and CBC16 were located within the Swan Estuary upstream and downstream from Claisebrook Cove on the western edge of the river and a comparison site (CBC17) was located approximately 1.5 km upstream from Claisebrook Cove also on the western edge of the Swan River.

Figure 1 Location of sites in the Swan Estuary.

Figure 2 Detailed view of sampling sites around Claisebrook Cove and potential contaminant sources.

2.2 Sediment toxicity and chemistry

Field sampling procedure

Samples were collected with Perspex^{TM} corers by scuba-assisted divers. Each sample comprised four litres of sediment collected from an area approximately 3 m x 3 m. Three litres of sediment was preserved in a food-standard zip-lock low-density polyethylene (LDPE) bag on ice for toxicity assessment by Ecotox Services Australasia, NSW (toxicity test methodology provided in Table 1 to Table 4). A 500 mL portion of sediment was preserved in a glass jar on ice for chemical analysis by the National Measurement Institute (NMI), WA; with a further 500 mL of sediment preserved in a food-standard LDPE bag on ice for particle-size analysis by CSIRO Minerals, WA (sediment chemistry and particle-size methodology provided in Table 5). Surficial sediment samples comprised the top 2 cm (according to Simpson et al. 2005) and the sub-surface samples comprised the 10 to 20 cm portion of the core.

Sediment toxicity test methodology

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;* the amphipod, *Grandidiella japonica;* the copepod, *Gladioferans imparipes;* and the fish, *Pagrus auratus.* All four test organisms selected were representative of those found in the Swan Estuary (SRRC 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 within the cove itself, but due to non-viable 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 substrate from time to time 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 with overlying

clean seawater, into which the mussel larvae were introduced. The fish larvae and the copepod both inhabit the water column. Therefore the test selected for these organisms was a sediment elutriate test, in which sediment was agitated in clean seawater and the organism 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 concentration 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 prior to the addition of larvae. 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.

Statistical analyses of toxicity data

Initially 100% test concentrations and whole-sediment were compared with controls using Bonferroni adjusted t-test (parametric data – confirmed using the Kolmogorov D test and Bartlett's test for normality and homogeneity of variance respectively). The results obtained using the Bonferroni t test were confirmed by performing an independent t test.

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

The concentration of the samples affecting 50% of the test population (EC50) 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 Observed Effect Concentration – NOEC) and the lowest concentration causing significant toxicity (Lowest Observed 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.

Sediment chemistry and particle-size methodology

Sediment samples were homogenised within a controlled laboratory environment according to method AS 4482.1-1997 (Standards Australia 1997). Table 5 lists the analytical methods and limits of reporting for each of the parameters.

Table 5 Sediment chemistry and particle-size methodology

Parameter	Description	Analysis method	Limit of Reporting
Particle-size analysis	Determination of the particle-size distribution of sediment. Particles grouped into the following size classes according to the Wentworth scale: <4 µm (clay) <62 µm (silt) <250 µm (fine sand) <500 µm (medium sand) <2000 µm (coarse sand) <10 000 µm (gravel)	Sieving followed by laser diffraction (Mudroch et al. 1997).	n/a
Moisture content	Determination of the percentage of water present in the sediment sample.	Gravimetric measurement of weight loss.	n/a
Bioavailable metals	Measurement of bioavailable metals suite: Al, As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, Zn Units: mg/kg dry sediment.	Analysis of dried sediment sample for a range of metals using a cold dilute acid extraction (0.5–1.0 M hydrochloric acid in a sediment:acid ratio of 1:50 for one hour – according to ANZECC & ARMCANZ 2000).	Lowest available (0.2 mg/kg for mercury; 0.5 mg/kg for other metals)
Polycyclic aromatic hydrocarbons (PAHs)	Measurement of PAH suite: Naphthalene Acenaphthylene Acenaphthene Fluorene Phenanthrene Anthracene Fluoranthene Pyrene Benz[a]anthracene Chrysene Benzo[b]and[k]fluoranthene Benzo[a]pyrene Indeno[1,2,3-cd]pyrene Dibenz[ah]anthracene Benzo[ghi]perylene Units: mg/kg dry sediment.	GC-MS, GC-FID analysis (USEPA 8080/8140 1983, 1996e; APHA 1998).	Lowest available (0.01 mg/kg)
Organochlorine (OC) pesticides	Measurement of OC pesticide suite: HCB HCH(BHC) Lindane (gamma-BHC) Heptachlor Heptachlor Epoxide Chlordane Alpha Endosulphan Beta Endosulphan Endosulphan Sulphate Aldrin Dieldrin Endrin p,p'-DDE p,p'-DDD p,p'-DDT Methoxychlor Total OCs Units: mg/kg dry sediment.	GC-MS, GC-ECD analysis (USEPA 8080/8140 1983, 1996e; APHA 1998).	Lowest available (0.01 mg/kg)

Parameter	Description	Analysis method	Limit of Reporting
Total organic carbon (TOC)	Measurement of TOC within the sediments, required for normalisation of organic compound data to 1% organic carbon in accordance with guidelines (ANZECC & ARMCANZ 2000). Units: mg/kg dry sediment.		n/a

Supporting in-situ water quality data

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 Instruments) hand-held meter, model no: 6600. This supporting data is provided Appendix B.

2.3 Bioaccumulation of contaminants by mussels

Field sampling procedure

Mussels *(Xenostrobus* sp.*)* were collected from six sites from the Swan River, the locations of which are shown in Figure 2. The mussels were sampled on the same date (2 July 2009) opportunistically at low tide, with most samples collected from approximately 0.5 to 1 m below the high water mark. At each site approximately 500 specimens were collected and placed into an LDPE bag and immediately chilled on ice. When the sampling program was complete, all specimens were stored in a freezer at approximately -20 ^{o}C for approximately three weeks, pending processing in the laboratory in accordance with the protocol for sample collection and storage recommended by the laboratory.

As shown in Figure 3, the samples were divided to form three subsamples for subsequent chemical analysis: the first for the determination of a suite of metals including aluminium (Al), arsenic (As), cadmium (Cd), chromium (Cr), cobalt (Co) copper (Cu), lead (Pb), manganese (Mn), mercury (Hg) and zinc (Zn); the second for determination of lipid and moisture content; and the third for the determination of organic compounds, including PAHs, petroleum hydrocarbons and OC pesticides. Each subsample consisted of 41–145 specimens, depending on the amount of tissue required for each determination, and ranging in length between 1.16 and 3.63 cm. This information is summarised in Appendix B. Each subsample of mussels was shucked separately using appropriate implements to avoid contamination to the determination for which they were intended. The organics subsample was shucked using only metal implements, with the mussel tissue placed into a glass jar; the metals subsample was shucked using plastic or teflon-lined implements and placed into a LDPE bag; and the moisture and lipid content subsample was shucked using only metal implements and placed into a LDPE bag.

The tissue was frozen as soon as possible after removal from the shell and was stored this way until immediately before processing at the laboratory (NMI) prior to chemical analysis. Processing entailed homogenising the entire sub-sample, then removing an aliquot sufficient for the respective analyses.

Chemical analysis of mussel tissue

A brief description of each analysis is as follows:

- the moisture content and lipid content were determined gravimetrically as the weight loss upon drying and the weight of material extracted by Soxhlet extraction using hexane respectively
- the organics were determined using gas chromatography – mass spectrometry techniques following extraction of the tissue using an organic solvent mixture and chromatographic cleanup
- the petroleum hydrocarbons were determined semi-quantitatively in a portion of the organic extract isolated at NMI using GC-MS techniques performed at the CSIRO Land and Water Perth Laboratory (Floreat, WA)
- the metals were determined using inductively coupled plasma techniques following acid digestion of the tissue.

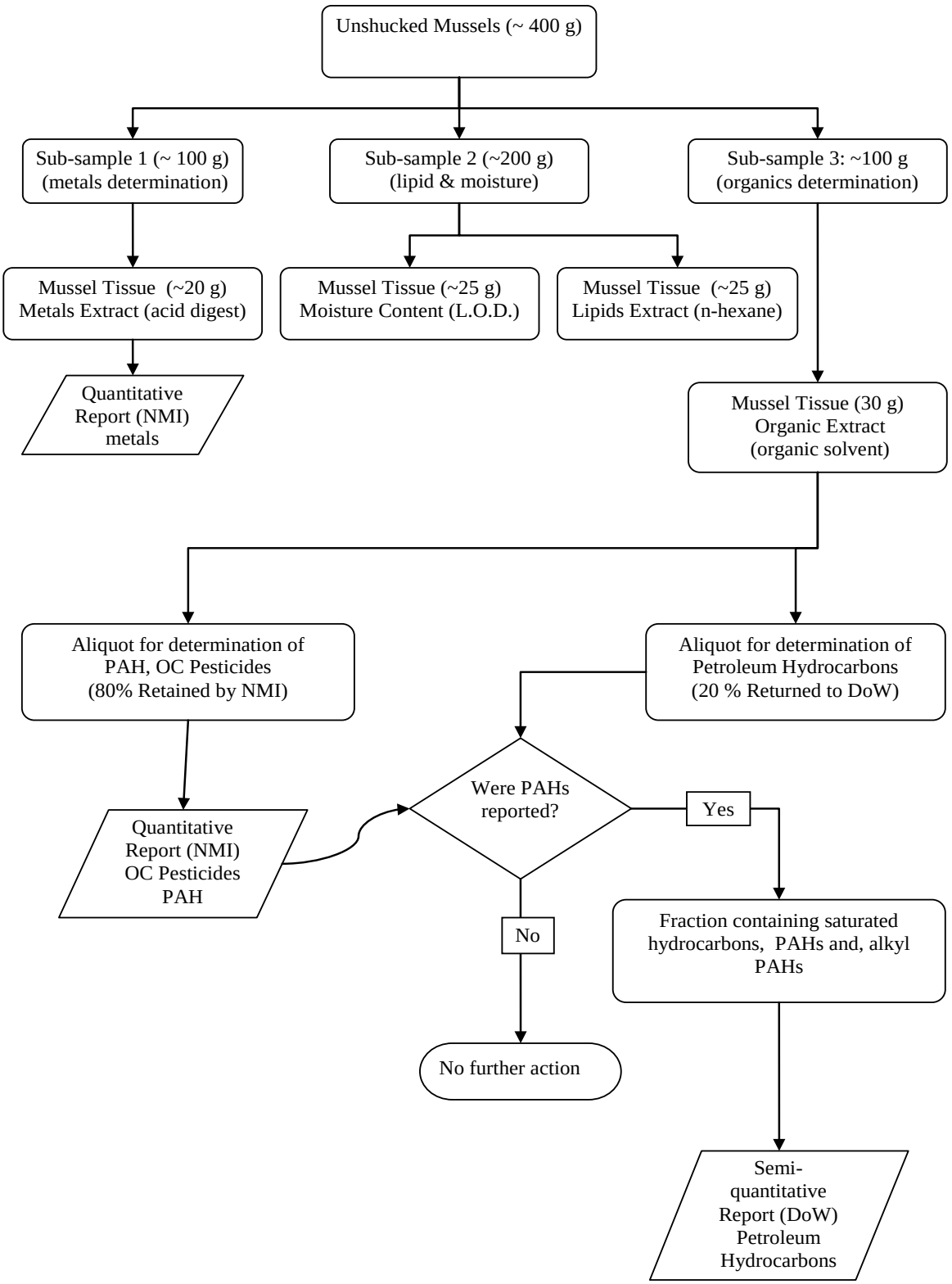

Figure 3 Flow chart for the collection and chemical analysis of mussels from the vicinity of Claisebrook Cove.

2.4 Application of guidelines

Sediment chemistry data were compared with the Interim Sediment Quality Guideline trigger values (ISQGs) from the 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. OC pesticide and PAH data were normalised to 1% organic carbon prior to comparison with the guidelines as prescribed by Simpson et al. (2005). For individual contaminants where ISQGs have not yet been established, Ontario Sediment Quality Guidelines (Ontario Ministry of Environment and Energy 1993) were applied.

There are no quality guidelines for mussel tissue analogous to the ISQGs to indicate ecological health. Instead, where available, the maximum levels (MLs), generally expected levels (GELs) and extraneous residue limits (ERLs) of these contaminants in molluscs – as specified in the Australia New Zealand Food Standards Code (FSANZ 2009) – are shown for comparison. The MLs are set to manage risk to public health and safety from dietary exposure, whereas the intent of GELs is to provide a benchmark against which to measure contaminant levels in food for metal contaminants that pose a low risk to the consumer. An ERL is defined as the maximum permitted limit of a pesticide residue arising from environmental sources other than the use of a pesticide directly or indirectly on the food.

While it is not the intent of this study to investigate the suitability of the Claisebrook Cove mussels for human consumption, comparison with the MLs, GELs and ERLs is useful in the absence of guidelines for ecological health.

3 Results

A summary of the sediment toxicity, sediment chemistry and mussel tissue bioaccumulation results follows:

Summary

For those toxicity test organisms and endpoints used in this study:

- toxicity was experienced in all four test organisms and the degree of toxicity experienced was dependent on site
- high-level toxicity was experienced at sites CBC01, CBC03, CBC05, CBC07, CBC08, CBC10 and CBC11
- the highest degree of toxicity was experienced at the two drain sites: Claisebrook Drain (CBC01) and Claisebrook Diversion Drain (CBC08)
- low-level toxicity was experienced at sites CBC09 and CBC18
- no toxicity was experienced at sites CBC02, CBC04 and CBC06.

For the contaminants in sediments targeted by this study:

- some bioavailable metals, organochlorine (OC) pesticides and polycyclic aromatic hydrocarbons (PAHs) were present in sediment collected from all sites
- most of the metals were detected above the limits of reporting at all sites (mercury was not present above the limits of reporting at any site)
- lead and zinc were present in concentrations higher than the low Interim Sediment Quality Guideline trigger values (ISQGs) at all sites except CBC03 and CBC08
- zinc was also present in concentrations above the high ISQG at CBC01, CBC02, CBC04 and CBC09
- copper exceeded the low ISQG at CBC02
- most of the PAHs were present in concentrations above the limit of reporting at all sites
- PAHs were present in concentrations above the low ISQGs at sites CBC05, CBC06, CBC07, CBC08 and CBC09
- PAHs were present in concentrations above the high ISQGs at sites CBC05 and CBC06
- the OC pesticide p,p'-DDE was present above the limits of reporting and the low ISQG at all sites except CBC08, CBC11 and CBC18
- the OC pesticide dieldrin was present above the limits of reporting and the low ISQG at CBC01, CBC02 and CBC09
- *trans*-chlordane was present above the limits of reporting and the low ISQG at CBC01.

For the contaminants in mussels targeted by this study:

- all metals except mercury were present in concentrations above the limits of reporting in mussels collected from all sites
- the concentrations of metals did not exceed the median generally expected levels with the exception of selenium in the sample (CBC13)
- PAHs were not detected above the limits of reporting in mussels collected from any site
- the OC pesticides dieldrin and p,p'-DDE were present in concentrations above the limits of reporting in mussels collected from all sites
- the concentrations did not exceed maximum levels (MLs) or extraneous residue limits (ERLs) for any of the contaminants for which these are specified in the Australia New Zealand Food Standards Code (FSANZ 2009).

Note: the experimental design incorporated the collection of surficial sediment samples from 12 sites and one sub-surface sediment sample from each of three of the 12 sites. All sediment samples referred to in the following sections are surficial samples unless specifically stated otherwise.

3.1 Sediment toxicity

Toxicity was evident for all test organisms and the degree of toxicity experienced was dependent on site (Table 6 and Figure 4 to Figure 7).

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.*

No toxicity was defined as no statistically significant effect (i.e. no statistically significant difference in response by the test organisms from the control organisms; $p>0.05$).

Low-level toxicity was defined as a statistically significant effect (statistically significant difference from the control organisms; $p<0.05$) observed with undiluted sediment elutriate concentration, but no such effect occurred when subsequent dilution-series testing was performed.

High-level toxicity was defined as a 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.

Dilution-series testing cannot be conducted for the amphipod test because the amphipod is a sediment dweller and is exposed to whole-sediment from which it is impossible to effectively perform a dilution-series. In this case, *low-level toxicity* was defined as a statistically significant effect (significantly lower mean percentage survival compared with the control; $p<0.05$) with mean percentage survival being > 50%. *High-level toxicity* was defined as a statistically significant effect (significantly lower mean percentage survival compared with the control: $p<0.05$) with mean percentage survival being $\leq$ 50%.

Toxicity comparisons across sites

Copepod survival was only affected for the sub-surface sample collected from site CBC08 and toxicity was reported as low-level (Figure 4) because subsequent dilution-series testing showed significant effects with the undiluted elutriates only (mean percentage survival 55% compared with 100% in the controls).

Low-level toxicity was also reported for samples collected from sites CBC05 and CBC09 for the amphipod test (mean percentage survival in whole-sediment was 72.5% and 65% respectively compared with 97.5% in the controls) (Figure 5).

Mussel development was affected for seven sites (Figure 6). Low-level toxicity was reported for samples collected from sites CBC07, CBC09, CBC18 and the sub-surface samples collected from CBC08 and CBC10 (mean percentage of normally developed mussels ranged between 28.4% and 45.8% for the undiluted sediment elutriate compared with 80.7% in the controls). High-level toxicity was reported for both the surface and subsurface samples collected from site CBC01 (mean percentage of normally developed mussels was 16.9% and 5.5% respectively in the

undiluted sediment elutriate). And subsequent dilution-series testing showed significant differences between the 25% sediment elutriate and the 50% sediment elutriate and the controls for the surface and sub-surface samples respectively (also refer to the dose-response plot – Figure 8).

Samples collected from sites CBC03, CBC05, CBC07, CBC10, CBC11 and the sub-surface sample from CBC08 were toxic to fish larvae (there was zero larval survival at all sites except CBC05 where larval survival was 5% compared with 95% survival in the controls). Toxicity was reported as high-level for all these sites (except CBC05), because subsequent dilution-series testing showed significant effects at sediment elutriate concentrations ranging between 12.5% and 50% (also refer to the dose-response plot – Figure 9).

A summary of the toxicity experienced across sites is presented in Table 6.

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

	Toxicity test			
	Copepod	**Amphipod**	**Mussel**	**Fish**
Site				
CBC01surface			**XX**	
CBC01sub-surface			**XX**	
CBC02surface				
CBC03surface				**XX**
CBC04surface				
CBC05surface		**X**		**XX**
CBC06surface				
CBC07surface			**X**	**XX**
CBC08surface				
CBC08sub-surface	**X**		**X**	**XX**
CBC09surface		**X**	**X**	
CBC10surface				**XX**
CBC10sub-surface			**X**	
CBC11surface				**XX**
CBC18surface (Ref)			**X**	
Control				

Blank cells = no toxicity

X = low-level toxicity

XX = high-level toxicity

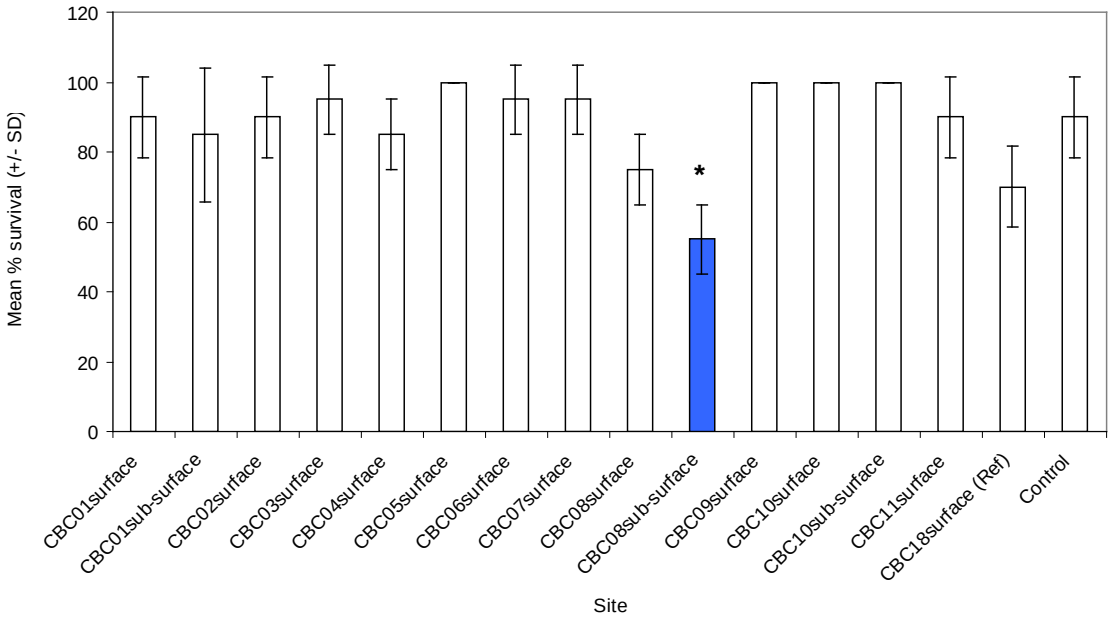

Figure 4 Mean percentage copepod survival after 48-hour exposure to sediment elutriates.

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

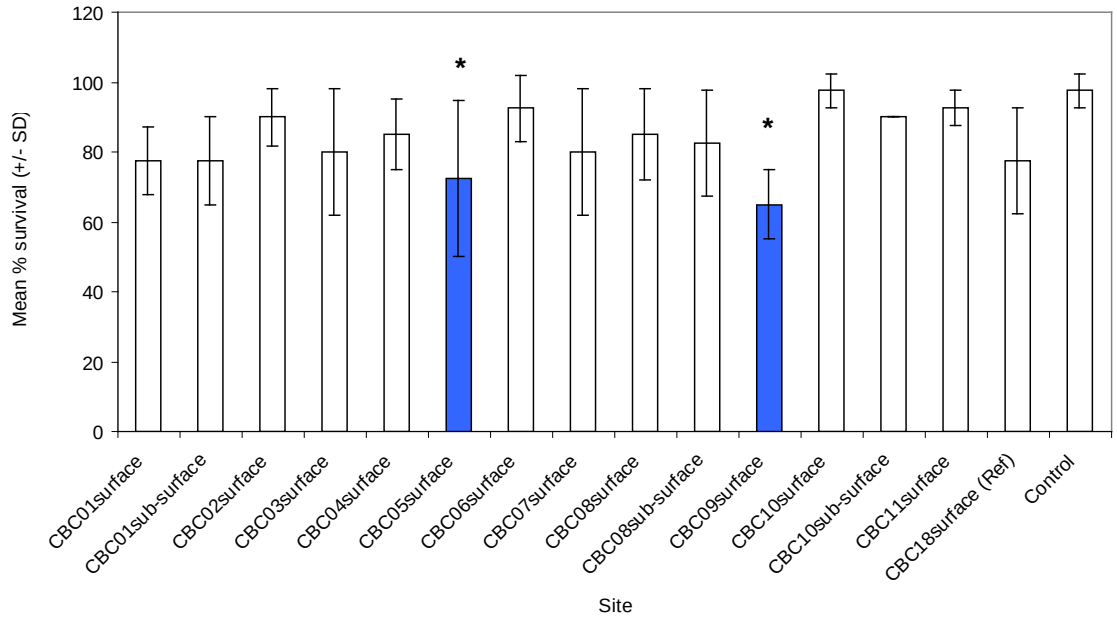

Figure 5 Mean percentage amphipod survival after 20-day exposure to whole-sediment.

*The asterisk * indicates significantly lower percentage survival 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.*

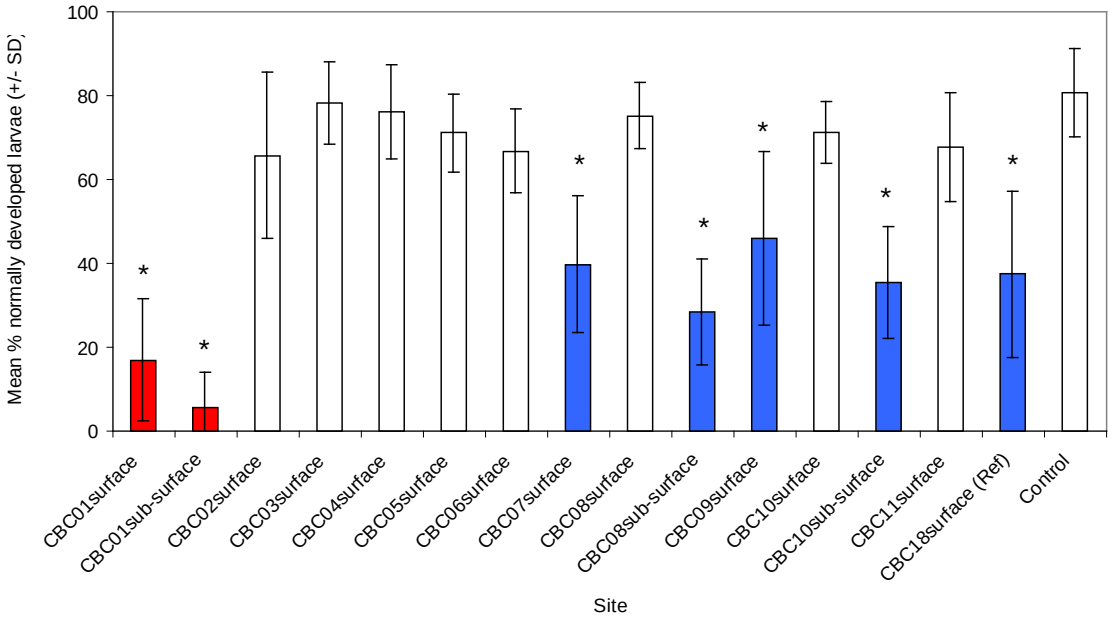

Figure 6 Mean percentage normally developed mussel larvae after 72-hour exposure to sediment elutriates.

*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. Red indicates high-level toxicity. White indicates no statistically significant difference in effect between field samples and control.*

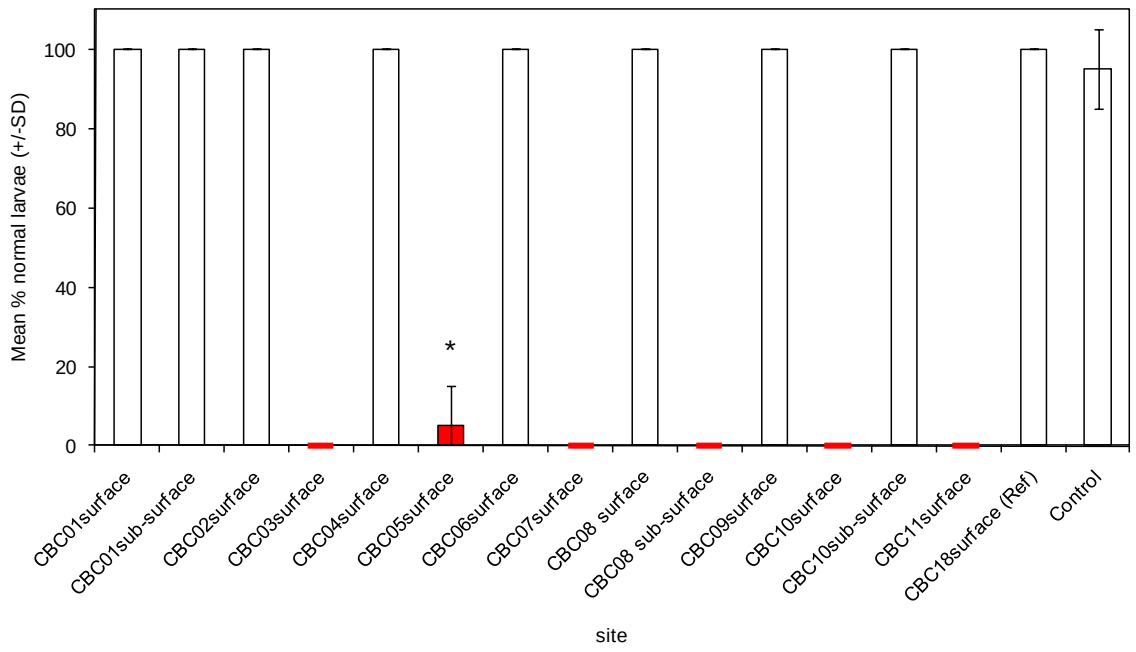

Figure 7 Mean percentage normal fish larvae after 96-hour exposure to sediment elutriates.

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

Dose-responses for sites where high-level toxicity was demonstrated

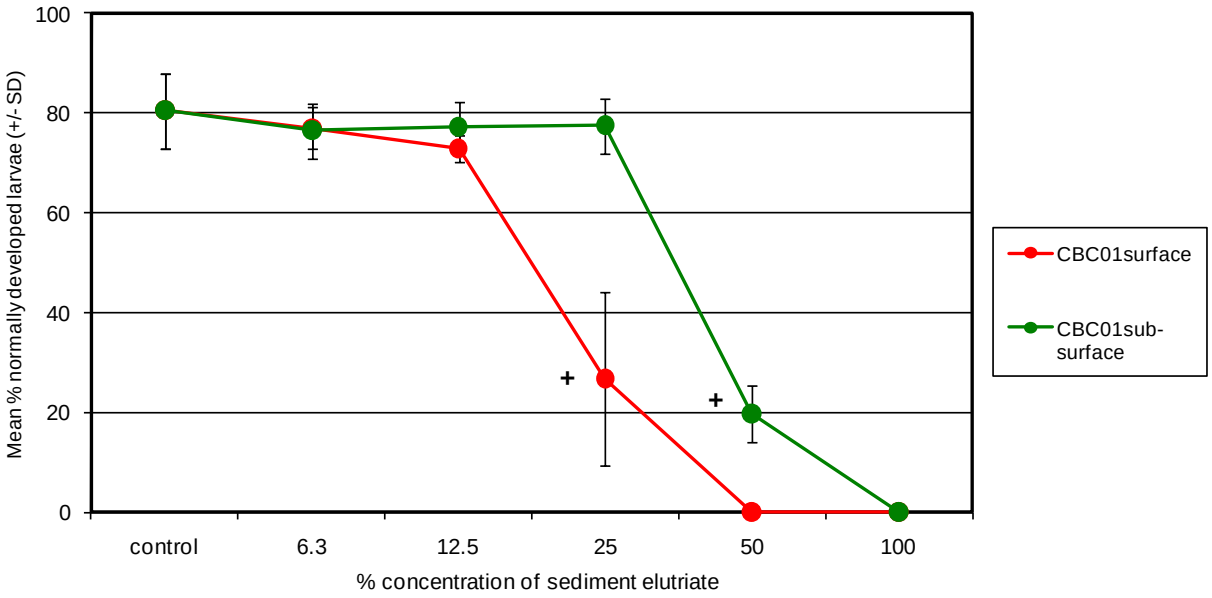

Figure 8 Dose-response plot for sites where high-level toxicity was experienced in mussel larvae.

The symbol $^{+}$ represents significantly lower percentage of normal larvae compared with the control (Dunnett's test, 1-tailed, p<0.05).

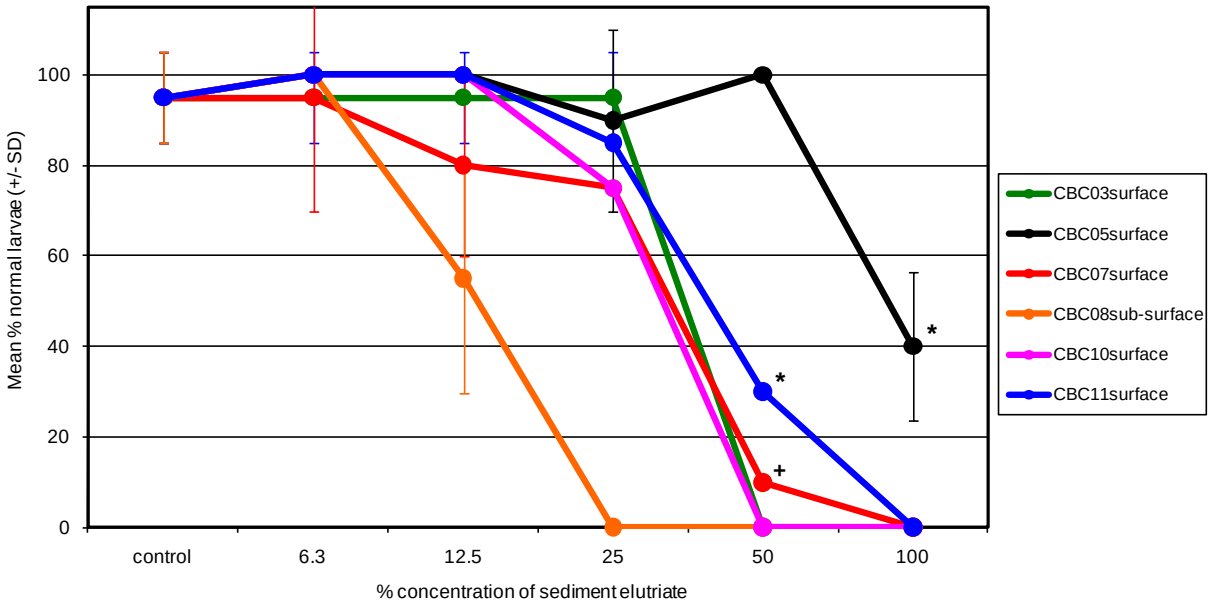

Figure 9 Dose-response plot for sites where high-level toxicity was experienced in fish larvae.

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

Toxicity data

Test data for sites where high-level toxicity was exhibited indicate that sediments collected from CBC01 (surface sample) and CBC08 (subsurface sample) caused the greatest toxicity to the test organisms used in this study (Table 7).

For the surficial sample collected from site CBC01, a 15% concentration of sediment elutriate (i.e. a 1 in 6.67 dilution of the elutriate) is likely to result in 10% mussel larvae abnormalities (relative to the control). A 22% concentration of sediment elutriate is likely to result in 50% mussel larvae abnormalities (relative to the control). From the test concentrations used in this study, a 12.5% concentration of sediment elutriate was shown to cause no observable effect; and the lowest concentration that induced a statistically significant effect was 25% (Table 7 and Figure 8).

For the subsurface sample collected from site CBC08, a 9.2% concentration of sediment elutriate is likely to result in 10% of fish mortalities (relative to the control). A 13% concentration of sediment elutriate is likely to result in 50% fish mortalities (relative to the control). From the test concentrations used in this study, a 6.3% concentration of sediment elutriate was shown to cause no observable effect; and the lowest concentration that induced a statistically significant effect was 12.5% (Table 7 and Figure 9).

Table 7 Toxicity test data for sites where high-level toxicity was exhibited

		Mussel		
Site	**72 hr EC/IC10 (%)**	**72 hr EC50 (%)**	**NOEC (%)**	**LOEC (%)**
CBC01surface	15.0 (12.5–16.8)	22.0 (20.4–23.1)	12.5	25
CBC01sub-surface	33.3^	43.5^	25	50

		Fish		
Site	**96 hr EC/IC10 (%)**	**96 hr EC50 (%)**	**NOEC (%)**	**LOEC (%)**
CBC03	26.7 (4.0–28.1)	35.4% (graphical method)	25	50
CBC05	60.3 (2.1–98.0)	89.1 (71.0–111.8)	50	100
CBC07	22.4 (12.2–28.4)	33.8 (26.0–41.0)	25	50
CBC08sub-surface	9.2 (7.8–12.4)	13.0 (11.2–15.2)	6.3	12.5
CBC10surface	20.1 (17.9–25.2)	30.0 (26.3–34.2)	25	50
CBC11	24.2 (14.8–30.3)	39.7 (32.1–47.9)	25	50

Highlighted rows indicate the sites with the highest sediment toxicity based on the test organisms used in this study

EC10 = Concentration of sediment elutriate which causes the effect in 10% of test organisms

IC10 = Concentration of sediment elutriate calculated by non-linear interpolation 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

3.2 Sediment chemistry

Chemistry data are presented for bioavailable metals, PAHs and OC pesticides in Table 8 to Table 10 respectively.

Bioavailable metals

For this discussion, the term bioavailable metals is operationally defined as those metals that are extracted from the sediment using a cold diluted acid solution, consistent with what is described in ANZECC and ARMCANZ (2000). In this way, the metals loosely bound to the surface of sediment particles are extracted (rather than those tightly bound in the mineral matrix) – this method being more indicative of the environmental contamination of the sediment.

Most of the metals assessed in this study were present in concentrations above the limit of reporting at all sites (Table 8). Mercury was not present above the limit of reporting at any site. Concentrations of zinc and lead exceeded ISQGs for all sites except CBC03 and CBC08. In the case of sites CBC01, CBC02, CBC04 and CBC09, the high ISQG was also exceeded for zinc. Copper exceeded the low ISQG at site CBC02.

PAHs

Most of the PAHs assessed in this study were present in concentrations above the limit of reporting at all sites (Table 9). At site CBC06, concentrations of every PAH assessed in this study exceeded ISQGs (for those PAHS where ISQGs are available). Acenaphthylene, pyrene and benz[a]anthracene concentrations also exceeded the high ISQG at this site.

At site CBC05, concentrations of most of the PAHs targeted by this study exceeded ISQGs. Pyrene concentrations also exceeded the high ISQG at this site.

Samples collected from sites CBC07, CBC09 and the surficial sample from CBC08 each had concentrations of a single PAH higher than the low ISQGs. Whereas the sub-surface sample collected from site CBC08 contained concentrations of most of the PAHs targeted by this study at levels higher than the low ISQGs.

OC pesticides

Of the suite of pesticides determined, only *trans*-chlordane, dieldrin and p,p'-DDE were present in concentrations above the limit of reporting. p,p'-DDE was present at most sites in concentrations that exceeded the low ISQG. Dieldrin was present at CBC01, CBC02 and CBC09 in concentrations that exceeded the low ISQG and the *trans*-chlordane concentration exceeded the low ISQG in the sub-surface sample only at site CBC01.

Table 8 Sediment metal concentrations (bioavailable).

*Limits of reporting for all metals except mercury: 0.5 mg/kg; limit of reporting for mercury: 0.1 mg/kg. n.d. = not detected in concentrations greater than the limit of reporting; n.a. = ISQG not available; * alternative guidelines for cobalt, manganese and selenium of 50, 1100 and 2 mg/kg respectively (Ontario Sediment Quality Guidelines 1993 & Lemly 1996) were also not exceeded.*

	Sediment metal concentrations (bioavailable) mg/kg											
	Aluminium	Arsenic	Cadmium	Chromium	Cobalt*	Copper	Lead	Manganese*	Mercury	Nickel	Selenium*	Zinc
Site												
CBC01$_{surface}$	2620	0.7	0.8	11.0	2.2	14.0	120.0	35.0	n.d.	5.1	0.8	740.0
CBC01$_{sub\text{-}surface}$	1820	1.1	0.7	6.8	1.2	32.0	130.0	18.0	n.d.	3.3	0.6	760.0
CBC02$_{surface}$	3900	3.4	0.7	16.0	5.0	72.0	130.0	110.0	n.d.	6.1	0.9	660.0
CBC03$_{surface}$	1360	2.0	n.d.	5.0	2.5	22.0	42.0	65.0	n.d.	2.0	n.d.	180.0
CBC04$_{surface}$	3370	9.6	n.d.	13.0	6.4	62.0	100.0	210.0	n.d.	4.8	0.9	430.0
CBC05$_{surface}$	3220	3.5	n.d.	11.0	5.5	36.0	87.0	170.0	n.d.	3.5	0.5	360.0
CBC06$_{surface}$	3070	3.9	n.d.	10.0	5.1	35.0	81.0	160.0	n.d.	3.4	0.6	340.0
CBC07$_{surface}$	2640	2.0	n.d.	8.7	4.0	29.0	67.0	110.0	n.d.	3.0	n.d.	300.0
CBC08$_{surface}$	170	0.7	n.d.	0.8	n.d.	6.9	10.0	39.0	n.d.	n.d.	n.d.	50.0
CBC08$_{sub\text{-}surface}$	150	n.d.	n.d.	0.7	n.d.	3.9	9.7	3.6	n.d.	n.d.	n.d.	30.0
CBC09$_{surface}$	2690	4.6	0.7	11	4.6	59.0	110.0	200.0	n.d.	4.1	0.6	460.0
CBC10$_{surface}$	3400	3.0	n.d.	12	5.3	27.0	72.0	290.0	n.d.	3.4	0.6	330.0
CBC10$_{sub\text{-}surface}$	3580	2.3	n.d.	12	5.6	28.0	76.0	170.0	n.d.	3.2	n.d.	350.0
CBC11$_{surface}$	2690	2.2	n.d.	8.6	4.1	25.0	54.0	170.0	n.d.	3.1	n.d.	260.0
CBC18$_{surface\ (Ref)}$	3180	6.4	n.d.	11	7.0	50.0	66.0	460.0	n.d.	3.5	n.d.	310.0
ISQG Low	n.a.	20.0	1.5	80.0	n.a.	65.0	50.0	n.a.	0.15	21.0	n.a.	200.0
ISQG High	n.a.	70.0	10.0	370.0	n.a.	270.0	220.0	n.a.	1.0	52.0	n.a.	410.0

Table 9 Sediment polycyclic aromatic hydrocarbon (PAH) concentrations.

Limit of reporting: 10 µg/kg; n.d. = not detected in concentrations greater than the limit of reporting; n.a. = ISQG not available.

	Sediment polycyclic aromatic hydrocarbon concentrations (µg/kg) normalised to 1% organic carbon*														
	Naphthalene	Acenaph-thylene	Acenaph-thene	Fluorene	Phenanthrene	Anthracene	Fluoranthene	Pyrene	Benz[a]a-nthracene	Chrysene	Benzo[b]&[k]-fluoranthene	Benzo[a]-pyrene	Indeno[1,2,3-cd]pyrene	Dibenz[ah]-anthracene	Benzo(ghi)-perylene
Site															
CBC01$_{surface}$	1.84	7.13	n.d	n.d	17.24	13.79	63.22	65.52	25.29	28.74	67.82	29.89	12.64	4.37	20.69
CBC01$_{sub-surface}$	3.00	5.91	n.d	n.d	18.18	14.55	46.36	51.82	20.91	22.73	49.09	21.82	9.09	3.27	11.82
CBC02$_{surface}$	3.77	8.99	n.d	4.78	23.19	18.84	94.20	133.33	55.07	28.99	66.67	37.68	17.39	5.51	21.74
CBC03$_{surface}$	4.38	12.19	n.d	n.d	21.56	11.56	75.00	121.88	56.25	31.25	56.25	37.50	19.38	6.88	19.69
CBC04$_{surface}$	4.23	26.92	n.d	3.27	38.46	32.69	169.23	250.00	107.69	78.85	230.77	92.31	8.08	2.88	7.88
CBC05$_{surface}$	100.00	583.33	91.67	83.33	250.00	291.67	1500.00	2916.67	1250.00	675.00	1083.33	833.33	216.67	83.33	216.67
CBC06$_{surface}$	336.36	1090.91	454.55	418.18	1454.55	1000.00	3909.09	7090.91	3000.00	1454.55	2545.45	2090.91	490.91	190.91	500.00
CBC07$_{surface}$	8.53	25.88	5.59	47.06	32.35	19.71	88.24	147.06	64.71	50.00	105.88	70.59	26.76	7.94	26.47
CBC08$_{surface}$	n.d	43.10	n.d	n.d	162.07	60.34	465.52	465.52	327.59	206.90	396.55	224.14	82.76	34.48	74.14
CBC08$_{sub-surface}$	n.d	176.47	n.d	n.d	317.65	170.59	1352.94	1470.59	1000.00	764.71	1470.59	882.35	352.94	129.41	323.53
CBC09$_{surface}$	28.00	74.00	11.40	13.80	102.00	52.00	300.00	500.00	220.00	158.00	320.00	220.00	72.00	32.00	72.00
CBC10$_{surface}$	6.00	16.00	n.d	4.25	20.00	13.25	65.00	107.50	40.00	30.00	70.00	42.50	15.75	5.25	16.75
CBC10$_{sub-surface}$	5.90	14.36	n.d	3.59	18.46	12.31	74.36	123.08	38.46	30.77	69.23	43.59	15.38	5.38	16.15
CBC11$_{surface}$	4.38	14.69	n.d	3.13	16.25	10.31	59.38	96.88	40.63	31.25	65.63	43.75	15.00	4.69	15.94
CBC18$_{surface\ (Ref)}$	n.d	5.94	n.d	n.d	9.06	4.38	29.06	37.50	16.56	15.31	31.25	18.13	7.50	n.d	7.81
ISQG Low	160	44	16	19	240	85	600	665	261	384	n.a.	430	n.a.	63	n.a.
ISQG High	2100	640	500	540	1500	1100	5100	2600	1600	2800	n.a.	1600	n.a.	260	n.a.

**Data normalised to 1% organic carbon according to Simpson et al. 2005.*

Table 10 Sediment organochlorine (OC) pesticide concentrations.

Limit of reporting: 10 μg/kg; n.d. = not detected in concentrations greater than the limit of reporting.

Sediment organochlorine pesticide concentrations (μg/kg) normalised to 1% organic carbon*			
	trans-chlordane	dieldrin	p,p'-DDE
Site			
CBC01$_{surface}$	n.d	4.94	4.25
CBC01$_{sub\text{-}surface}$	1.64	2.55	4.27
CBC02$_{surface}$	n.d	3.04	4.20
CBC03$_{surface}$	n.d	n.d	n.d
CBC04$_{surface}$	n.d	n.d	4.23
CBC05$_{surface}$	n.d	n.d	14.17
CBC06$_{surface}$	n.d	n.d	24.55
CBC07$_{surface}$	n.d	n.d	3.53
CBC08$_{surface}$	n.d	n.d	n.d
CBC08$_{sub\text{-}surface}$	n.d	n.d	n.d
CBC09$_{surface}$	n.d	2.40	9.80
CBC10$_{surface}$	n.d	n.d	2.50
CBC10$_{sub\text{-}surface}$	n.d	n.d	3.08
CBC11$_{surface}$	n.d	n.d	n.d
CBC18$_{surface\ (Ref)}$	n.d	n.d	n.d
ISQG – LOW	0.50	0.02	2.20
ISQG – HIGH	6.00	8.00	27.00

**Data normalised to 1% organic carbon according to Simpson et al. 2005; only OC pesticides that were present in concentrations greater than the limit of reporting are presented.*

3.3 Sediment particle size

All sediment samples consisted of particles from a range of size categories. The dominant fraction was silt for most sites. The exceptions were both surface and sub-surface samples collected at CBC08 where the dominant fractions were sand. At CBC03 and the sub-surface sample at CBC01, the dominant fractions were approximately evenly split between the silt and sand categories.

Table 11 Sediment particle size

Site	Clay (<4 µm)	Silt (4-62 µm)	Fine sand (62–250 µm)	Medium sand (250–500 µm)	Coarse sand (500–2000 µm)	Gravel (2000–10000 µm)
	Proportion of sediments (% by weight)					
CBC01surface	17.05	48.04	11.73	0.08	10.60	12.50
CBC01sub-surface	10.57	29.31	19.72	27.20	12.70	0.50
CBC02surface	27.56	50.26	2.58	0.00	4.30	15.30
CBC03surface	18.84	30.85	5.30	6.61	32.40	6.00
CBC04surface	37.42	59.07	2.61	0.00	0.90	0.00
CBC05surface	31.24	63.61	3.76	0.00	0.70	0.70
CBC06surface	29.60	50.54	13.21	2.15	4.20	0.30
CBC07surface	28.51	45.01	1.98	0.00	0.60	23.90
CBC08surface	1.93	2.30	11.49	65.48	18.40	0.40
CBC08sub-surface	0.84	1.05	3.44	36.67	56.90	1.10
CBC09surface	25.74	57.38	15.68	0.00	0.60	0.60
CBC10surface	37.14	58.39	1.87	0.00	0.10	2.50
CBC10sub-surface	36.85	60.41	1.14	0.00	0.30	1.30
CBC11surface	28.75	47.60	1.24	0.00	1.30	21.10
CBC 18surface (Ref)	41.21	47.60	1.59	0.00	3.50	6.10

Blue text indicates dominant fraction(s)

3.4 Bioaccumulation of contaminants by mussels

Metals

The concentrations of various metals in the tissue collected from the mussel specimens are shown in Table 12.

The concentrations of all of the contaminants for which MLs are specified; that is, (inorganic) arsenic, cadmium, lead and mercury, were below the ML in all samples. It should be noted that in this study total arsenic, including organic and inorganic arsenic, was determined rather than inorganic arsenic for which the ML is specified.

Because the total arsenic concentration is less than the ML for inorganic arsenic, the concentration of inorganic arsenic in the mussel samples cannot exceed this ML.

The concentration of copper did not exceed the median GEL in any sample, while the median GEL for selenium (0.5 mg/kg) was only exceeded in the sample from CBC13. Food-standard guideline values are not available for the other metals determined, namely aluminium, cadmium, cobalt, manganese nickel and zinc.

As discussed previously (see Section 2.4), GELs provide a benchmark for contaminants that pose a low risk to the consumer. When the value exceeds the 90th percentile, FSANZ recommends that further investigation should be undertaken to determine whether or not the samples have consistently high values, to find an explanation for these values and to implement management to reduce the concentrations. At CBC13, the measured selenium concentration (0.57 mg/kg) was below the 90th percentile GEL concentration (1 mg/kg) so it is unlikely that it poses a threat to the human consumer. This risk is further mitigated through warnings by Western Australia's Department of Health against consuming shellfish from the estuary and rivers.

PAHs

The concentrations of 16 PAHs (those that the US Environmental Protection Agency has designated as priority pollutants) were determined in the mussel tissue. The limit of reporting was not exceeded for any of these compounds. The limit of reporting was 0.01 mg/kg for all analytes except benzo[b]fluoranthene and benzo[k]fluoranthene which were summed and therefore had a collective limit of reporting of 0.02 mg/kg.

Petroleum hydrocarbons

Although no PAHs were reported in any of the mussel tissue samples, the organic extract from each was subjected to further semi-quantitative analysis to determine the presence or otherwise of other petroleum hydrocarbons in the mussel tissue (i.e. contrary to the flowchart shown in Figure 3). These hydrocarbons included saturated hydrocarbons, alkylnaphtahlenes, alkylphenanthrenes and alkyldibenzothiophenes. Although all of these compounds were present in extracts from the mussels from all sites, they were also present in the procedural blank and therefore were artefacts of the chemical analytical procedure. These compounds could not be unequivocally attributed to an environmental source, so attempts to identify and (semi-) quantify were abandoned.

OC pesticides

The concentrations of 16 OC pesticides in the mussel tissue samples are shown in Table 13. Eight of these pesticides have ERLs prescribed by the Australia New Zealand Food Standards Code (FSANZ 2009), which are also shown in Table 13. Of the pesticides determined, only dieldrin and p,p'-DDE were present in concentrations that exceeded the limit of reporting. Both pesticides were present in all samples; however, the highest concentration of each (0.0052 mg/kg for dieldrin and 0.0041 for

p,p'-DDE occurring in the sample from CBC17, the site most remote from Claisebrook Cove) was two and three orders of magnitude below the respective ERLs.

It should be noted that the pesticide formulation known as DDT contains several components, including p,p'-DDT, o,p'-DDT, p,p'-DDE and p,p'-DDD. Furthermore, p,p'-DDE and p,p'-DDD are degradation products of p,p'-DDT. The sum of the concentration of these components is usually assigned as the total DDT concentration. It is this summed concentration to which the ERL refers.

Lipid and moisture content

The lipid and moisture content was determined for each of the mussel tissue samples: the results are shown in Appendix D. These were determined so that concentrations of contaminants may also be expressed relative to dry weight and to lipid weight.

Table 12 Metal concentrations in mussel tissue (mg/kg, wet weight) compared with maximum levels and generally expected levels prescribed by the Australia New Zealand Food Standards Code.

Instances where the limit of reporting was not exceeded are indicated by the < symbol.

	Metal concentrations in mussel tissue mg/kg (wet weight)											
	Aluminium	Arsenic	Cadmium	Chromium	Cobalt	Copper	Lead	Manganese	Mercury	Nickel	Selenium	Zinc
Limit of reporting→	1	0.5	0.01	0.05	0.01	0.01	0.01	0.01	0.01	0.01	0.05	0.01
Site												
CBC12	61	0.6	0.21	0.17	0.16	2.1	0.52	28	<0.01	0.26	0.34	8.3
CBC13	53	0.75	0.27	0.15	0.11	2.2	0.32	12	<0.01	0.26	0.57	9.6
CBC14	38	0.55	0.26	0.11	0.08	1.8	0.16	6.3	<0.01	0.25	0.39	7.1
CBC15	12	0.46	0.27	0.06	0.06	1.7	0.08	3.3	<0.01	0.22	0.36	6
CBC16	50	0.61	0.22	0.13	0.09	1.8	0.29	6.4	<0.01	85	0.48	7.6
CBC17	18	0.55	0.25	0.05	0.07	1.7	0.1	4.9	<0.01	0.36	0.45	7.1
GEL (median)						5					0.5	
GEL (90th percentile)						30					1	
Maximum levels		1[1]	2				2		0.5[2]			

Notes: 1. The maximum level is for inorganic arsenic; 2.The maximum level for mercury is the mean of 10 samples.

Table 13 Organochlorine (OC) pesticide concentrations in mussel tissue.

Instances where the limit of reporting was not exceeded are indicated by the < symbol.

	Organochlorine pesticide concentrations in mussel tissue (mg/kg, wet weight)															
	HCB	BHC (Total α-, β-, δ-)	γ-BHC (Lindane)	Heptachlor	Heptachlor epoxide	Chlordane (total)	α-Endosulphan	β-Endosulphan	Endosulphan sulphate	Aldrin	Dieldrin	Endrin (total)	p,p'-DDE	p,p-DDD	p,p-DDT	Methoxychlor
Site																
CBC12	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	**0.0023**	<0.001	**0.0015**	<0.001	<0.001	<0.001
CBC13	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	**0.0046**	<0.001	**0.0032**	<0.001	<0.001	<0.001
CBC14	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	**0.0036**	<0.001	**0.0033**	<0.001	<0.001	<0.001
CBC15	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	**0.0041**	<0.001	**0.0021**	<0.001	<0.001	<0.001
CBC16	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	**0.0033**	<0.001	**0.0023**	<0.001	<0.001	<0.001
CBC17	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	**0.0052**	<0.001	**0.0041**	<0.001	<0.001	<0.001
ERL[1]	0.1	0.01	1	0.05		0.05				0.1	0.1		1[2]			

Notes: 1. Where available, the extraneous residue limits (ERLs) for the various pesticides as prescribed by the Australia New Zealand Food Standards Code (FSANZ 2009) are also shown. 2. The ERL for DDT includes the sum of p,p'-DDT, o,p'-DDT, p,p'-DDE and p,p'-DDD concentrations.

4 Discussion

4.1 Sediment toxicity

Toxicity was demonstrated at most sites in this study to varying degrees. Seven out of 12 sites exhibited high-level toxicity. However, samples collected downstream from the two drains (Claisebrook Drain and Claisebrook Diversion Drain) at sites CBC01 and CBC08 respectively, clearly exhibited the greatest toxicity. This indicates that the two drains are at least in part the sources of contamination to the Claisebrook Cove area.

Toxicity at the two drain sites

Of the two drains, sediments collected from site CBC08 downstream from the Claisebrook Diversion Drain, which discharges into the Swan Estuary rather than Claisebrook Cove, exhibited both the greatest toxicity across test organisms (EC10 = 9.2%) and toxicity to the greatest number of testing organisms. These sediments were toxic to three of the four testing organisms: copepod adults, mussel larvae and fish larvae. Notably, only the subsurface sample collected at this site resulted in toxicity and not the surficial sample. This is probably because this site is subject to a degree of tidal flow and wave activity (being located in the Swan Estuary rather than in the cove), resulting in the constant shifting and renewal of the surficial (top few cm) sediments as compared with the subsurface fraction (10–20 cm in this study).

Sediments collected from site CBC01 adjacent to Claisebrook Drain, which discharges into Claisebrook Cove, exhibited the next greatest toxicity (EC10 = 15%), although of the four organisms tested, toxicity was only experienced by mussels. At this site (CBC01), mussels exposed to both the surface and sub-surface samples experienced high-level toxicity. This was most likely due to the site being located in the furthest reach of the cove, where flushing (apart from drain discharge itself) is minimal due to the cove's sheltered nature. Therefore, the surficial sediments (top 2 cm) were unlikely to be replaced with cleaner sediments or flushed with cleaner seawater as regularly as those in the adjacent Swan Estuary or even in the wider areas of the cove.

Given that toxicity was experienced across a wider range of organisms for samples collected at site CBC08 (Claisebrook Diversion Drain), one would expect different contaminant(s) (or combinations of contaminants) to be responsible for differences in the toxicity exhibited between the two sites. Additionally, greater concentrations of contaminants with toxic potential may be associated with Claisebrook Diversion Drain (CBC08) than Claisebrook Drain (CBC01). This would be expected given the diversion drain was constructed during site re-development to carry most of the stormwater away from Claisebrook Drain and discharge it directly to the estuary; whereas the flow from Claisebrook Drain is now related mainly to overflow events (EPA 1992).

Based on the chemistry data collected in this study, the toxicity of sediments at CBC01 (Claisebrook Drain) may be at least partly attributable to the metals zinc and lead, both of which exceeded high ISQGs at this site. However, metals are less likely to be contributing to the toxicity at site CBC08 because although present in the sediment, no metal exceeded the guidelines. In fact this was one of the only sites in the entire study where this was the case. Similarly for site CBC01, the toxicity experienced may be at least partly attributable to the OC pesticides *trans*-chlordane, dieldrin and p,p'-DDE, each of which was present in concentrations that exceeded low ISQGs at this site. No OC pesticides were detected in the sediment collected at the site downstream from the Claisebrook Diversion Drain (CBC08).

Although PAHs from historic information (e.g. Bowman Bishaw Gorham 1992) are known to be associated with the area, and were present at all sites tested in this study, they were not present in concentrations that exceeded guidelines at any site within the cove – so may not be responsible for toxicity in samples collected from site CBC01 (downstream from Claisebrook Drain). However, they were found in concentrations that exceeded low ISQGs at site CBC08 (Claisebrook Diversion Drain), so may be at least in part contributing to the toxicity experienced there.

The final point of note between these two drain sites is that the CBC08 sample was comprised predominantly of coarse sand. Thus on a volume-by-volume basis comparatively fewer binding sites (number of sites is relative to surface area of particle size) were available for contaminants such as metals and OC pesticides, than the sample collected from site CBC01 which was comprised of a much greater proportion of silt (with a comparatively greater number of binding sites). Given that samples from both sites exhibited high-level toxicity, this could mean that either the compounds bound up in the sediment from CBC08 had greater toxic potential than those from CBC01 (which could be explained by the nature of the discharge from the drain); or that even though there were relatively fewer binding sites for the contaminants, the types of contaminants were less tightly bound and more easily released into the porewater and therefore more readily available for uptake by the testing organisms.

Toxicity at non-drain sites

Within the cove and parallel to the barrier wall on the cove's northern edge (and the southern boundary of the historic gasworks site), samples collected from sites CBC02 and CBC04 did not cause any toxicity to test organisms. This indicates that the zone of impact (in terms of toxicity experienced by the test organisms used in this study) from Claisebrook Drain does not extend 150 m through the cove as far as site CBC02. This could be because the volume of drain discharge is not sufficient to be affecting the surficial sediments further down the cove, or that there is sufficient flushing from the estuary and subsequent dilution to prevent an accumulation of toxic contaminants to sufficiently high concentrations to affect these organisms (as measured by these endpoints).

Interestingly, high-level toxicity was experienced by fish larvae exposed to sediments from site CBC03. As introduced above, sediment collected from the sites either side

(CBC02 and CBC04) did not result in toxicity, which would suggest that toxicity at CBC03 is not due to contamination from surface flow from Claisebrook Drain or from upstream in the Swan Estuary. One possible explanation for this localised effect is that it is an artefact of repairs to the wall on the northern edge of Claisebrook Cove. These repairs were being conducted on the section of wall immediately adjacent to site CBC03 and were underway at the time of sampling. Works of this kind may be the cause of the observed toxicity in a number of ways: existing contaminated sediments may have been disturbed and mobilised; new material such as building sand (supported by the larger particle sizes present at this site) may have been introduced from outside the area; and/or contaminated material may have escaped from the historic gasworks site during the repair-works to the barrier wall. Whatever the source, the toxicity observed at site CBC03 could not be explained by the contaminants directly assessed within this study; and an agent that appears to only affect the fish (of the four organisms tested), is responsible.

Beyond the cove and within the Swan Estuary, the toxicity observed in sediments from sites CBC05, CBC07, CBC09 and CBC10 may be at least partly attributable to the OC pesticides p,p'-DDE and in the case of site CBC09, dieldrin, which exceeded the low ISQGs at these sites. Lead and zinc may also be contributing to the toxicity at these sites (and CBC11) because they also exceeded guidelines.

It is important to note that the three contaminant groups assessed in this study is not exhaustive and that many more contaminants are expected to be associated with the sediments both within the cove and the adjacent estuary, given the urbanised nature of the Claisebrook catchment and the general area in this part of the estuary (e.g. Kesteven 2000; Lord 1999). Therefore, in cases where metals, OC pesticides and PAHs have been suggested as being responsible for some of the toxicity observed, it should be considered they are most likely to be acting within a complex mixture of contaminants and that the combined effects of different contaminants (and reactions between different contaminants) should not be ruled out. This is emphasised by the fact that there is not always a clear link between high levels of these contaminants being recorded in the sediments and toxicity being experienced. For example, in some cases, toxicity was not demonstrated for a site (with the test organisms used in this study), despite relatively high concentrations of a contaminant being present (e.g. zinc exceeded high ISQGs at CBC04 where no toxicity was demonstrated).

Spatial distribution of contaminants in the sediments

PAHs

Perhaps most striking of all is the spatial distribution of PAHs in the area. In addition to ISQGs being exceeded in samples collected from site CBC08 as previously discussed, ISQGs were also exceeded for at least one (and in some cases all of the PAHs assessed in this study for which ISQGs are available) at sites CBC05, CBC06, CBC07 and CBC09. This is consistent with earlier findings (Nice 2009) where site CBC07 (the Claisebrook site) had consistently the highest concentrations of PAHs from 20 sites tested throughout the Swan Canning system. Of note is that the high

ISQGs in the current study were also exceeded for one of the PAHs at CBC05 and four of the PAHs at CBC06.

Sites CBC05, CBC06, CBC07, CBC08 and CBC09 are all located in the Swan Estuary, within the remediation zone (Figure 10) where known PAH-contaminated sediments were replaced with clean fill in September 1994 (Tingay & Associates 1994, cited by Trayler & McKernan 1997). This would indicate that either the contaminated sediments were not completely removed in 1994 or that there has been an input of PAHs to this area of the Swan Estuary since 1994. The source of this particular contaminant group is unlikely to be Claisebrook Drain or the cove itself because although many PAHs were present within the cove, the concentrations measured were comparatively lower (did not exceed any guidelines) and there was no clear gradient in PAH concentration from the drain. A proportion of the PAH contamination may be attributable to boat fuel (e.g. oil and diesel) from recreational boats using the area. However, such a high accumulation of PAHs from boat fuel alone is unlikely. It is possible that a source is the Claisebrook Diversion Drain, and that the PAH contamination is being transported upstream to sites CBC05 and CBC06, in which case one would also expect similar or greater concentrations downstream at site CBC09, which was not the case. It is also possible that the PAH contamination at sites CBC05, CBC06, CBC07 and CBC08 is from an alternative source upriver from the Claisebrook area. However, if this were the case, one would expect similar concentrations at sites CBC10 and CBC11. A further, more likely possibility is that the source of the PAHs was the neighbouring historic contaminated site (the former East Perth Gasworks) known to have contained high concentrations of these compounds (EPA 1992).

Also of note is that for sites CBC05, CBC06, CBC07, CBC08 and CBC09 (located in the remediation zone within the estuary), the majority of the PAHs exceeding guidelines were from the low molecular weight category of PAHs (i.e. naphthalene, acenapthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene and pyrene). Low molecular weight PAHs typically degrade more rapidly in water and sediments than their high molecular weight counterparts and so are less persistent in the environment. This further suggests a relatively recent source of PAH contamination to the area, given that the surficial sediments within this zone are often disturbed by wave and tidal action and recreational activity, which would likely accelerate their degradation. It is not possible to estimate the timing of the contamination event from this information without knowledge of the original composition of the contaminants. In the case of acenapthylene and pyrene, the high ISQG was also exceeded. Acenaphthylene in particular is not commonly found in fuel products such as gasoline and diesel, giving less credence to boat fuel as a possible source of the PAHs.

Although PAHs may be in part contributing to the toxicity experienced at sites CBC05 and CBC07 (as well as lead, zinc and p,p,-DDE as previously discussed), this contaminant group is known more for long-term chronic effects (Varanasi et al. 1985) and its constituents are typically not as acutely toxic as the OC pesticides (high molecular weight PAHs in particular). This study did not target long-term chronic

effects, so it is not possible to predict the magnitude of the potential environmental impact of the PAHs from these toxicity results alone. This also explains why in the case of site CBC06, toxicity was not demonstrated with the toxicity tests employed here, yet PAHs were present at the highest levels and in many cases exceeded both low and high ISQGs. Exceeding the low ISQG indicates that adverse biological effects are likely to occur and exceeding the high ISQG indicates that these effects are likely to occur more frequently (ANZECC & ARMCANZ 2000).

Although not demonstrated in this study, it is likely that PAHs recorded at these concentrations will be causing significant environmental harm (in the form of long-term chronic effects) to organisms associated with the sediments (living in and/or feeding on) in the area of the Swan Estuary (former remediation zone) adjacent to the historic contaminated site (e.g. carcinomas in wild fish populations – Murchelano & Wolfe 1985).

Figure 10 Approximate extent of the former remediation zone (according to Trayler & McKernan 1997).

OC pesticides

All three OC pesticides detected in this study are present in concentrations that exceed low ISQGs. A potential source of these contaminants detected within Claisebrook Cove is Claisebrook Drain. However, the concentrations of p,p'-DDE at sites CBC05 and CBC06 located in the Swan Estuary are between three and six times those concentrations reported within the cove, which suggests a different source for these sites and others located within the estuary. OC pesticides were not present above limits of reporting at the Claisebrook Diversion Drain site, so this drain is unlikely to be a source of OC pesticides to this part of the Swan Estuary.

The most widely reported OC pesticide in this study (p,p'-DDE) is a metabolite of DDT, and is more resistant to degradation than the parent compound (Porter et al. 2005), which provides an explanation for its presence in the estuary sediments, even though the use of DDT has been banned in Australia since 1987 (DEWHA 1987).

Dieldrin, which was only present in concentrations above the limit of reporting at two sites within Claisebrook Cove and one site in the adjacent Swan Estuary, has also been banned since 1987 (DEWHA 1987). However, it is highly persistent in the environment, which again may explain its presence in this study.

A broad range of toxic effects has been reported for aquatic organisms exposed to the OC pesticides (including immune system damage as well as endocrine disrupting, teratogenic, carcinogenic and mutagenic effects; e.g. Arukwe 2006; Lundberg et al. 2006; Lundholm 1997; MAFF 1981; WFPHA 2000). Both dieldrin and p,p'-DDE also have the potential to bioaccumulate (Connolly & Glaser 2002; WHO 1989).

The findings presented here agree with those in an earlier study (Nice 2009), where dieldrin and p,p'-DDE were present in the sediments at the Claisebrook site at concentrations higher than the low ISQGs.

Metals

The metals exceeding guidelines in this study, namely lead, zinc and copper (again consistent with the Nice 2009 study), are known to cause a range of toxic effects in aquatic organisms (e.g. Heinz et al. 1999; King et al. 2006; Martinez-Tabache et al. 2000); and lead and copper have the potential to bioaccumulate in aquatic organisms (Ali & Fishar 2005; Hoffman et al. 2000). These metals are typical constituents of stormwater containing road runoff (e.g. Rate et al. 2000). However, around the Claisebrook area there is evidence to suggest that relatively high background levels of lead and zinc exist in the sediments (Kesteven 2000) from previous land uses including but not limited to the former East Perth Gasworks. Others include the former East Perth Power Station, railway lines, transport depots, automotive services including engine and body works, metal works, scrap metal yards and textile industries – many of which discharged into Claisebrook Drain (DEP 2000; Thurlow et al. 1986). Although many of these land uses are no longer in existence, and the sites have been remediated, there is a strong likelihood that pollutants from these

industries (such as lead and zinc) are still present in the local environment (Kesteven 2000).

Reference site

It should be noted that in addition to laboratory controls for each test, a reference site was included in this study. This site was not considered pristine, but rather included in the study as a point of reference or comparison for sites associated with Claisebrook Cove. Site CBC18 was selected as the reference site because although contaminants were present (as would be expected in an urbanised estuary system) they did not exceed any guidelines in an earlier study (Nice 2009). In the current study, OC pesticides and many of the PAHs were not detected at this reference site (those PAHs that were present were comparatively low concentrations compared with the other sites and in many cases orders of magnitude lower than the guidelines). Lead and zinc were detected at levels that exceeded the low ISQG (these were reported at the majority of sites), and may be responsible for the low-level toxicity reported at this site for the mussel test. Note that only low-level toxicity was reported for this site (i.e. mussel larval development was affected when the sediment sample elutriates remained undiluted). However, no toxicity was observed with subsequent dilution testing compared with the majority of other sites (CBC01, CBC03, CBC05, CBC07, CBC08, CBC10 and CBC11); and the low-level toxicity was observed for only one test as compared with multiple tests for the majority of other sites (CBC05, CBC07, CBC08, CBC09, CBC10 and CBC11). Interestingly, the fish larvae, which generally appeared to be the most sensitive of test organisms in this study, remained unaffected at CBC18.

4.2 Bioaccumulation of contaminants by mussels

Metals

Some perspective to the bioaccumulation of metals in mussels may be gained by comparing the results from the present study with those from three previous studies summarised in Table 14. Perhaps the most relevant of these studies is that by the Swan River Trust (1993) where the concentrations of chromium and zinc were determined in the mussel, X*enostrobus securis* collected from the Swan Estuary in East Perth and Perth. Comparison with the current study shows that the chromium concentration is approximately one order of magnitude less in the current study. The concentration of zinc is of the same order of magnitude.

Less relevant due to the difference in mussel species examined *(Mytilus edulis*), the study by Chegwidden (1980) measured metals in mussels from four sites in the Swan and Canning rivers. Comparison with these results reveals that:

- the concentrations of cadmium (mean = 0.25 mg/kg) and copper (mean = 1.8 mg/kg) in this study are similar to those measured previously

- the concentrations of lead and zinc are approximately one order of magnitude less in the contemporary samples
- the concentrations of nickel are similar with the exception of the sample from CBC16 which contained 85 mg/kg (i.e. the maximum value of the range – see also Table 12), inflating the value of the mean (It is not clear what caused the elevated concentration of nickel in this sample.)
- chromium, while previously undetected is present in the contemporary samples, albeit at low concentrations (i.e. one to three times the limit of reporting).

Arguably least relevant, yet still useful is comparison with the study by Burt et al. (1995) where metals were determined in *Mytilus edulis* from 52 sites offshore from Perth (i.e. different mussel species with the study not conducted in the Swan River). The most notable features of this comparison are found in the concentrations of:

- aluminium and manganese which are approximately one order of magnitude higher in the Claisebrook Cove mussels than in those from offshore
- arsenic and zinc wherein the reverse is observed
- cadmium, chromium, copper, lead and mercury which are similar between the two studies
- nickel which is similar with the exception of the sample collected from CBC16.

Table 14 A summary of metal concentrations in mussel tissue (mg/kg, wet weight) collected from Claisebrook Cove and the adjacent Swan Estuary compared with previous studies local to the Perth metropolitan area.

		Metal concentration (mg/kg, wet weight) in mussel tissue											
		Al	As	Cd	Cr	Co	Cu	Pb	Mn	Hg	Ni	Se	Zn
Study / Species/ Location/													
Claisebrook Cove 2009 (this study) *Xenostrobus sp.* Swan River	Mean (n=6)	39	0.58	0.25	0.12	0.085	1.8	0.23	6.35	<0.01	0.26	0,42	7.4
	Median (n=6)	44	0.59	0.25	0.11	0.095	1.9	0.25	10.15	<0.01	14	0.43	7.6
	Range (min–max)	12–61	0.46–0.75	0.21–0.27	0.05–0.17	0.06–0.16	1.7–2.2	0.08–0.52	3.3–28	<0.01	0.22–85	0.34–0.57	6–9.6
SRT 1993 *Xenostrobus securis* Swan River	Mean (n=40)				2.08								20.4
	Median (n=40)				1.1								17
	Range (min–max)				0.20–17								8.8–72
Chegwidden 1980 *Mytilus edulis* Swan River	Mean (n=20)			0.25	<0.05 (n=19)		1.85	1.24			0.3		51.5
	Median (n=20)			0.25	<0.05		1.95	1.15			0.3		54.2
	Range (min–max)			0.2–0.3	<0.05		1.1–2.5	0.5–3			0.3–0.7		27.6–76.0
Burt et al. 1995 *Mytilus edulis* Perth metropolitan – offshore	Mean (n=52)	4.4	4.4	<0.1	<0.1		1.3	<0.5	0.82	<0.25	<0.1		34
	Median	4	4.0				1.1		0.80				32
	Range (min–max)	0.6–15	0.5–11				0.2–8.4		0.11–1.4				3–100

OC pesticides

As discussed earlier (see Section 3.4), of the OC pesticides determined, only dieldrin and DDT exceeded the limit of detection. Both were present in concentrations at least two orders of magnitude less than the ERLs. Further, no gradient in concentration with increasing distance from either of the two drains was apparent – in fact the sample collected from the most remote location at CBC17 contained the highest concentration of both pesticides.

Other local studies where concentrations of dieldrin or DDT have been reported in mussel tissue are rare. In the report by Burt et al. (1995) discussed above, DDT and dieldrin were determined in *Mytilus edulis* at 52 sites offshore from the Perth metropolitan area. The limit of reporting (0.001 mg/kg) for DDT was exceeded in only 19 of these samples, with the maximum concentration of 0.004 mg/kg reported. The limit of reporting for dieldrin (0.001 mg/kg) was not exceeded in any sample. In a similar study using *Mytilus edulis* collected in 1991 from coastal waters off Perth, Burt and Ebell (1995) reported DDT concentrations ranging from <0.001 to 0.002 mg/kg. Other studies of accumulation of DDT and dieldrin in *Mytilus edulis* in waters offshore from the Perth metropolitan area include the Perth Long-term Ocean Outlet Monitoring Program (Kinhill 1995; 1998) in which the maximum measured concentration of dieldrin was 0.014 mg/kg and the concentration of DDT failed to exceed the limit of reporting (0.002 mg/kg). These concentrations are comparable with those measured in the contemporary samples from Claisebrook Cove (0.0015–0.0041 mg/kg).

Other studies where the DDT congeners have been determined in *Mytilus edulis* have been reviewed by Burt and Ebell (1995). Locations include Corio Bay, Victoria with concentrations ranging from <0.001 to 0.021 mg/kg (Fabris et al. 1992); California Harbour, US, 0.007 to 0.130 mg/kg and north California coast, 0.001 to 0.007 mg/kg (Riseborough et al. 1983); middle and north Adriatic, 0.036 to 0.065 mg/kg (Picer et al. 1978); and Scotland <0.003 to 0.064 mg/kg (Cowan 1981). In general, these concentrations are higher than those observed in the present study.

Concentrations for the OC pesticides and the metals may also be expressed per unit dry weight or lipid weight, calculated from the moisture content and lipid content presented for each sample in Appendix D. Although not applied here – because both the Australia New Zealand Food Standards Code and the previous local studies discussed above expressed the concentrations in terms of wet weight of mussel tissue – this information might be useful for comparison with future studies or other unpublished data not canvassed in this report.

PAHs

As described in Section 3.4, none of the 16 PAHs determined were present in concentrations exceeding the limit of reporting (0.01 or 0.02 mg/kg). Although PAHs were included in the study by Burt et al. (1995), comparison with the current study is difficult since the analytical technique used in the previous study was apparently

more sensitive. In the previous study by Burt et al., concentrations of 11 individual PAHs in 49 samples of mussel tissue were presented, namely naphthalene, acenaphthylene, acenaphthene, fluorene, anthracene, fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo[a]pyrene and benzo[ah]anthracene, each with a limit of detection of 0.001 mg/kg (wet weight), one order of magnitude lower than for the current study. Also, in the study by Burt et al. (1995), the individual PAHs exceeded the current limit of detection in only 11 instances with naphthalene accounting for seven of these. Anthracene, fluoranthene and pyrene co-occurred in one of these samples, while chrysene was detected in isolation in a separate sample. In planning the bioaccumulation module of the present study, it was envisaged that more sensitive chemical preparative and analytical techniques would be used to decrease the limit of reporting, but as discussed earlier (Section 3.4), contamination of the tissue extracts during the original analysis of the PAHs precluded further treatment.

5 Conclusions

From the information presented here we conclude that both the Claisebrook Drain and the Claisebrook Diversion Drain are sources of toxic contaminants to the Swan Estuary system. The evidence suggests that different contaminants (or combinations thereof) are responsible for the toxicity observed for samples collected from these two sites.

Although the test organisms experienced the greatest degree of toxicity when exposed to samples collected from the two drain sites, it is likely there are other sources of toxic contaminants to this area of the Swan Estuary. For example, there appeared to be an additional source of toxicity within Claisebrook Cove adjacent to the barrier wall on the cove's northern edge in the vicinity of the repair works that were underway at the time of sampling. If the repair works resulted in introduction of toxic material to the surficial sediments of the cove, the source may no longer be present (although this has not yet been tested).

The toxicity experienced by test organisms in this study is not completely explained by the metals, PAHs and OC pesticides targeted. While members of these contaminant groups are considered in many cases to be contributing to the toxicity, they are most likely acting in conjunction with a range of other contaminants typical of an urbanised estuary – particularly given the nature of the current and historic land uses in the Claisebrook catchment and this portion of the Swan Estuary in general.

A range of PAHs were present at concentrations of concern in the Swan Estuary in the former remediation zone. Although these contaminants are probably contributing to some of the toxicity experienced in this study, it is not possible from this study alone to predict the magnitude of their potential environmental impact – given that they tend to cause long-term chronic effects, which were not measured here. Although PAHs were recorded in the sediments at concentrations likely to be causing significant environmental harm to biota associated with the sediment and/or the sediment-water interface, the PAHs targeted by this study did not accumulate in mussels living closer to the top of the water column (in concentrations above the limits of reporting for PAHs).

It was evident from the spatial distribution of PAH contamination that a source other than the Claisebrook Drain and the Claisebrook Diversion Drain was responsible. Claisebrook Cove is subject to multiple landuse impacts, typical for an inner city area. However, based on the results of this study, it was concluded that the most likely dominant source of PAH contamination to this portion of the Swan Estuary was the neighbouring historic contaminated site (i.e. the former East Perth Gasworks).

The metals and OC pesticides recorded in the sediment at concentrations of concern (lead, zinc and p,p'-DDE in particular) were fairly evenly distributed throughout the study area and were not attributable to any one source, although Claisebrook Drain (discharging within Claisebrook Cove) did appear to be a potential current source of these contaminants (and also dieldrin and *trans*-chlordane), along with general runoff in the area and the likely historic signature of these contaminants within the

sediments. The metals and OC pesticides were considered to be contributing to some of the toxicity experienced in this study and were also found to be bioaccumulating in the mussels collected from the study area. However, the resulting effects of this level of bioaccumulation are unknown, since environmental guidelines for bioaccumulation are not yet available. Comparison with previous studies of these contaminants in mussels elsewhere in the Swan Estuary, albeit in a different species (*Mytilus edulis*), revealed that the concentrations were generally comparable with the contemporary observations. Although perhaps not relevant to the ecological health of Claisebrook Cove, it should be noted that the concentrations of these contaminants did not exceed the Australia New Zealand Food Standards Code (FSANZ 2009).

The importance of considering the subsurface fractions of the sediment in this type of investigation has also been demonstrated, because in some instances they are more environmentally relevant (in terms of biota exposure) and often yield different results to the surficial sediments if, for example, they are located in areas of sediment disturbance and renewal.

Finally, overall, the spatial distribution of the sites where toxicity was observed, and the concentrations of potentially toxic contaminants in the sediments reported in this study, warrant further investigation. Recommendations for further action follow in Section 6 and a conceptual diagram of the potential contaminant sources, receptors and exposure routes is provided (Figure 11).

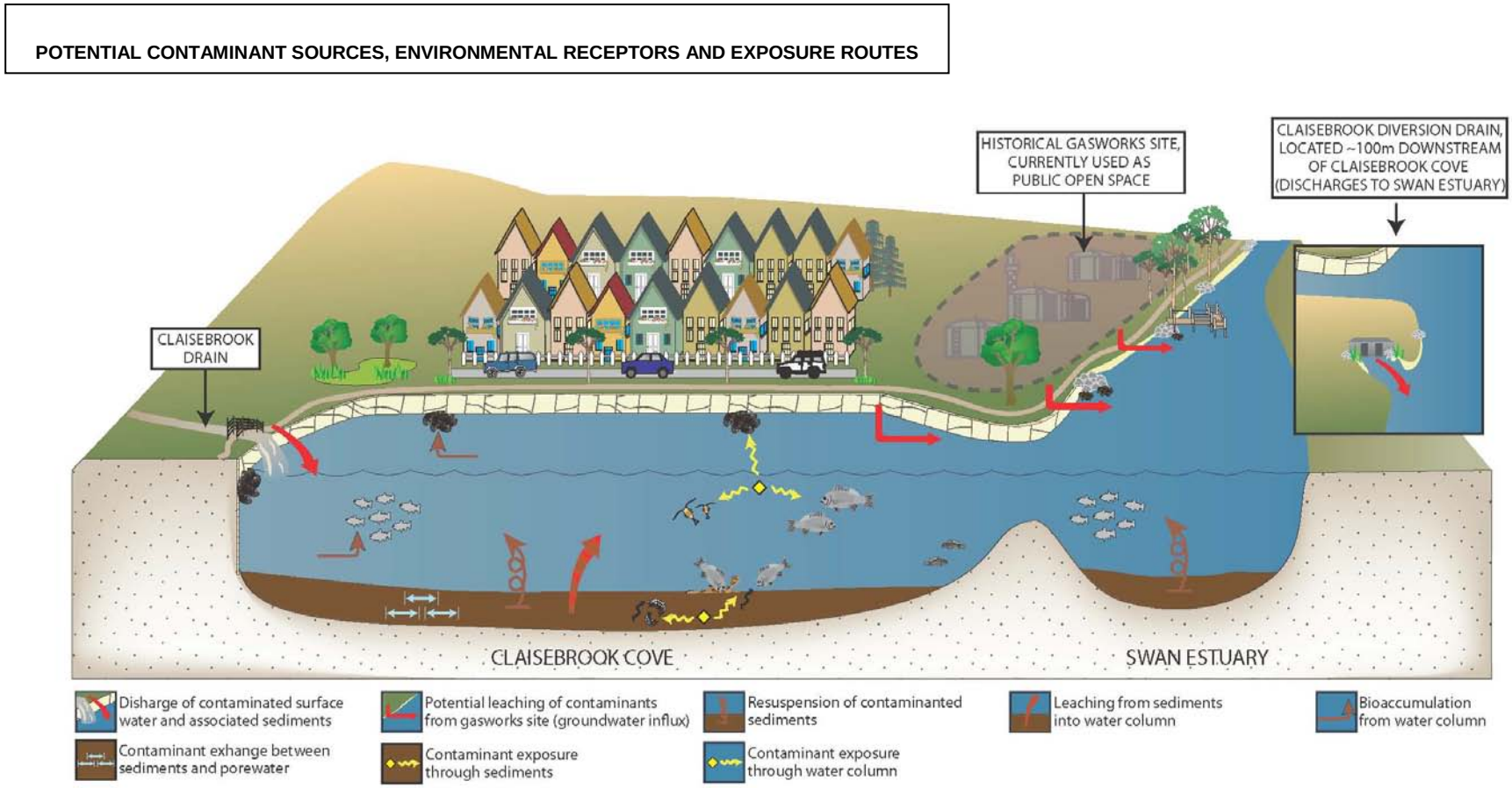

Figure 11 Claisebrook Cove and the Swan Estuary - Conceptual diagram of potential contaminant sources, environmental receptors and exposure routes.

6 Recommendations

Table 15 outlines the key issues arising from this investigation, their significance and the subsequent recommended actions.

Table 15 Key issues, their significance and recommended actions arising from this investigation.

Key issues arising from this study that need to be addressed	Significance	What should be investigated?	Recommended actions	Component(s) of Swan Canning system to be examined
1) High levels of toxicity were reported for several sites. In particular, the two drain sites: Claisebrook Drain and Claisebrook Diversion Drain. Different contaminants (or combinations thereof) are responsible for differing toxicity between the two drain sites.	Suggests direct impacts to biota inhabiting the receiving environment.	1) Which particular contaminants of stormwater and associated sediments are responsible for the toxicity observed?	1) Conduct Toxicity Identification and Evaluation (TIE) tests on stormwater from both drain sites to determine the specific chemical group(s) responsible for the toxicity observed. This will help further characterise the stormwater/sediments and help target those contaminant groups responsible for the toxicity observed.	Drain water.
		2) What are the likely source(s) and/or at what point along the drain line are these contaminants entering the drain?	2) Conduct an in-line assessment of organic contaminants present in stormwater over a period of time using Passive Sampler technology (e.g. Foulsham et al. 2009). This study will establish concentrations of contaminants in the stormwater that accumulate over a given period. Passive Samplers could be deployed at both the drain outfalls and at strategic positions along the Claisebrook drain line to trace the general location at which certain contaminants are entering the drain systems.	Drain water.
	Water from the Claisebrook Diversion Drain is used for irrigation.		3) Department of Health to be informed of findings of this report, particularly regarding potential quality of irrigation water.	N/A
2) High concentrations of PAHs in the sediments (exceeding guidelines) at several sites within the Swan Estuary adjacent to Claisebrook Cove (including the former remediation zone). It was evident from the spatial distribution of PAH contamination that a source other than the two drains may be at least partly responsible.	Suggests either historic contamination in the sediments of the remediation zone or an ongoing source of contamination to the formerly remediated zone.	1) Is the high level of PAHs recorded in the Swan Estuary sediments (in the formerly remediated zone) purely historic or is there ongoing contamination from adjacent land?	1a) Conduct a groundwater investigation to determine whether PAHs are leaching to the estuary from the adjacent historic contaminated site.	Groundwater.
			1b) Department of Environment and Conservation to be informed of findings of this report, particularly regarding the level of contamination in the formerly remediated zone of the estuary and the possibility of ongoing contamination from the adjacent historic contaminated site.	N/A
		2) Are there also high levels of PAHs in the water column?	2) Conduct a water quality survey in the Swan Estuary using Passive Sampler technology.	Estuary water.

	Suggests direct impacts to biota living in these estuarine sediments.	3) Is there a measurable impact on sediment infauna in-situ? Have sediment infauna populations recovered since remediation in 1994?	3) Conduct a survey following the methods of Bouckaert (1996) and Trayler and McKernan (1997) to assess the recolonisation of the former remediation area. The study should target sediment invertebrates and assess factors such as species richness, abundance and community composition. The study should compare current data to that collected in the two former studies to determine whether the site has recovered or whether there is still an obvious impact to sediment infauna. Organisms should be collected from both inside the remediation area and multiple reference locations and data compared with the two historic datasets (1996 & 1997).	Estuary sediment invertebrates.
3) Some metals and OC pesticides were recorded at concentrations of concern in the sediments throughout the study area. These contaminants were considered to be contributing to some of the toxicity experienced by the testing organisms in this study.	Suggests direct impacts to biota inhabiting the receiving environment.	1) Is there a measurable impact on sediment infauna in-situ?	1) Incorporate additional sites (to capture sites of concern from this study) into the estuary sediment infauna study recommended for Issue 2 above.	Estuary sediment invertebrates.
4) The metals and two OC pesticides (dieldrin and p,p'-DDE) were also found to be accumulating in the mussels collected from the study area.	Suggests other animals inhabiting the receiving environment may also be bioaccumulating such contaminants.	1) Are black bream collected from Claisebrook Cove bioaccumulating metals and OC pesticides?	1) Black bream collected from the cove to be investigated for bioaccumulating contaminants known to be present in the cove (particularly since these organisms are sediment foragers where many of these contaminants are bound). Note: any fish study conducted for Claisebrook Cove should incorporate a tracking component to inform on time the fish spend in the cove.	Estuary fish.
	If these are recreational fish species this may have human health as well as ecological health implications.		2) Department of Health to be informed of findings of this report, particularly regarding the potential for accumulated contaminants in recreational fish and/or shellfish species and the compliance of the mussel tissue with the Australia New Zealand Food Standards Code.	N/A

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^{o}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^{o}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^{o}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 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 5–8 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^{o}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^{o}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 euthanized 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 Supporting mussel data

Table 16 A summary of the number of specimens and shell lengths of mussels (Xenostrobus sp.) collected from the vicinity of Claisebrook Cove in July 2009 for various subsequent chemical analyses.

Site	Subsample	No. of specimens	Mean shell length (cm)	Range shell length (cm) (min–max)
CBC12	Organics	70	2.21	1.62–2.69
	Metals	68	2.27	1.60–2.86
	Moisture & lipids	101	2.29	1.70–3.17
CBC13	Organics	91	2.00	1.16–2.95
	Metals	73	2.17	1.43–3.12
	Moisture & lipids	108	2.12	1.48–3.06
CBC14	Organics	41	2.68	2.07–3.55
	Metals	32	2.67	1.96–3.28
	Moisture & lipids	123	2.19	1.44–3.63
CBC15	Organics	92	2.08	1.45–3.23
	Metals	41	2.35	1.80–2.93
	Moisture & lipids	109	2.20	1.56–2.98
CBC16	Organics	91	2.36	1.37–3.15
	Metals	61	2.35	1.90–2.94
	Moisture & lipids	145	2.29	1.61–3.20
CBC17	Organics	106	2.18	1.56–2.91
	Metals	59	2.36	1.79–2.84
	Moisture & lipids	125	2.25	1.64–2.99

Appendix C In-situ water quality data

Table 17 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 17 In-situ water quality data

Site code	Temperature (^{o}C)	Salinity (ppt)	pH	Dissolved oxygen (%)	Dissolved oxygen (mg/L)
CBC01	16.7	29.8	7.7	58	4.7
CBC02	16.1	31.6	7.9	71	5.8
CBC03	15.8	32.1	7.9	75	6.1
CBC04	15.6	32.4	7.9	78	6.3
CBC05	15.6	32.6	8.0	84	6.8
CBC06	15.6	32.7	8.0	84	6.8
CBC07	15.8	32.9	8.0	85	6.9
CBC08	15.9	32.1	7.9	73	5.9
CBC09	15.7	32.7	8.0	82	6.7
CBC10	15.8	32.9	7.9	81	6.6
CBC11	15.8	33.2	8.0	87	7.0
CBC18	16.3	35.2	8.0	85	6.8

Appendix D Bioaccumulation data

Table 18 Polycyclic aromatic hydrocarbon (PAH) concentrations in mussel tissue.

Instances where the limit of reporting was not exceeded are indicated by the < symbol.

	Polycyclic aromatic hydrocarbon concentrations in mussel tissue (mg/kg, wet weight)														
	Naphthalene	Acenaphthylene	Acenaphthene	Fluorene	Phenanthrene	Anthracene	Fluoranthene	Pyrene	Benz(a)anthracene	Chrysene	Benzo[b]&[k] –fluoranthene	Benzo[a]-pyrene	Indeno[1,2,3-d]pyrene	Dibenz[ah] anthracene	Benzo[ghi] -perylene
Site															
CBC12	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	<0.01	<0.01	<0.01	<0.01
CBC13	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	<0.01	<0.01	<0.01	<0.01
CBC14	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	<0.01	<0.01	<0.01	<0.01
CBC15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	<0.01	<0.01	<0.01	<0.01
CBC16	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	<0.01	<0.01	<0.01	<0.01
CBC17	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	<0.01	<0.01	<0.01	<0.01

Table 19 Moisture content and lipid content of mussels collected from the vicinity of Claisebrook Cove in July 2009.

Site	Lipid content (% w/w wet weight)	Moisture content (% w/w wet weight)
CBC12	0.3	92.0
CBC13	0.7	89.0
CBC14	0.5	89.8
CBC15	0.4	90.4
CBC16	0.6	88.6
CBC17	0.8	88.4

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)
EPRA	East Perth Redevelopment Authority
ESA	Ecotox Services Australasia
FSANZ	Food Standards Australia New Zealand
MAFF	Ministry of Agriculture Fisheries and Food (UK)
PSEP	Puget Sound Estuary Program
SRRC	Swan River Reference Committee
SRT	Swan River Trust
WFPHA	World Federation of Public Health Associations
WHO	World Health Organization

Glossary

Bioaccumulation	The accumulation of substances, such as metals, pesticides or other compounds in an organism.
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).
ERL	Extraneous Residue Limit: the maximum permitted limit of a pesticide residue, arising from environmental sources other than the use of a pesticide directly or indirectly on the food, expressed in milligrams of the chemical per kilogram (mg/kg) of the food (Standard 1.4.2 – FSANZ 2009)
GELs	Generally Expected Levels. GELs are not legally enforceable, but they provide a benchmark against which to measure contaminant levels in food (FSANZ 2009)
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].
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.
IC10	Concentration of sediment elutriate calculated (by non-linear interpolation) to cause the described effect in 10% of test organisms.
Limit of reporting	The lowest concentration at which an analyte will be reported after taking into account interferences and instrumental limits of detection.
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.
ML	The maximum level of a specified contaminant, or specified natural toxicant, which is permitted to be present in a nominated food, expressed, unless otherwise specified, in milligrams of the contaminant or the natural toxicant per kilogram of food (mg/kg). The ML must be calculated for the edible content of the food that is ordinarily consumed (Standard 1.4.1 – FSANZ 2009).
NOEC	No observable effect concentration: the highest tested concentration at which organisms are unaffected compared with control organisms.
No toxicity	No statistically significant effect (i.e. no statistically significant difference in response by the test organisms from the control organisms; $p>0.05$).
PAH	Polycyclic aromatic hydrocarbon.
Pesticide	Substance or mixture of substances intended for preventing, destroying, repelling or mitigating pests such as insects.
OC	Organochlorine.
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 upright position in water column) was used as a measure of toxicity.

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