Western Port Toxicant Study Stage 4

Assessment of pesticide risks in catchments of north-eastern Western Port

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Executive Summary

The Western Port catchment comprises an area of approximately 3,433 km^2, including approximately 2,232 km of rivers and creeks (Keough et al., 2011). The unique marine environment of Western Port is a natural asset providing habitat for fish, invertebrates, marine mammals, birds, plants and other marine life and is a Ramsar listed area. Its importance regionally is observed through its use as a popular destination for recreational activities and for agricultural production. Overall this region accounts for 32 per cent of Victoria's total gross value for agricultural production (EPA, 2001). Research and monitoring programs conducted over the last 4 years have shown the occurrence of pesticides, in particular herbicides and fungicides, in Western Port's waterways at concentrations that could pose a risk to resident flora and fauna. Studies, in the north-western part of the bay indicated that the primary land uses contributing these pesticides are related to agriculture, notably intensive farming such as market gardens. Based on these studies, monitoring other agriculturally dominated catchments of Western Port was recommended to provide a broader understanding of the risks of pesticides across the region.

There are several large rivers and drain systems in the north-east of Western Port that flow into the bay, including the Bunyip River, Yallock Cut, Cardinia Creek, Deep Creek and Toomuc Creek. The lower reaches of these systems are dominated by intensive agriculture, including market gardens. In addition, Cardinia Creek, the Bunyip River and Yallock Cut catchments are significant contributors of sediments into the bay (Wilkinson et al 2016), which may impact key environmental values in this region of the bay. Furthermore, this area is a major high tide roost for migratory wader birds and home to vulnerable and endangered fishes (DSE 2003).

To date no extensive monitoring of pesticide concentrations in the north-eastern catchments has been undertaken, accordingly Melbourne Water commissioned this assessment of pesticide inputs in sediment and waters in these catchments over a 12-month period.

Objectives

- To determine spatial and temporal occurrence of pesticides in sediments and waters of streams dominated by agricultural land uses in the north-east of Western Port.
- To assess biological impacts of pesticides in water and sediment to flora and fauna in these catchments.

A multiple lines of evidence approach was used to monitor and assess risks from pesticides. This provided details about the spatial and temporal variability in pesticides potentially being transported into the north-east of Western Port and whether they are at concentrations likely to pose risks to flora and fauna and whether biological impacts are being observed.

Methods

Sediments and water were collected between June 2017 and May 2018 from seven sites from two sub-catchments (Lower Bunyip sub-catchment and the Cardinia, Toomuc, Deep and Ararat Creeks sub-catchment) situated in the north-east, which represented areas of significant agricultural land use. Sediment was collected at each of the sites twice, spring 2017 and autumn 2018 and analyzed for a suite of pesticides as well as nutrients. In addition, the toxicity of the sediment was assessed using a local amphipod. *In situ* assessments were carried out at these times to determine the impacts of these waters on flora (algae) and fauna (glass shrimp). Water samples were collected, and passive samplers were deployed at each location four times throughout the study period (Spring 2017, Summer 2017, Autumn 2018 and Winter 2018) to assess seasonal fluctuations in pesticide class and concentration.

Key Findings

- There is pesticide contamination, particularly the herbicides diuron and simazine, and fungicides, across the two catchments, both individually and as complex pesticide mixtures.
- Concentrations of individual pesticides and mixtures of PSII herbicides exceeded the Australian and New Zealand TVs (ANZECC and ARMCANZ, 2000) at some of the sites, predominantly Drain One Creek and Lower Gum Scrub Creek.
- There were seasonal patterns of contamination that were pesticide-class specific.
- Nutrient levels were elevated at several sites according to State Environment Protection Policy (SEPP) Waters (2018) guidelines for estuarine systems or lowlands of Western Port.
- Water and sediment at several sites resulted in significant biological impairment in plants and invertebrates, most notably in the Autumn 2018 sampling period.

Recommendations

Several recommendations for further monitoring and research are proposed based on the outcomes from the current study. In priority order, they include:

1. Sourcing of Pesticides

- Determine the sources of pesticides in Lower Gum Scrub Creek, Yallock Cut Creek, Drain One Creek and Deep Creek.

2. Transport pathways, pesticide persistence and management actions

- Determine the major transport pathways for pesticides to Western Port Creeks (e.g.: groundwater, surface water runoff, aerial deposition, dissolved or sediment bound). Key pesticides for initial focus: diuron, simazine, atrazine, metolachlor, tebuconazole, iprodione.
- Determine if pesticide detections are related to recent application and subsequent runoff or due to persistence?
- Determine and assess management actions to reduce pesticide inputs?

3. Herbicide and Fungicide Threats to Western Port

- Determine concentrations of herbicides, singly and in mixtures, that present a concern for local flora and fauna e.g.: plants, frogs, fish. Initial focus on key herbicides detected, e.g.: diuron, simazine, atrazine, metolachlor
- Determine whether concentrations of fungicides are a concern for local flora and fauna. Initial focus on development of assessment methods with local species and key fungicides detected, e.g.: tebuconazole, iprodione.
- Investigate the effect of nutrient enrichment on pesticide toxicity. Initially, focus should be on herbicide and nutrient interactions on local flora.

4. Nutrient threats to Western Port

- Determine the source and transport pathways and total loads of nutrients throughout the year and the risk they pose for increased nuisance algal growth and eutrophication.
- Assess the impacts of nutrients on freshwater and estuarine biota. Initial focus on characterisation of soft sediment chemistry and microfauna/flora using eDNA techniques.
- Determine and assess management actions to reduce nutrient inputs?

Introduction

The Western Port catchment lies 70 kilometres south-east of the city of Melbourne, Victoria, and covers an area of approximately 3,721 square kilometres, including approximately 2,232 kilometres of rivers and creeks (Keough et al., 2011). The river and creek networks support many economic, social and ecological values in the region including water supply, flood mitigation, primary production, lifestyle and recreational activities and are home to numerous flora and fauna species, including many vulnerable and endangered species such as the growling grass frog, southern toadlet, Australian grayling and dwarf galaxias (Melbourne Water 2018; City of Casey 2017). The marine ecosystem within Western Port is also of regional, national and international importance supporting numerous habitats including mangroves, saltmarsh, seagrass, reef and mud flats.

Based on the new Healthy Waterways Strategy (Melbourne Water 2018), the Western Port catchment is made up of twelve sub-catchments (Figure 1). Much of the northern catchments have been modified to support rural and green wedge land uses, with approximately 70% of the catchment being agricultural, comprised of horticulture, dryland grazing and dairying. Forests and reserves occupy a further 20%, while the remaining 10% is made up of remnant vegetation and urban areas. Over the last 15 years there has been significant urban growth throughout the catchment (128 – 172 % between 1996 and 2010 (ABS, 2011)), with further expansion likely to continue. The northern catchments are an agriculturally important region of Victoria, accommodating 70% of Victoria's broiler chicken industry and 40% of Victoria's market gardens; accounting for over 30% of Victoria's gross value agricultural production (DPCD, 2011; EPA 2001).

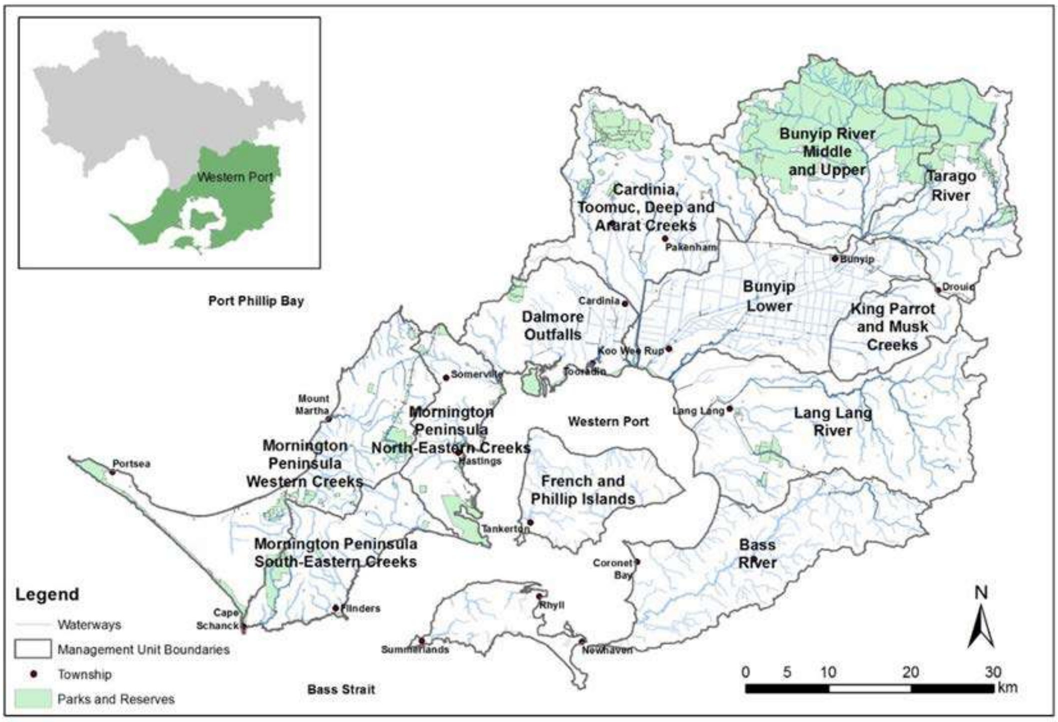

Figure 1: Western Port catchment and its twelve sub-catchments.

In 2011, the Western Port knowledge review recommended a study to understand whether toxicants pose a risk to the aquatic ecosystems of Western Port (Keough et al 2011). Since 2011, research and monitoring programs assessing the risks from toxicants in waterways of Western Port have shown the occurrence of elevated concentrations and complex mixtures of pesticides, which could pose risk to the environmental, social and economic values of Western Port and associated waterways. Programs to date have focused on creeks in the sub-catchments of Mornington Peninsula North-eastern Creeks and Dalmore Outfalls in the north west of Westernport. These sub-catchments are dominated by agricultural, including market gardens, livestock and poultry, orchards and rural residential land-uses. There has also been significant urban growth in the sub-catchments.

The research and monitoring programs since 2011 have detected some 44 different pesticides in surface waters and sediments, with concentrations of several individual pesticides exceeding ANZECC/ARMCANZ (2000) guideline values, which is a concern for the health of local flora and fauna species. Key pesticides detected include the herbicides prometryn, metolachlor, simazine, diuron, linuron and atrazine; fungicides boscalid, iprodione, metalaxyl and dimethomorph; and insecticides fenamiphos, chlorpyrifos, dimethoate and diazinone. In general, lower numbers of pesticides have been detected in sediments; however, concentrations are generally greater than those detected in surface waters.

Based on these previous findings it was recommended that monitoring of systems in agriculturally dominated catchments in the north-east of Western Port be undertaken to provide a broader understanding of the risks of pesticides across the region. Like the north-west, the north-east catchments of Cardinia, Toomuc, Deep and Ararat Creeks and Bunyip River Lower are agriculturally dominated sub-catchments and studies of these systems will contribute important information of the effects of land-use and catchment management on water quality in creeks and estuaries that lead to the bay. There are several streams in the sub-catchments that enter the bay in a radial fashion. The larger stream networks are the combined Bunyip-Tarago system, Cardinia Creek, the combined Toomuc and Deep creek system, Yallock Cut and Lylls Inlet.

The Cardinia, Toomuc, Deep and Ararat Creeks and Bunyip Lower sub-catchments were substantially modified in the 1880s -1930s, with the flow of many creeks diverted into artificial drains to provide drainage for intensive agricultural land which extends over the old bunyip swamp lands (DSE 2003). Prior to this drainage work, few streams discharged directly into Western Port in the North. The construction of these drains provides flood protection and drainage to the area, but also allows transport of sediment and anthropogenic contaminants, once contained in the swamp, to be transported to Western Port Bay (EPA 2001). Known as the Koo-Wee-Rup horticultural district, this region has the second highest rate (per hectare) of agricultural production in Victoria (DPCD, 2011). Primary crops are vegetables, including celery, leeks, herbs, silver beet, radishes, spring onions, leafy greens and asparagus (City of Casey 2017; Victoria Places 2015). Other land use includes dryland grazing, animal production and rural residential.

Based on the Healthy Waterways Strategy, environmental water quality in Cardinia, Toomuc, Deep and Ararat Creeks and Lower Bunyip management units are currently considered low to moderate (Melbourne Water 2018). Three of the waterways draining the area, Cardinia Creek, the Bunyip River and Yallock Cut are significant contributors of sediments into the bay (Wilkinson et al 2016). These systems, discharging into a region of the bay where significant losses in seagrass have occurred in recent decades, are major high tide roosts for migratory wader birds, including the Greenshank, Curlew Sandpiper, Red-necked Stint, Sharp-tailed Sandpiper, Eastern Curlew and Masked Lapwing (DSE 2003) and are also home to vulnerable and endangered species such as the Australian grayling and dwarf galaxias (Melbourne Water 2018).

To date, no extensive monitoring and assessment of pesticide risks and water quality in the North-eastern sub-catchments has been undertaken. Performance objectives in the recent Healthy Waterways Strategy have identified a need to reduce threats across rural and urban land from toxicants such as pesticides and to ensure no polluted sediments enters drains and watercourses to protect Ramsar values in Western Port and reduce threats to seagrass. To do this, we need an understanding of whether toxicants pose a threat in these systems.

This report details the results of spatial and temporal investigations of pesticides and nutrients in waterways of north-east Western Port. The study approach involved seasonal monitoring of pesticide and nutrient concentrations in seven creeks across two sub-catchments of Cardinia, Toomuc, Deep and Ararat Creek and Bunyip Lower. Secondly an examination of in stream health through the deployment of *in situ* bioassays of caged flora and fauna and an assessment of sediment toxicology through laboratory bioassays was undertaken. The overall focus of the study was to assess seasonal and spatial variability in pesticides and nutrients and determine whether ecological impacts to flora and fauna were occurring.

Study Objectives

The objectives of this project are to investigate temporal pesticide occurrence and concentrations across north-eastern catchments of Western Port and assess risks to resident flora and fauna. More specifically to:

- Determine spatial and temporal occurrence of pesticides in sediments and waters of streams dominated by agricultural land uses in the north-east of Western Port.
- Conduct investigations to assess for biological impacts in these catchments.

To monitor and assess risks from pesticides a multiple lines of evidence approach was applied. This provided details about the spatial and temporal variability in pesticides potentially being transported into the north-east of Western Port, whether they are at concentrations likely to pose risks to flora and fauna and whether biological impacts are being observed.

Methods

Study Area

The Western Port catchment is comprised of twelve sub-catchments, which drain into Western Port Bay (Figure 1). As part of this program seven sites were monitored from two sub-catchments situated in the north-east: Lower Bunyip sub-catchment and the Cardinia, Toomuc, Deep and Ararat Creeks sub-catchment (Figure 2), which represented areas of significant agricultural land use. Two sites were situated in the Lower Bunyip sub-catchment: Yallock Cut and Bunyip River, while Cardinia Creek, Lower Gum Scrub Creek, Toomuc Creek, Deep Creek and Drain One were situated in the Cardinia, Toomuc, Deep and Ararat Creeks sub-catchment (Figure 2).

A reference site (Cardinia Creek at Chasemore Road, Cardinia) was used during the deployment periods where *in situ* cages were applied. This site has had little to no pesticides detected previously and is the collection site for invertebrates for A3P stocks.

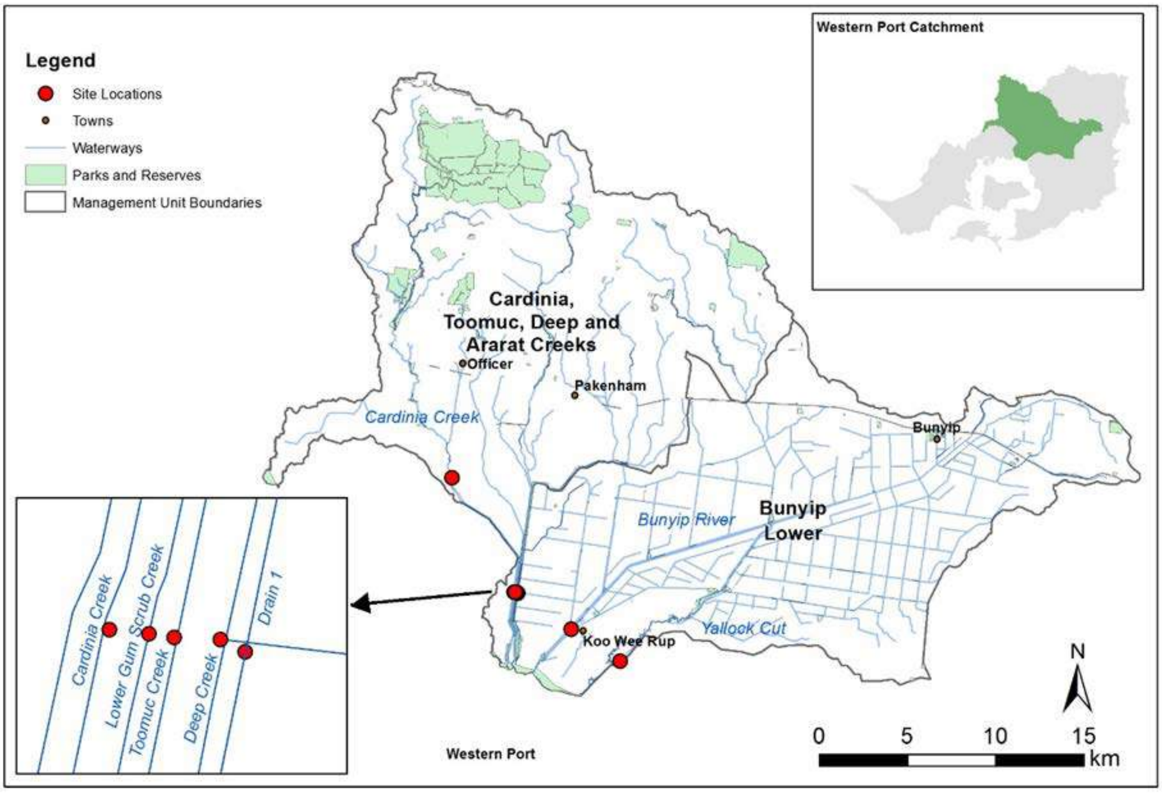

Figure 2: Locations of monitoring sites within the Cardinia, Toomuc, Deep and Ararat Creek and Bunyip Lower sub-catchments for the pesticide assessment program in north-eastern catchments of Western Port.

Catchment Land-Use

Upstream catchment areas for each site were determined using the Melbourne Water catchment layers, waterway drainage lines and topography in ArcGIS 10.3. Catchment land-use was determined from the Victorian Land-use Information System (2010). Land-use was reclassified into nine broader land-use categories (Animal Production, Grazing Pastures), Horticulture (vegetables and herbs), Irrigated Pastures and Fruits, Native Forests, Parks and Recreation, Roads, Urban and Waterways) extracted by tabulating areas within each watershed and summarised for each watershed as a percentage of the total catchment area.

Sample Collection

Chemistry

Surface Water Physico-chemistry

Physico-chemical measurements were determined *in-situ* using a multi-parameter water quality analyser (HANNA 9829) for temperature, dissolved oxygen (DO) and electrical conductivity (EC) calibrated prior to each sampling event. Turbidity was determined on site using a turbitimeter (HANNA HI93414-01) calibrated using certified formazin standards.

Passive Sampling

Passive samplers were deployed at each site on four occasions, representing summer, autumn, winter and spring, to measure the concentrations of pesticides over extended periods. Sampler units consisted of an Empore™ SDB-XC disk deployed in a Chemcatcher® housing (Kingston et al 2000), with a polyethersulfone (PES) diffusion-limiting membrane covering the Empore™ disk surface. Empore™ disks and PES membranes were conditioned with methanol followed by deionised water, then stored in deionised water at 4°C until deployment at field sites. Chemcatchers® were deployed in cages (plastic mesh pockets 15cm x 15cm) attached to steel star pickets with cable ties for 3-weeks (~21 days). Following retrieval, the samplers were filled with site water and transported to the laboratory on ice. Empore™ disks and PES membranes were then removed from the Chemcatcher® housing and dried at 35°C for one hour. Empore™ disk were then packaged, labelled and sent to National Measurement Institute (NMI), Sydney, for extraction and chemical analysis.

Grab Water Sampling

Grab water samples were collected at each site at the same time as the passive samplers were deployed. Samples were collected from approximately 0.2m below the water surface in the centre of the channel, near the deployed passive samplers. For pesticides, samples were collected directly into 250 mL amber glass bottles, while nutrient samples were collected directly into two sets of 125 mL plastic bottles, with one preserved with sulfuric acid to pH <2. Samples were then transported back to University of Melbourne on ice and stored in the dark at 4⁰C before analysis.

Grab samples for pesticide analysis were analysed by National Measurement Institute, Sydney. Analysis of nutrients in grab samples was undertaken by Australian Laboratory Services (ALS), Melbourne. Samples were analysed for total phosphorus (TP), Total nitrogen (TN), Total Kjeldahl Nitrogen (TKN), ammonium nitrogen as N (NH4-N) and the sum of nitrate and nitrite (NOx). All were determined by direct colourimetry by discrete analyser (APHA 2005) (See Appendix 2 for detection limits).

Sediment Sampling

Sediment samples were collected at each site on two occasions, spring and autumn, to assess seasonal and temporal differences in pesticide classes and concentrations. Depositional sediments (top 0 - 2 cm layer) were collected using a shovel, wet sieved (< 63µm), and the fine fraction retained in 250mL pre-cleaned glass jars with a Teflon coated lid for pesticide analysis. Pesticide analysis in fine sediments was undertaken by National Measurement Institute, Sydney.

Whole sediments were also collected for analysis of particle size. Particle size was determined by laser diffraction for sand, silt and clay (<2mm) and by wet sieving for gravel (>2mm).

Chemical Analysis

Pesticides on Empore^{TM} disks, from the water grab and sediment samples were extracted and then analysed using a multi-residue GC-MS/MS method (USEPA SW 846). A total of 117 pesticides were analysed, including 14 herbicides, 76 insecticides, 23 fungicides (See Appendix 1 for pesticide names and detection limits).

Ecotoxicological Assessment

Several ecotoxicological tools were applied during autumn and spring sampling events to determine if faunal and floral assemblages were being impacted in the Lower Bunyip and Cardinia, Toomuc, Deep and Ararat Creeks sub-catchments. Tools included *in situ* field caging experiments with microalgae (*Scenedesmus* sp.) and Australian glass shrimp (*Paratya australiensis*) and sediment toxicology using the local amphipod species *Austrochiltonia subtenuis* as the indicator organism.

In situ Field Caging Experiments

Australian glass shrimp, *Paratya australiensis*

A standardised cage toxicity test, developed at CAPIM, using the Australian glass shrimp, *P. australiensis*, was used to assess *in situ* toxicity of waters to faunal species. In short, 240 field collected *P. australiensis* from a non-contaminated site in the Western Port catchment (Cardinia Creek at Chasemore Road, Cardinia) were randomly assigned to cages (white PVC 90 mm x120 mm tube with PVC caps attached to each end and 250 µm mesh covering three side windows (40 x 50 mm) to allow water and oxygen to flow through the cage) and deployed at each site. Each cage contained five *P. australiensis* individuals, a piece of cotton gauze (approximately 16 cm^2) to provide habitat and six pellets of 'Shrimps natural sinking pellets' (Sera, North America) for food. Five cages were deployed at each site, attached to star steel pickets at no more than 30cm below the water surface for 10 days. At the termination of exposure, cages were retrieved and the number of surviving *P. australiensis* was determined.

Microalgae, *Scenedesmus* sp.

A standardised cage toxicity test, based on methods developed by Moreira-Santos et al (2004), with modifications undertaken by CAPIM, using the green microalgae, *Scenedesmus sp.*, was used to assess *in situ* toxicity of waters to floral species. In short, *Scenedesmus* sp. cells immobilised in alginate beads (alginic acid sodium salt from brown algae, SIGMA Life Sciences) were randomly assigned to specially made exposure or control cages and deployed at each site. Control cages consisted of clear polycarbonate 50 mL centrifuge tubes filled with MLA algal growth medium (Bolch and Blackburn 1996). Exposure cages consisted of clear polycarbonate 50 mL centrifuge tubes with two rectangular holes cut in the sides and covered with 125 µm nylon mesh to allow water exchange through the cage. Each control and exposure cage contained 20 algal beads and were deployed, in triplicate at each site, attached to star steel pickets at no more than 20cm below the water surface. The cages remained *in situ* for 10 days. At the end of 10 days, the beads were collected and transported to the University of Melbourne laboratory for biomass determination. Algal cell biomass was determined by *in vivo* fluorescence measurements of dissolved algal beads. The cell biomass was used to assess impacts to the test endpoint of algal growth. Final cell biomass was expressed as a percentage of the site control to eliminate, to the extent possible, differences in abiotic site conditions.

Sediment Toxicology

Standard sediment toxicity tests (based on the procedures of the US EPA (2000) and Environment Canada (2013) with modifications to meet the requirements of Australian species) using the freshwater amphipod, *Austrochiltonia subtenuis*, were used to assess toxicity of depositional sediments. In short, on day 0, ten juvenile amphipods (pass 297 µm sieve and retain on 212 µm sieve) were added to beakers containing ca. 50 g (wet weight) of fine sediment (<63 µm) and 200 mL of artificial water. A reference sediment (Bittern Reservoir, Victoria), collected at the same time as site sediments, was also prepared and tested as per site sediments as an external control. All treatments were run in quadruplicate, with the 8 replicates of the external control. All beakers were aerated and maintained in a temperature controlled incubator (21°C) under a 16:8 h light:dark cycle for 14 days. During this period, overlying water was renewed once a week and amphipods fed (0.5 ml Yeast Trout Chow and Cerophyll^{TM} + 0.9 mg Tetramin® commercial fish food) three times per week. The test was terminated at 14 days, where amphipods were removed by sieving the water and sediment (250µm) and the test endpoints of survival and growth (as determined by head length measurements) were determined. If there was less than 50% survival of the amphipods at a site, growth was not measured and excluded from further analyses.

General water quality parameters electrical conductivity (EC), pH, dissolved oxygen (DO), and ammonia (NH^{3+}) concentration were measured at each water renewal and at the end of the test using a TPS Water Quality meter.

Data Analysis

Pesticide data was summarised to assess presence/absence, frequency of detection and risks to aquatic life. Assessment of risks to aquatic life was undertaken by comparison of pesticide concentrations detected to the Australian and New Zealand Water Quality Guidelines (ANZECC/ARMCANZ 2000) Trigger Values (TVs) for the protection of aquatic ecosystems. Nutrient and physico-chemical data was summarised and compared to State Environment Protection Policy (SEPP) Waters (2018) guideline TVs for estuarine systems or lowlands of Western Port or to ANZECC/ARMCANZ (2000) TVs for the protection of aquatic ecosystems.

Differences in survival of *P. australiensis* and *A. subtenuis*, growth of *A. subtenuis* and cell biomass of *Scenedesmus* sp. were assessed using one-way analysis of variance (ANOVA) followed by a Dunnett multiple comparison test to determine which sites significantly differed from the control or reference site. Survival and cell biomass data was arcsine square root-transformed prior to analysis. All data was checked for normality and homogeneity of variances prior to ANOVA. If data did not meet the assumptions of the parametric ANOVA, a Kruskal-Wallis test was applied to assess for differences between medians. Statistical significance was set at $p < 0.05$. Analyses for *in situ* ecotoxicological tests were performed using SPSS Statistics Version 21 software, while sediment toxicological analysis was performed using Minitab software version 17.

For the *in situ* algal bioassay, inhibition of growth (based on cell biomass) was determined for each site. Inhibition was calculated using the following equation:

% inhibition = ((site control biomass – site biomass)/site control biomass) * 100

Weight of Evidence Approach

We applied a WoE determination for causality based on the sediment quality triad approach as outlined in Chapman et al (2002) and Burton et al (2002).

Results

Land Use

Land-use within the catchments of the 7 sites is detailed in Table 1 and Figure 3. Grazing pastures is the predominant land-use across the catchments of all creeks (29-86%), except for Drain One, where Horticulture (vegetables and herbs) was the predominant land-use. The catchments of Cardinia, Lower Gum Scrub, Toomuc and Deep Creeks have significant urban areas (23-33%) in the upper reaches, followed by native forest in the top of the catchments (Table 1; Figure 3). Horticulture and irrigated pastures comprise a small area of the catchments (<5%). These land-use activities are based in the mid to lower sections of the creeks and directly abound the stream sides (Figure 3). Horticulture in this area is predominantly asparagus crops (visual observation over study period). In the mid to upper reaches of Lower Gum Scrub and Cardinia Creeks and Drain one there is a small amount of land used for animal production (3-5%; Table 1).

Bunyip River and Yallock Cut catchments have low urban (<10%) and horticultural or irrigated pasture (<2.2%) land-use. Yallock Cut and Bunyip catchments are predominantly Grazing pastures for cattle and horses (86% and 58% respectively). Yallock Cut has a small amount of native forest (3.5%), while remaining land-use in the Bunyip River catchment is native forests (17%) and Parks and recreation (14%) (Table 1; Figure 3 and visual observation).

Table 1: Land use (%) in the catchments of the seven sites monitored during the study period (Victorian Land-use Information System, 2010).

Catchments	Cardinia Creek	Lower Gum Scrub Creek	Toomuc Creek	Deep Creek	Drain One	Bunyip River	Yallock Cut
Animal Production	2.7	5	0.6	0.8	4.7	0.8	0.6
Grazing Pastures	28.6	52.8	52.7	55.3	41.7	57.7	86.1
Horticulture (vegetables and herbs)	0.4	1.6	0.2	0.7	49	1.4	0.2
Irrigated Pastures and Fruits	0.5	2.5	0.6	4.5	0.2	0.8	0
Native Forests	17.6	8	10.5	6.2	0	17.3	3.5
Parks and Recreation	3.4	0.8	0.7	1.3	0	13.5	0.4
Roads	4.2	3.7	4.3	5	1.5	2.9	0.9
Urban	33.2	23.7	29.9	24.7	0.5	5.4	8.2
Waterways	9.4	1.9	0.3	1.6	2.4	0.2	0.1
Catchment Area (km^2)	138.9	36.9	68.5	74.1	18.8	959.4	109.6

Rainfall

Figure 4 depicts rainfall over the sampling period (Kooweerup rainfall gauge, Melbourne Water), July 2017 to May 2018. Highest rainfall occurred in winter (47.4 mm total, 1.58 mm daily average) and spring (40.4 mm total, 1.39 mm daily average) sample periods. During summer and autumn there was less rainfall (summer: 29.6 mm total, 1.02 mm daily average; autumn: 32.8 total, 1.09 mm daily average), however there were storm events with greater rainfall volumes during these seasons.

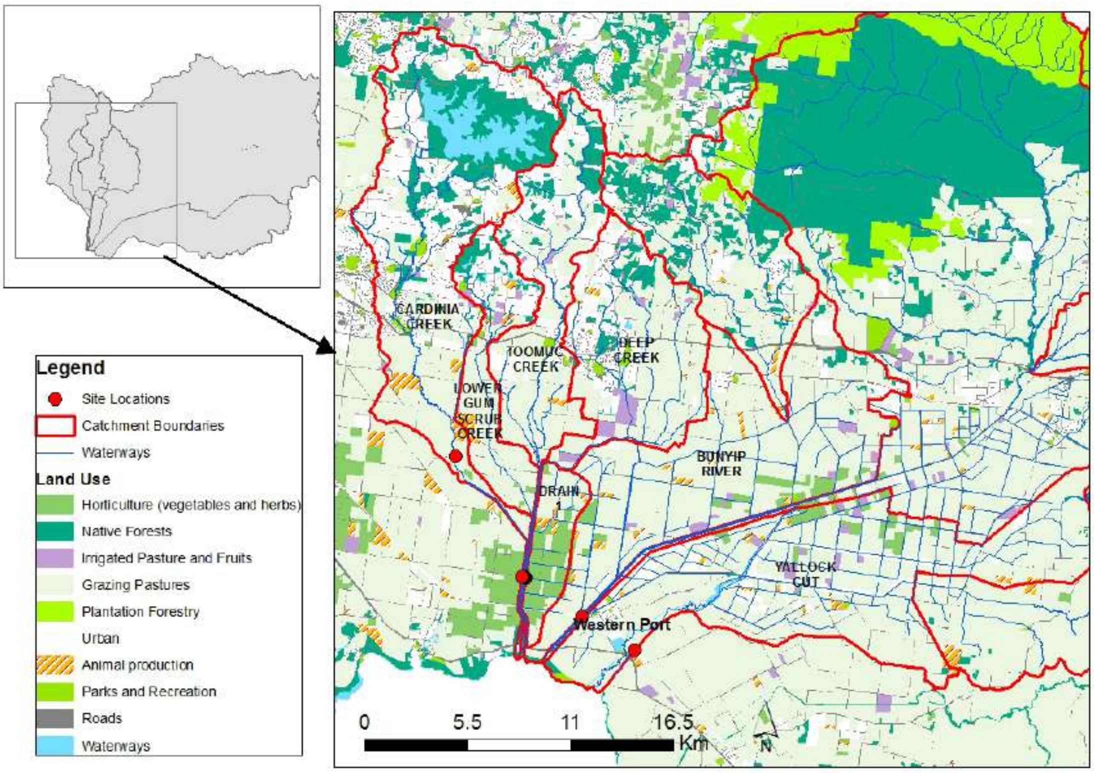

Figure 3: Land use in the catchments for the seven sites monitored during the study period. Note that not all of the Yallock Cut and Bunyip River catchments are shown.

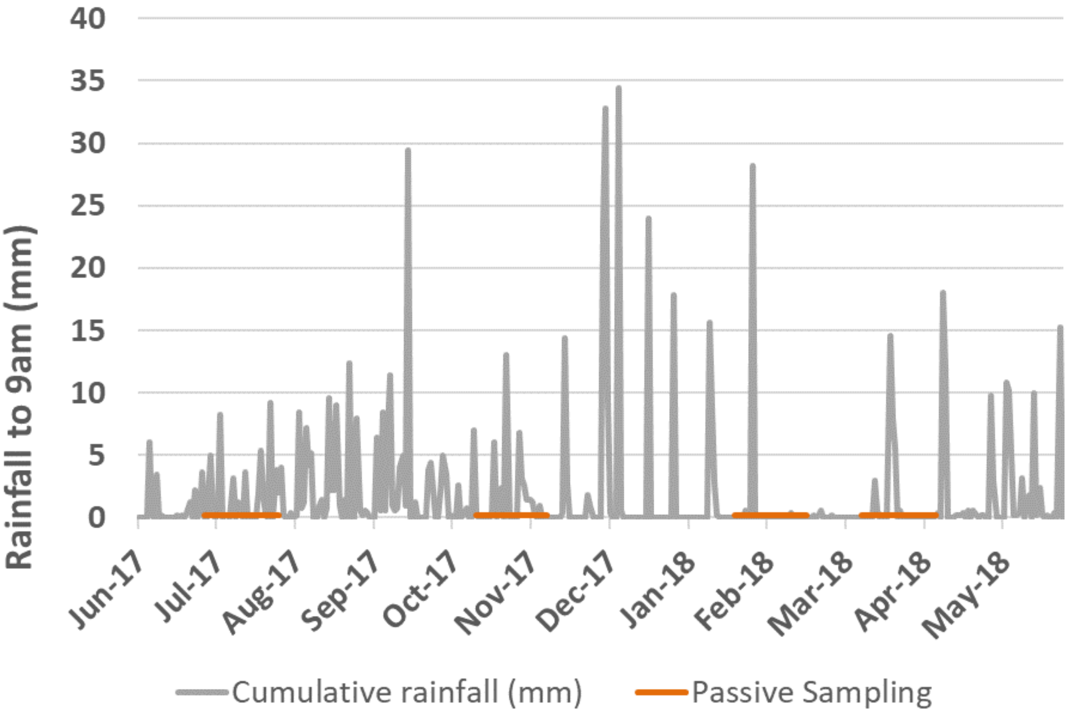

Figure 4: Monthly rainfall over the sampling period from June 2017 through May 2018 (based on Kooweerup rainfall gauge, Melbourne Water). Sample deployment periods in each of winter, spring, summer and autumn are indicated by the orange line on the x-axis.

Chemistry

Surface Water Physico-Chemistry

In situ physico-chemistry measurements (water temperature, dissolved oxygen, pH, electrical conductivity, turbidity) for each season and site are summarised in Figure 5. Surface water temperatures (SWT) varied by season, however were generally similar amongst sites within a season (Figure 5a). Highest SWTs were recorded in summer (mean 23.4°C), followed by autumn (mean 19.9°C) and spring (mean 18.4°C). SWT during winter were the coolest (mean 9.5°C).

Electrical conductivity (EC) varied seasonally and amongst sites (Figure 5b). Most sites were tidally influenced, based on field observations; the exceptions were the site in Yallock Cut as it was situated above a small pool and weir, preventing estuarine influence, and the reference site on Cardinia Creek. Highest ECs were measured during summer and autumn, notably at sites in Lower Gum Scrub Creek, Toomuc Creek, Deep Creek and Drain One Creek. Sites in Cardinia Creek, Bunyip River and Yallock Cut had more consistent and lower EC measurements across seasons.

Dissolved oxygen (DO) levels ranged from 29 to 272 % and were generally similar across seasons, however varied among sites (Figure 5c). Toomuc Creek had the lowest DO levels across all sites and seasons (range 29.2-86.7%), while highest levels were measured in Drain One during each season (range 68.4-271.8%). SEPP Waters (2018) guideline range for acceptable DO in estuaries is 80-110%, while in freshwaters is 75-110%. Dissolved oxygen levels were often outside these acceptable ranges at many sites on at least one occasion during spring, summer and autumn indicating at times there are potential DO issues in these creeks.

In situ pH measurements were generally consistent across seasons and among sites, ranging from 6.13 to 8.84 (Figure 5d). The pH values were generally within SEPP Waters (2018) guideline range for fresh and estuarine systems (fresh 6.7-7.7; estuarine 7-8).

Turbidity varied amongst sites and across seasons, ranging from a low of 0.2 NTU to a high of 370 NTU (Figure 5e). The highest turbidity was measured at the site in Drain One during winter (370 NTU). In Bunyip River, Cardinia Creek, Toomuc Creek and Drain One (except for winter) turbidity was stable across seasons. However, in Deep Creek, Lower Gum Scrub Creek and Yallock Cut turbidity varied with season. The turbidity in these systems generally exceeded the SEPP Waters (2018) guideline values for fresh and estuarine systems (fresh 25; estuarine 10).

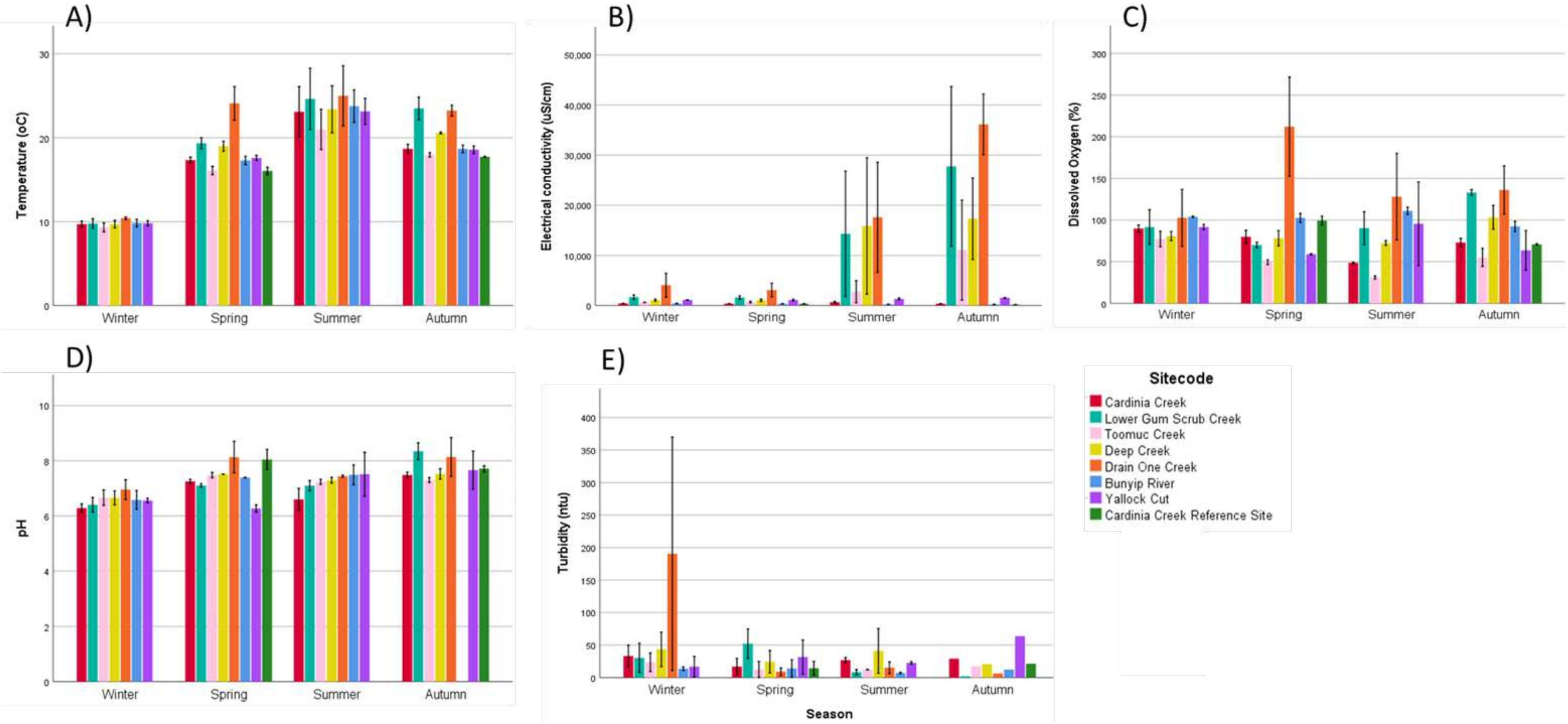

Figure 5: Seasonal i*n situ* physico-chemistry at seven study sites and the reference site in north-east catchments of Western Port. A) surface water temperature B) electrical conductivity C) dissolved oxygen D) pH, electrical and E) turbidity.

Pesticides

Surface Water Samples

Twenty different pesticides and caffeine were detected in surface waters during the sampling period. Figures 6 and 7 show the total number of pesticides (grouped according to their target pest) at each site that were above the limit of reporting (LOR), based on grab and passive samples, respectively. Yallock Cut, Deep Creek, Drain One and Lower Gum Scrub Creek all had the greatest number of pesticides detected (8 in each), and had the greatest variety of pesticide classes in both grab and passive samples (insecticides, herbicides and fungicides). Of the seven impact sites investigated, the sampling site in the Bunyip River had the least number of pesticides detected by both sampling methods and furthermore, only insecticides were detected. At the reference site in Cardinia creek, one insecticide was detected in passive samplers. In general, passive samplers detected a greater number of pesticides at each site than grab sampling (Figures 6 and 7).

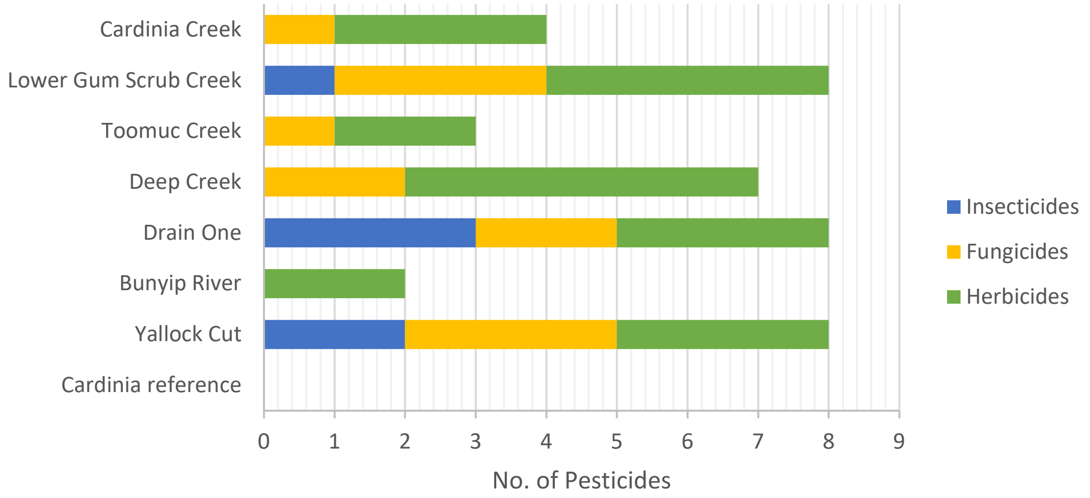

Figure 6: Pesticide richness detected in grab water samples at each site across all seasons. Pesticides have been grouped based on their target organisms: green = herbicide, yellow = fungicide and blue = insecticide.

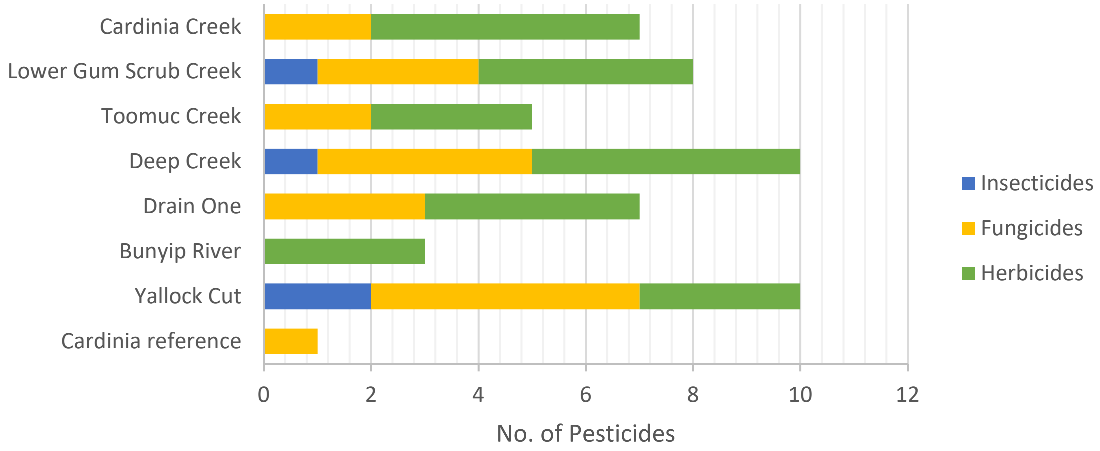

Figure 7: Pesticide richness detected in passive samplers (Empore^{TM} disks) at each site across all seasons. Pesticides have been grouped based on their target organisms: green = herbicide, yellow = fungicide and blue = insecticide.

Figures 8 and 9 show the total number of pesticides detected seasonally that were above the LOR, in grab and passive samples, respectively. Based on grab samples, winter had the greatest number of pesticides detected and greatest diversity of pesticides. However, for passive samples the greatest number and diversity of pesticides were detected in spring. Herbicides and fungicides made up the greatest number of detected pesticides, with few insecticides being detected. The lowest number and variety of pesticides were detected in summer using grab samples, consisting of an insecticide and two herbicides (Figure 8). In general, passive samplers detected a greater number of pesticides each season than grab sampling, except in winter (Figures 8 and 9).

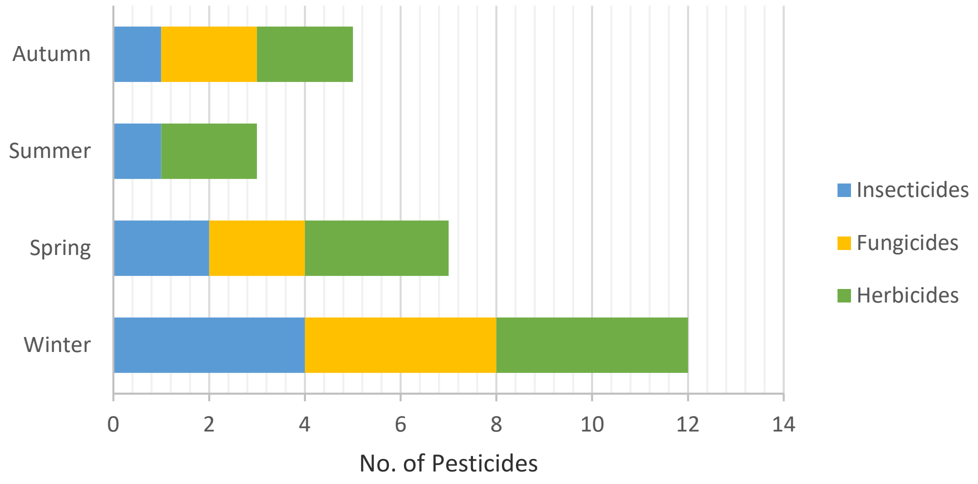

Figure 8: Seasonal pesticide richness detected in grab water samples. Pesticides have been grouped based on their target organisms: green = herbicide, yellow = fungicide and blue = insecticide.

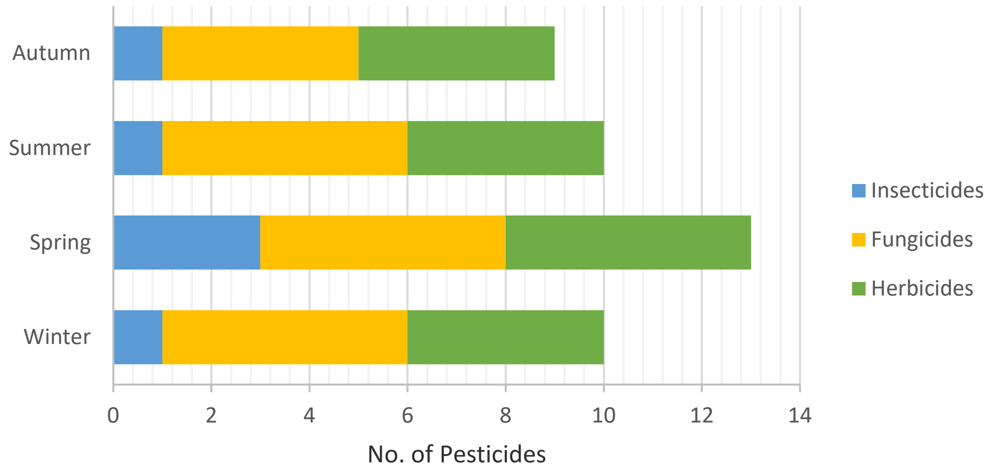

Figure 9: Seasonal pesticide richness detected in passive samplers (Empore^{TM} disks). Pesticides have been grouped based on their target organisms: green = herbicide, yellow = fungicide and blue = insecticide.

To assess the most common pesticides entering the sites, the frequency of pesticide detection was calculated for grab and passive samples (Figures 10 and 11). For both sample methods herbicides were the most frequently detected pesticide group (occurring in 62% and 91% of grab and passive samples, respectively), followed by the insecticides (36% and 65% of grab and passive samples, respectively). Insecticides were detected in <25% of grab and passive samples.

From the 47 grab samples analysed (Figure 10), 13 different pesticides were detected, with the herbicides simazine and diuron detected most frequently (occurring in ≥40% of samples). The herbicide metribuzin and fungicide tebuconazole were also frequently detected, occurring in >20% of samples.

Seventeen different pesticides were detected from the 30 passive samples analysed (Figure 11). Simazine was the most frequently detected pesticide (occurring in 79% of samples) followed by the herbicide diuron, and fungicides tebuconazole and iprodione, which were all detected in more than 45% of samples. The herbicides atrazine and metolachlor were also frequently detected, occurring in 29% and 21% of samples, respectively. Of the 20 pesticides detected, seven were only detected in passive samples (prometryn, pyrimethanil, propiconazole I and II, procymidone, DEET and malathion), while three were exclusively detected in grab samples (p,p-DDT, p,p-DDD and p,p-DDE) (Figures 10 and 11).

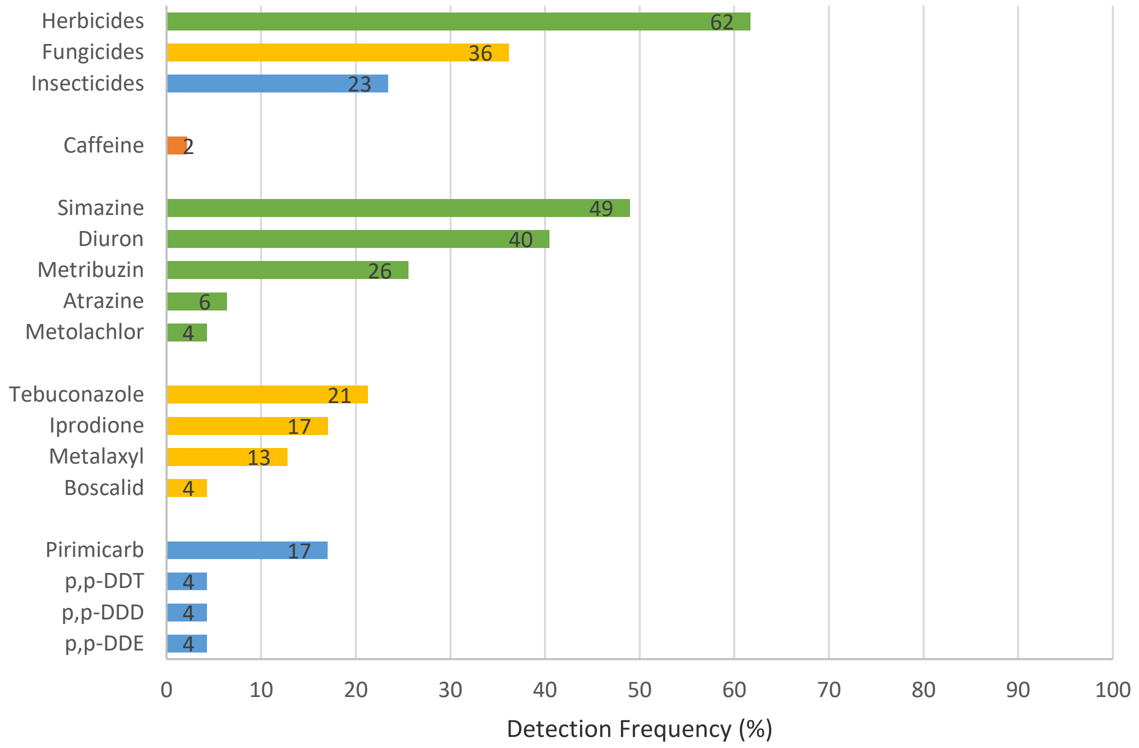

Figure 10: Frequency (%) of pesticide detections from grab water samples collected across all sites and seasons. Green bars represent herbicides, yellow bars fungicides, blue are insecticides and orange are miscellaneous.

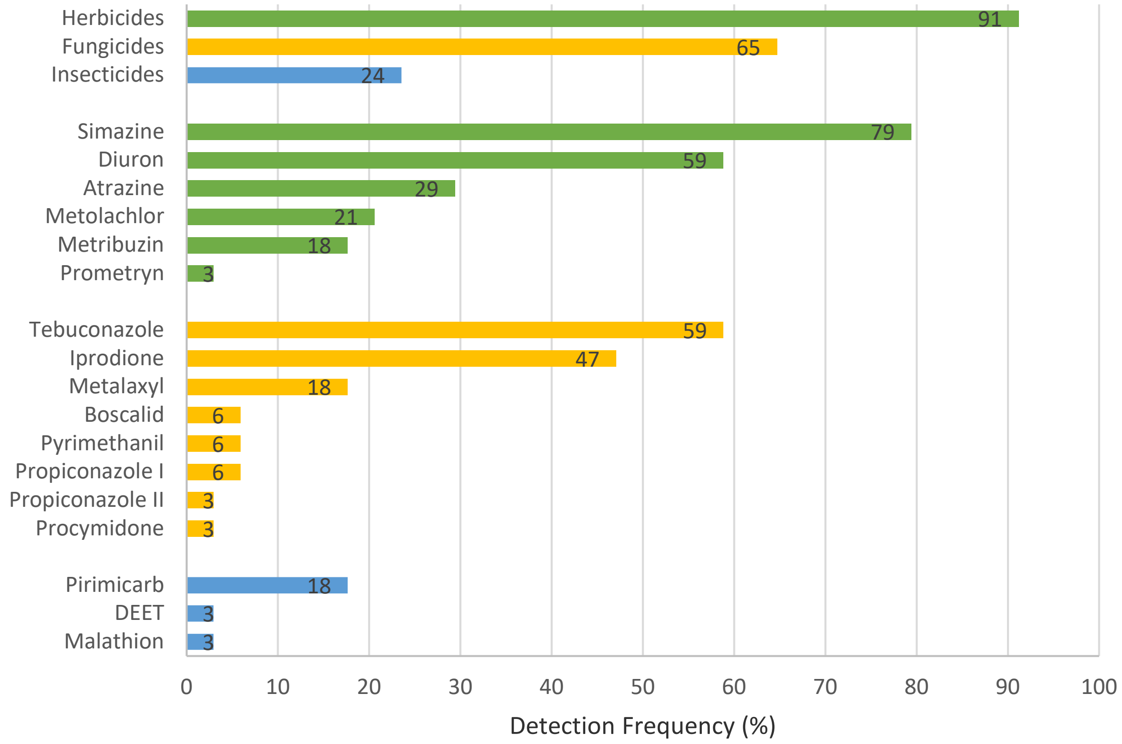

Figure 11: Frequency (%) of pesticide detections from passive samplers (Empore^{TM} disks) across all sites and seasons. Green bars represent herbicides, yellow bars fungicides and blue bars are insecticides.

A summary of the concentrations and mass of pesticides detected both spatially and temporally in the grab water samples and on the passive samplers are shown in Figures 12 and 13 and compared with available water quality guideline values in Tables 2 and 3. Concentrations of pesticides detected ranged from the LOR (0.01µg/L) to a maximum of 3.6 µg/L. The highest concentration measured was for the herbicide diuron (3.6 µg/L) during spring at the Drain One site, followed by the fungicide iprodione (2.4 µg/L), detected during winter at the site in Lower Gum Scrub creek (Table 2). Concentrations of diuron and *p,p*-DDT exceeded default TVs for fresh and/or marine waters in 47% and 100% of samples where they were detected (as indicated in bold in Table 2). The concentrations of total pesticides detected in grab water samples varied by site and season, ranging from 0.015 to 3.13 µg/L (Figure 12). The highest total pesticide concentrations were at site Drain One in spring (3.13 µg/L), followed by Lower Gum Scrub Creek in winter (2.17 µg/L) and Yallock Cut in summer (1.14 µg/L). For all sites, the lowest pesticide concentrations were detected during autumn sampling. The exception was the site in the Bunyip River, where the highest concentration of total pesticides was detected (0.06 µg/L) during autumn.

Masses of pesticides accumulated on Empore^{TM} passive sampler disks ranged from the LOR (0.01 µg) to 4.5 µg (Table 3). The greatest mass accumulated was for the fungicide iprodione (4.5 µg) during winter at the site in Lower Gum Scrub creek. For all other pesticides detected on Empore^{TM} disks, masses were <1 µg. The total mass of pesticides detected in Empore^{TM} disks varied by site and season and ranged from 0.01 µg to 4.92 µg (Figure 13). The greatest mass of total pesticides was detected on disks from Lower Gum Scrub Creek during winter (4.92 µg). Greatest masses of total pesticides were also detected during winter in Cardinia and Toomuc Creeks, while were in spring in Deep and Drain One Creeks and during summer in Yallock Cut (Figure 13).

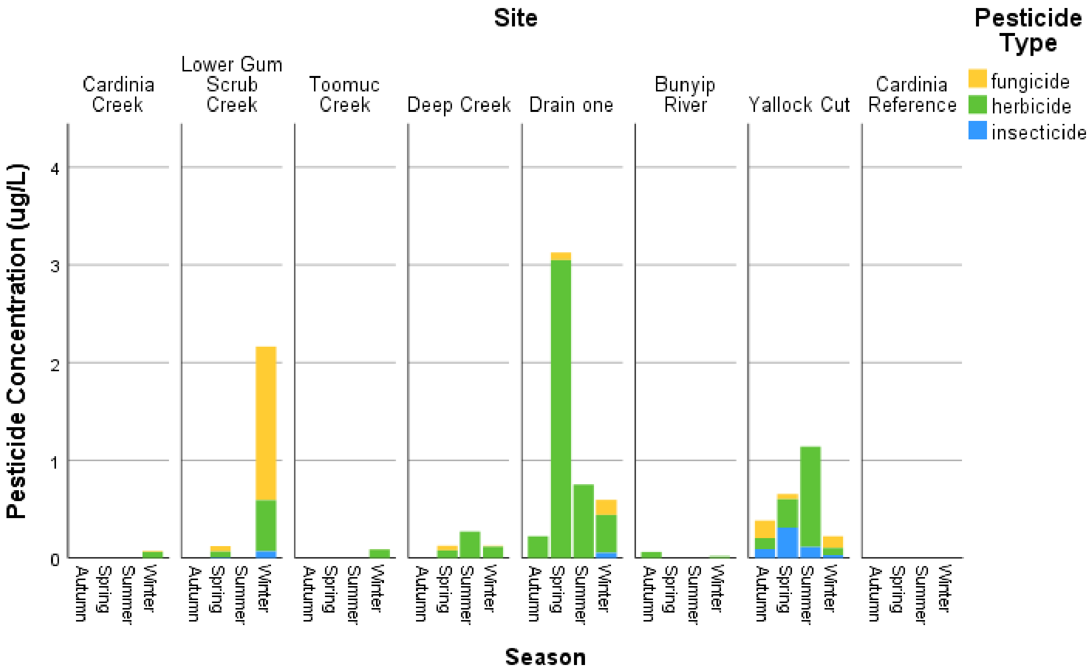

Figure 12: Total pesticide concentrations (µg/L) detected seasonally in grab water samples at each site. Pesticides have been grouped based on their target organisms: green = herbicide, yellow = fungicide and blue = insecticide.

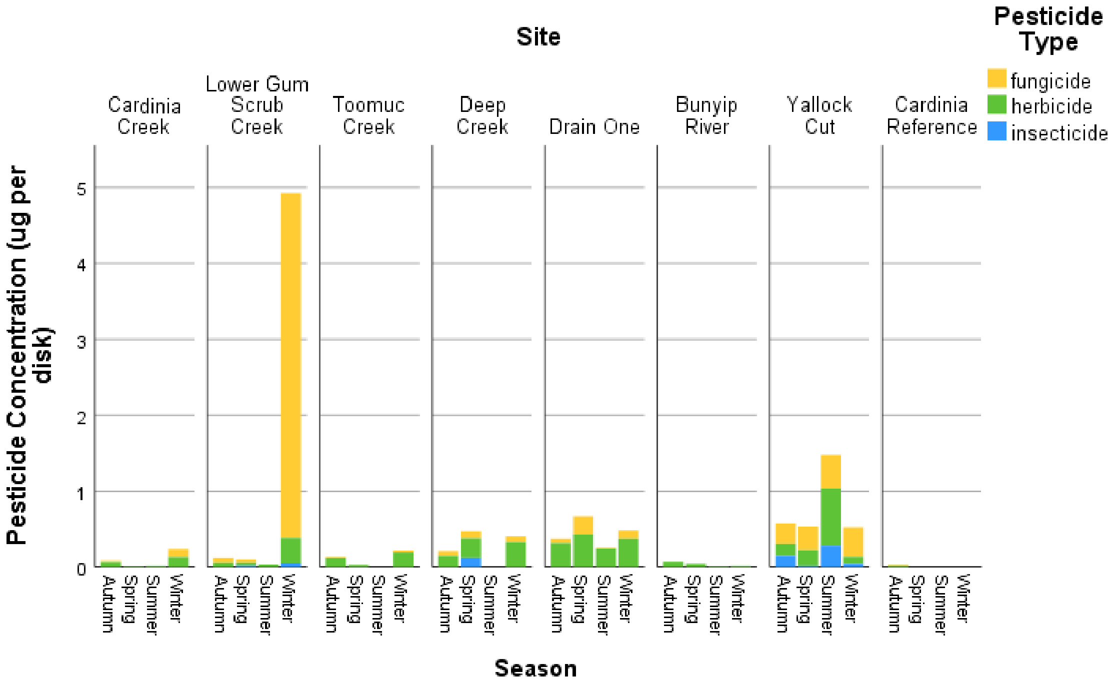

Figure 13: Total pesticide concentrations (µg) detected seasonally in passive samplers (Empore^{TM} disks) at each site. Pesticides have been grouped based on their target organisms: green = herbicide, yellow = fungicide and blue = insecticide.

Table 2: Pesticide concentrations (µg/L) for the pesticides detected (of 117 analysed) in grab water samples from 7 sites sampled seasonally in north-eastern Western Port. Figures in bold exceed current water quality guidelines.

Site	Season	Insecticides					Fungicides				Herbicides					Misc.
		Malathion	p,p-DDE	p,p-DDD	p,p-DDT	Pirimicarb	Boscalid	Metalaxyl	Iprodione	Tebuconazole	Metolachlor	Atrazine	Metribuzin	Diuron	Simazine	Caffeine
LOR		0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.1
Cardinia Reference	Spring	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Autumn	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bunyip River	Winter	-	-	-	-	-	-	-	-	-	-	-	-	-	0.029	-
	Spring	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Summer	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Autumn	-	-	-	-	-	-	-	-	-	0.057	-	-	-	-	-
Drain one	Winter	-	-	-	-	-	-	0.077	-	0.034	-	-	0.039	0.069	0.055	-
		-	0.012	0.02	**0.036**	-	-	0.088	-	0.048	-	-	0.11	**0.25**	0.074	-
		-	0.016	0.021	**0.038**	-	-	0.13	-	0.083	-	-	0.14	**0.34**	0.098	-
	Spring	-	-	-	-	-	-	-	-	-	-	-	-	**3.6**	0.29	0.13
		-	-	-	-	-	-	0.15	-	-	-	-	-	**2**	0.21	-
	Summer	-	-	-	-	-	-	-	-	-	-	-	-	**0.53**	0.22	-
	Autumn	-	-	-	-	-	-	-	-	-	-	-	-	**0.22**	-	-
Deep Creek	Winter	-	-	-	-	-	-	-	-	-	-	0.022	0.064	-	0.024	-
		-	-	-	-	-	-	-	-	0.016	-	-	0.055	-	0.068	-
	Spring	-	-	-	-	-	-	-	0.096	-	0.012	-	-	0.034	0.075	-
		-	-	-	-	-	-	-	-	-	-	-	-	-	0.034	-
	Summer	-	-	-	-	-	-	-	-	-	-	-	-	-	0.27	-
	Autumn	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Toomuc Creek	Winter	-	-	-	-	-	-	-	-	-	-	-	0.031	-	0.018	-
		-	-	-	-	-	-	-	-	0.0138	-	-	0.046	-	0.07	-
	Spring	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Summer	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Autumn	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lower Gum Scrub Creek	Winter	-	-	-	-	0.014	0.021	-	2.4	-	-	0.017	0.16	**0.61**	0.022	-
		-	-	-	-	0.12	0.023	-	0.69	0.016	-	-	0.067	0.15	0.018	-
	Spring	-	-	-	-	0.016	-	-	0.087	-	-	-	-	0.067	-	-
		-	-	-	-	-	-	-	0.044	-	-	-	-	0.053	-	-
		-	-	-	-	-	-	-	0.038	-	-	-	-	0.051	-	-
	Summer	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Autumn	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cardinia Creek	Winter	-	-	-	-	-	-	-	-	-	-	0.014	0.018	-	0.022	-
		-	-	-	-	-	-	-	-	0.015	-	-	0.032	-	0.038	-
	Spring	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Summer	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Autumn	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Yallock Cut	Winter	-	-	-	-	0.031	-	-	0.039	0.092	-	-	-	0.032	0.041	-
		-	-	-	-	0.023	-	-	0.041	0.067	-	-	0.03	0.018	0.036	-
	Spring	0.1	-	-	-	-	-	-	-	-	-	-	-	**0.22**	0.14	-
		-	-	-	-	0.52	-	0.11	-	-	-	-	-	0.11	0.11	-
	Summer	-	-	-	-	0.11	-	-	-	-	-	-	-	**0.4**	0.63	-
	Autumn	-	-	-	-	0.086	-	0.05	-	0.13	-	-	-	0.11	-	-
Water Quality Guideline value			0.03		0.01							13		0.2	3.2	

- Indicates not detected
LOR = limit of reporting

Table 3: Mass of detected pesticides (µg sampler) accumulated in Chemcatcher™ samplers deployed seasonally at 7 sites in north-eastern Western Port.

Site	Season	Insecticides			Fungicides								Herbicides					
		Malathion	DEET	Pirimicarb	Procymidone	Propiconazole II	Propiconazole I	Pyrimethanil	Boscalid	Metalaxyl	Iprodione	Tebuconazole	Prometryn	Metribuzin	Metolachlor	Atrazine	Diuron	Simazine
LOR		0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Cardinia Reference	Spring	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Autumn	-	-	-	-	-	-	-	-	-	-	0.011	-	-	-	-	-	0.019
Bunyip River	Winter	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.015
	Spring	-	-	-	-	-	-	-	-	-	-	-	0.016	-	0.029	-	-	-
	Summer	-	-	-	-	-	-	-	-	-	-	-	-	-	0.011	-	-	-
	Autumn	-	-	-	-	-	-	-	-	-	-	-	-	-	0.073	-	-	-
Drain One	Winter	-	-	-	-	-	-	-	-	-	0.045	0.075	-	0.15	-	0.016	0.025	0.12
		-	-	-	-	-	-	-	-	-	0.041	0.07	-	0.14	-	0.015	0.18	0.096
	Spring	-	-	-	-	-	-	-	-	0.19	-	0.047	-	-	-	-	0.079	0.35
	Summer	-	-	-	-	-	-	-	-	0.012	-	0.012	-	-	-	-	0.14	0.14
		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.094	0.11
	Autumn	-	-	-	-	-	-	-	-	0.014	-	0.041	-	-	-	-	0.22	0.096
Deep Creek	Winter	-	-	-	-	0.015	0.014	-	-	-	0.02	0.024	-	0.086	-	0.042	0.024	0.18
	Spring	-	0.12	-	-	-	0.023	-	-	-	0.05	0.025	-	-	0.031	0.07	0.036	0.12
	Summer	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Autumn	-	-	-	-	-	-	-	-	-	0.02	0.028	-	-	-	-	0.029	0.087
		-	-	-	-	-	-	-	-	-	0.037	0.047	-	-	0.014	-	0.035	0.13
	Winter	-	-	-	-	-	-	-	-	-	0.01	0.012	-	0.058	-	0.02	-	0.12
	Spring	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.029
	Summer	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Autumn	-	-	-	-	-	-	-	-	-	-	0.012	-	-	-	-	-	0.12
Lower Gum Scrub Creek	Winter	-	-	0.055	-	-	-	-	0.028	-	4.5	0.014	-	0.18	-	0.022	0.093	0.032
	Spring	-	-	0.019	-	-	-	-	-	-	0.042	-	-	-	-	-	0.028	0.012
		-	-	0.018	-	-	-	-	-	-	0.039	-	-	-	-	-	0.028	0.013
	Summer	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.013	0.021
	Autumn	-	-	-	-	-	-	-	0.011	-	0.029	0.021	-	-	-	-	0.015	0.044
Cardinia Creek	Winter	-	-	-	-	-	-	-	-	-	0.09	0.017	-	0.044	-	0.018	0.012	0.055
	Spring	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.011
	Summer	-	-	-	-	-	-	-	-	-	-	-	-	-	0.015	-	-	-
	Autumn	-	-	-	-	-	-	-	-	-	-	0.015	-	-	0.044	-	-	0.024
Yallock Cut	Winter	-	-	0.047	-	-	-	-	-	-	0.22	0.17	-	-	-	0.01	0.027	0.049
	Spring	0.015	-	-	-	-	-	0.056	-	0.19	0.03	0.033	-	-	-	-	0.069	0.14
	Summer	-	-	0.28	0.011	-	-	0.04	-	0.089	0.075	0.23	-	-	-	0.041	0.33	0.38
	Autumn	-	-	0.15	-	-	-	-	-	0.11	0.05	0.11	-	-	-	0.056	0.051	0.049

- Indicates not detected
LOR = limit of reporting

Sediment Samples

Four different pesticides were detected in sediments during the sampling period (Figure 14). Yallock Cut and Drain One had the greatest number of pesticides detected (two per site), and the greatest diversity of pesticide types. Lower Gum Scrub Creek and Toomuc Creek had a single pesticide detected that was either a herbicide or insecticide. No pesticides were detected in sediments at the sites in Cardinia Creek, Deep Creek, Bunyip River or the Cardinia Creek reference site.

Pesticides detected in sediment varied by season (Figure 15). Insecticides were detected in sediment collected in autumn and spring; whereas fungicides were only detected in autumn and herbicides in spring.

To assess the most common pesticides occurring in sediments collected in the current study, the frequency of pesticide detection was calculated (Figure 16). All three pesticide groups were detected in 13% of samples. Only one herbicide (diuron) and one fungicide (tebuconazole) were detected in sediment; while two insecticides, pirimicarb and bifenthrin, were detected.

A summary of the concentrations of pesticides detected both spatially and temporally are shown in Table 4. Concentrations of pesticides ranged from the 0.012 µg/Kg to a maximum of 0.046 µg/Kg. The highest concentration measured was for the herbicide diuron (0.046 µg/Kg) during spring at the Drain One site. The other pesticides detected included the fungicide tebuconazole and insecticides bifenthrin and pirimicarb which were all detected at concentrations ≤0.016 µg/Kg.

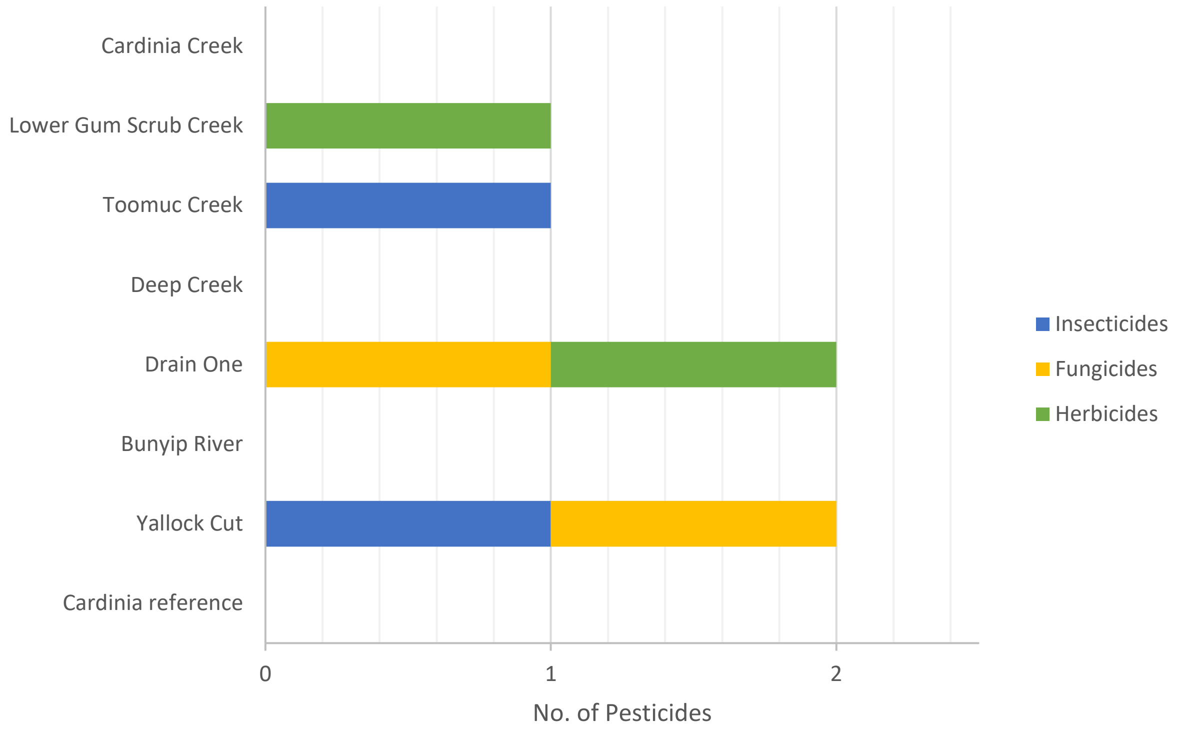

Figure 14: Pesticide richness detected in sediment samples at each site. Pesticides are grouped based on their target organisms: green = herbicide, yellow = fungicide and blue = insecticide.

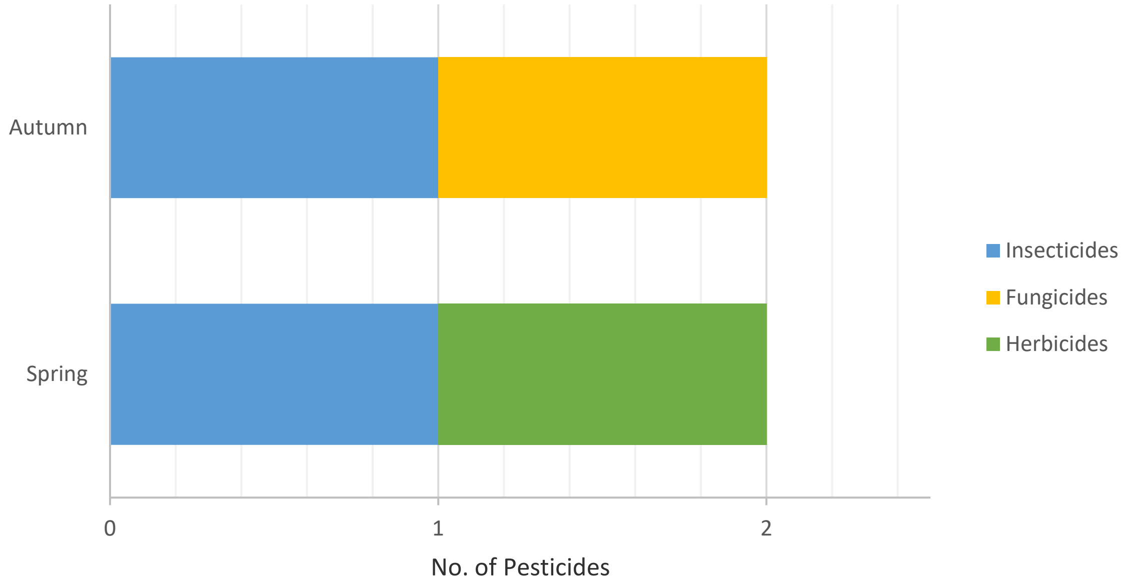

Figure 15: Seasonal pesticide richness detected in sediment samples. Pesticides have been grouped based on their target organisms: green = herbicide, yellow = fungicide and blue = insecticide.

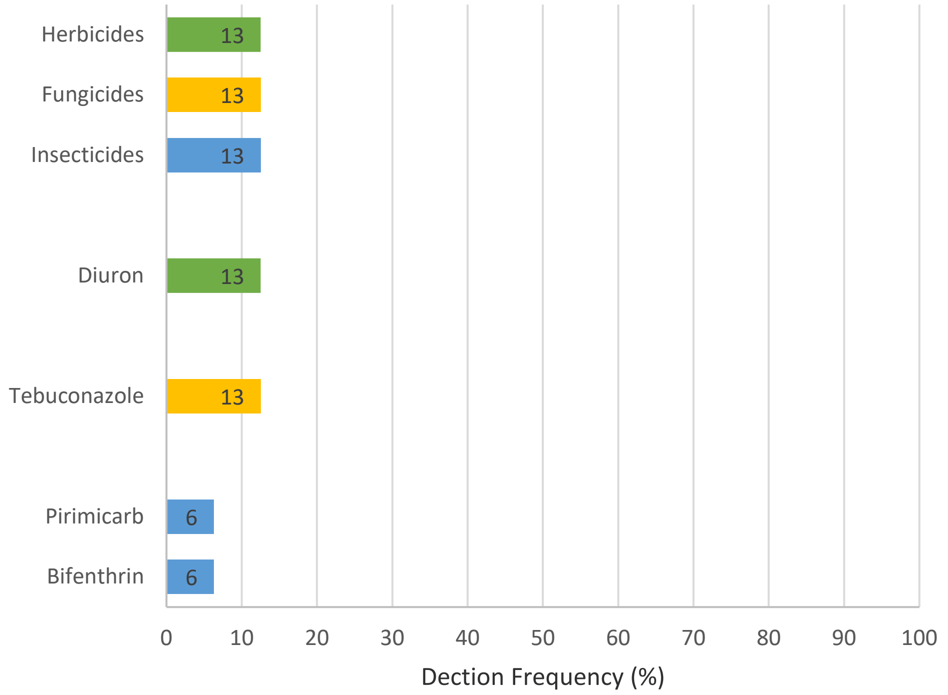

Figure 16: Frequency (%) of pesticide detections from sediment samples collected across all sites. Green bars represent herbicides, yellow bars fungicides and blue are insecticides.

Table 4: Pesticide concentrations (mg/Kg) for the pesticides detected (of 117 analysed) in sediments from 7 sites sampled during spring and autumn in North-eastern Western Port.

Site	Season	Insecticides		Fungicides	Herbicides	Total Organic Carbon (%)
		Bifenthrin	Pirimicarb	Tebuconazole	Diuron	
LOR		0.01	0.01	0.01	0.01	
Cardinia Reference	spring	-	-	-	-	1.7
Bunyip River	spring	-	-	-	-	2.9
	autumn	-	-	-	-	2.2
Drain One	spring	-	-	-	0.046	3.0
	autumn	-	-	0.015	-	2.1
Deep Creek	spring	-	-	-	-	2.4
	autumn	-	-	-	-	1.9
Toomuc Creek	spring	-	-	-	-	3.1
	autumn	0.012	-	-	-	3.6
Lower Gum Scrub Creek	spring	-	-	-	-	3.1
		-	-	-	0.015	3.4
	autumn	-	-	-	-	2.6
Cardinia Creek	spring	-	-	-	-	2.0
	autumn	-	-	-	-	1.7
Yallock Cut	spring	-	0.016	-	-	1.1
	autumn	-	-	0.016	-	2.0

- Indicates not detected
LOR = limit of reporting

Sediment Particle Size

The total organic carbon (TOC) content and particle size of sediments from the seven study sites are summarised in Tables 4 and 5 respectively. The TOC content in sediments ranged between 1.1 to 3.6% (Table 4) and varied seasonally and amongst sites. At the sites in Cardinia, Lower Gum Scrub, Deep and Drain One Creeks and the Bunyip River higher TOC were detected in spring, while in Yallock Cut and Toomuc Creek higher TOC was detected in autumn. Toomuc Creek and Lower Gum Scrub creek had the highest levels of TOC in autumn and spring, respectively (Table 4).

Sediments from sites in Lower Gum Scrub, Cardinia and Deep Creeks and Yallock Cut were predominantly silt (< 63 µm; >62%). Toomuc Creek and Bunyip River sediments were predominantly sand (49% and 75% respectively), while sediment from Drain One was a mixture of silt (<63 µm; 51%) and sand (46%) (Table 5).

Table 5: Particle size of sediments from seven study sites in north-east Western Port catchments.

Site	Clay (<2 µm)	Silt (<63 µm)	Sand (63 - 2,000 µm)	Gravel (>2,000 µm)
Cardinia Creek	6.9	76.5	16.6	0
Lower Gum Scrub Creek	4.48	69.72	25.8	0
Toomuc Creek	1.62	39.48	58.9	0
Deep Creek	5.85	66.25	27.9	0
Drain One	3.52	50.58	45.9	0
Bunyip River	0.57	7.44	75.2	16.8
Yallock Cut	6.75	62.25	31	0

Nutrients

Surface Water Samples

Nutrient concentrations measured in grab water samples seasonally are summarised in Figure 17. Total phosphorus (TP) concentrations varied seasonally and amongst sites, ranging from 0.02 to 1.65 mg/L (Figure 17a). Highest TP concentrations were generally detected during summer and autumn, with the highest concentration measured in Cardinia Creek (1.65 mg/L). Total phosphorus concentrations exceeded the SEPP Waters (2018) trigger values for fresh and estuarine systems (0.055mg/L and 0.09 mg/L, respectively) in 63% of samples. During summer and autumn, TP concentrations were up to 18 times the trigger values in Cardinia, Lower Gum Scrub, Toomuc, Deep and Drain One Creeks (Figure 17a).

Total nitrogen (TN) concentrations varied seasonally and amongst sites, ranging from 0.1 to 7.5 mg/L (Figure 17b). Highest TN concentrations were generally measured in summer for all sites, except in Drain One and Yallock Cut where the highest concentrations were detected in spring. Total nitrogen concentrations exceeded the SEPP Waters (2018) trigger values in 37% of samples (fresh TV 1.1mg/L; estuarine TV 1.0 mg/L). Total nitrogen concentrations were up to 7 times the trigger values at sites in Drain One and Yallock Cut during spring and summer and Cardinia Creek during summer. Generally, nitrate and nitrite (NOx) were the dominant nitrogen sources, followed by Total Kjeldahl Nitrogen (TKN) and ammonia. An exception to this was in Cardinia Creek and Lower Gum Scrub Creek in summer where TKN was the dominant nitrogen source, followed by NOx and ammonia (Figures 17c, d and e).

Ammonia concentrations varied seasonally and amongst sites, ranging from 0.02 to 0.12 mg/L (Figure 17e). In general, the highest concentrations of ammonia were detected during autumn and summer. The study site in Drain One generally had the highest ammonia concentration each season, except during summer, where ammonia concentrations were greatest in Cardinia Creek. Ammonia concentrations didn't exceed the ANZECC/ARMCANZ (2000) trigger values for fresh and marine waters of south-eastern Australia (fresh TV 0.9mg/L; marine TV 0.91 mg/L).

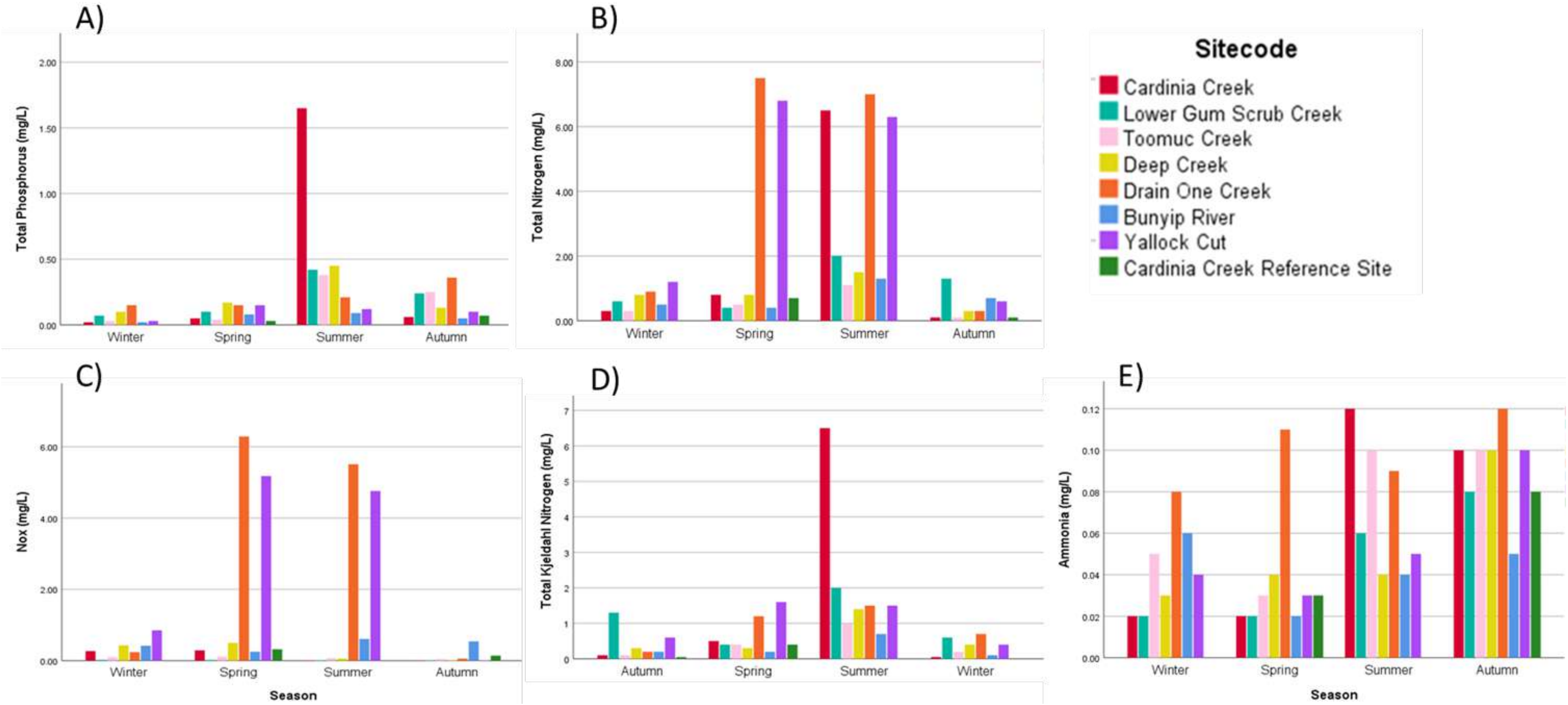

Figure 17: Seasonal nutrient concentrations in surface waters from seven study sites and the reference site in north-eastern catchments of Western Port. A) Total Phosphorus, B) Total Nitrogen, C) Nitrate + Nitrite (NOx), D) Total Kjeldahl nitrogen (TKN) and E) Ammonia.

Ecotoxicological Assessment

Aquatic Field Caging Experiments

Australian glass shrimp, *Paratya australiensis* responses

Survival of *Paratya australiensis* deployed for 10 days in spring 2017 and autumn 2018 is shown in Figure 18. For both deployments survival of shrimp at the reference site was >80% suggesting that caging did not affect survival.

In spring, mean survival of *P. australiensis* at the reference site was 100%, while at the study sites ranged 92-100% (Figure 18a), with the lowest survival recorded at Bunyip River. There were significant differences in survival of *P. australiensis* between the sites (Kruskal-wallis, H=14.97, DF = 7, p = 0.036). However, pairwise comparisons indicated there were no significant differences between the survival of *P. australiensis* at sites and the reference site in Cardinia Creek (p >0.05). In autumn, survival of *P. australiensis* varied significantly between the sites ($F_{(7,32)}$ = 8.935, p <0.05 - Figure 18b). There was 100% mortality at Drain One, Toomuc Creek and Lower Gum Scrub Creek and there was reduced survival at the other sites compared to the reference site (Cardinia Creek); however, this was only statistically significant for Deep Creek (Figure 18b).

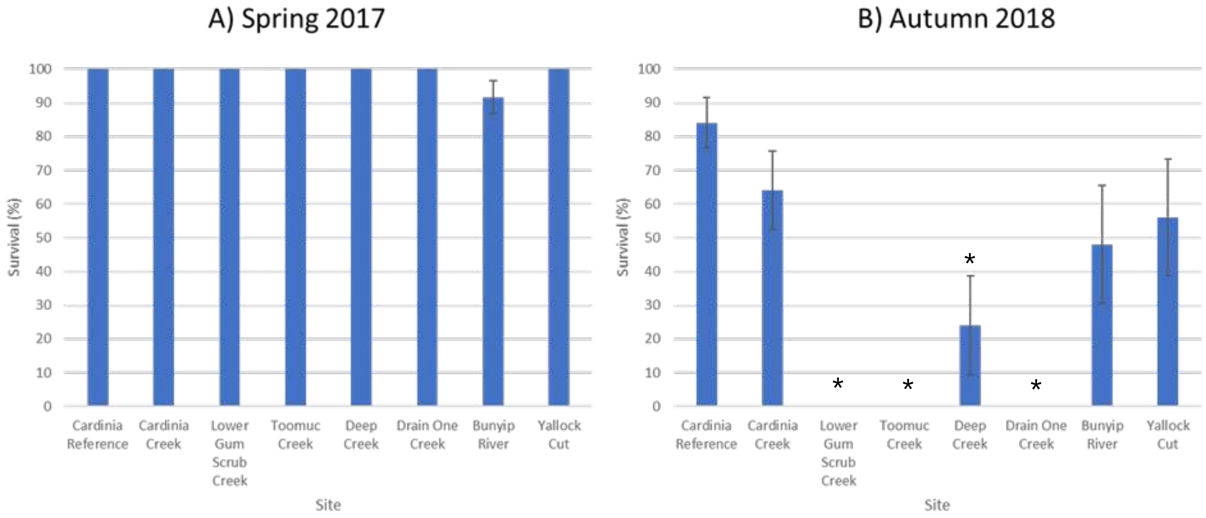

Figure 18: Mean (± SEM) survival of *Paratya australensis* collected after 10 days deployment in cages at sites during A) spring and B) autumn. N = 5. Asterisk denotes sites that differ significantly to reference site (CAR-REF), *p* <0.05.

Microalgae, *Scenedesmus* sp. responses

Final cell biomasses (as a percentage of each sites control) of immobilised *Scenedesmus* sp. following 10 days deployment at study and reference sites during spring 2017 and autumn 2018 are shown in Figure 19. In spring 2017, there were significant differences in final cell biomass of immobilised *Scenedesmus* sp. (as a percentage of each site control) between sites (ANOVA, $F_{(7,35)}$ = 24.617, p <0.05). At the study sites in Bunyip River, Lower Gum Scrub Creek and Deep Creek cell biomasses were significantly reduced compared to *Scenedesmus* sp. deployed at the reference site in Cardinia Creek (Figure 19a). Inhibition of *Scenedesmus* sp. growth at these three sites ranged 12% to 47% (Table 6). At the site in Drain One 37% inhibition of algal growth was observed, however there was high variability in the response (Table 6). At Cardinia reference site, Cardinia creek, Toomuc Creek and Yallock Cut in autumn growth of *Scenedesmus* sp. was stimulated by 23-88% (Table 6).

In autumn 2018, there were significant differences in final cell biomass of immobilised *Scenedesmus* sp. (as a percentage of site control) between sites (ANOVA, $F_{(7,34)} = 8.534$, $p < 0.05$). At all the study sites, except for Toomuc Creek, cell biomasses were reduced compared to that in immobilised *Scenedesmus* sp. deployed at the reference site in Cardinia Creek, however this was only statistically significant at Lower Gum Scrub Creek (Figure 19b). The greatest inhibition of *Scenedesmus* sp. growth was observed at Lower Gum Scrub Creek (58%), however inhibition of *Scenedesmus* sp. growth ranged between 28-54% in Drain One, Deep Creek, Bunyip River and Yallock Cut (Table 6).

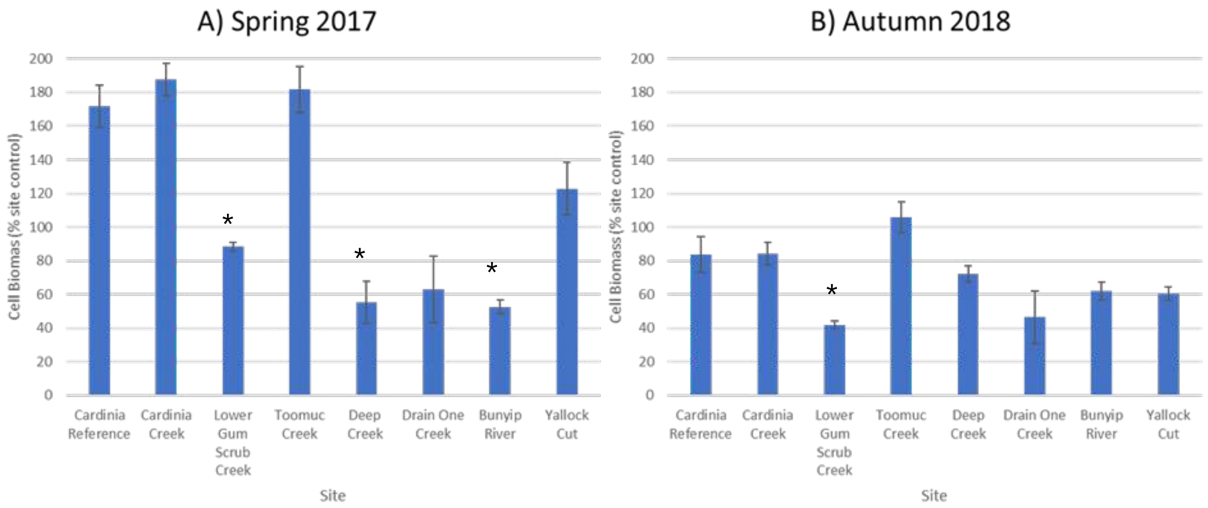

Figure 19: Mean (± SEM) cell biomass of immobilised *Scenedesmus* sp. following 10-day *in situ* exposure at study sites and Cardinia Creek reference site during A) spring 2017 and B) autumn 2018. N = 3. * denotes significant differences between study site and reference site survival, *p* <0.05.

Table 6: Mean (± SEM) inhibition of immobilised *Scenedesmus* sp. growth (relative to each site deployed control) following 10-day *in situ* exposure at study sites and Cardinia Creek reference site during spring 2017 and autumn 2018. N = 3. '+' = stimulation of growth relative to each site deployed control.

Season	Site							
	Cardinia Creek	Lower Gum Scrub Creek	Toomuc Creek	Deep Creek	Drain One Creek	Bunyip River	Yallock Cut	Cardinia Reference
Spring	+88 ± 10	12 ± 3	+82 ± 14	45 ± 12	37 ± 20	47 ± 4	+23 ± 16	+72 ± 12
Autumn	16 ± 7	58 ± 2	+6 ± 9	28 ± 5	54 ± 16	38 ± 5	39 ± 4	16 ± 10

Sediment Toxicology - *Austrochiltonia subtenuis*

Survival

Survival of *A. subtenuis* exposed to sediments collected from study sites and an external reference site during spring 2017 and autumn 2018 is depicted in Figure 20. There was no significant difference in 14 day survival between the study sites and the control site, Bittern in spring 2017 (ANOVA, $F_{(7,28)} = 2.34$, $p > 0.05$ - Figure 20a). *Austrochiltonia subtenuis* exposed to sediment from Bunyip River had the lowest survival (with mean survival of 75%). Mean survival at all the other sites was 90% or over. There were significant differences in *A. subtenuis* survival in sediment collected in autumn 2018 (ANOVA, $F_{(7,28)} = 4.23$, $p < 0.05$ - Figure 20b). Survival of amphipods exposed to sediment from

Toomuc Creek and Yallock Cut was significantly lower than *A. subtenuis* survival in the control sediment (Figure 20b), with mean survival of 47.5 and 52 %, respectively. Survival of *A. subtenuis* exposed to sediment from Cardinia Creek and Lower Gum Scrub Creek was also reduced, but this was not significantly different to survival of *A. subtenuis* exposed to Bittern Reservoir sediment. Survival of *A. subtenuis* exposed to sediment from the other study sites was above 80%.

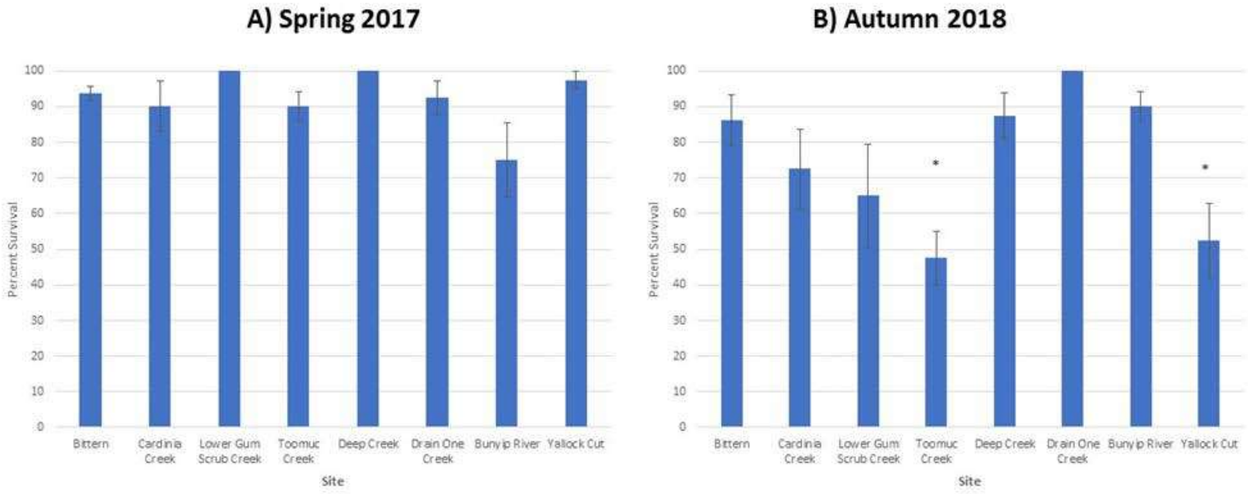

Figure 20: Mean (± SEM) survival of *Austrochiltonia subtenuis* following laboratory exposure to site sediments collected during A) spring 2017 and B) autumn 2018. N = 4 for sites, N = 8 for controls. * denotes significant differences between study site and control site survival, *p* <0.05.

Growth (as measured through head length)

Growth, as measured through head lengths, of *A. subtenuis* exposed to sediments collected from study sites and an external reference site during spring 2017 and autumn 2018 is depicted in Figure 21. There were significant differences in growth of *A. subtenuis* exposed to Drain One sediment compared to those exposed to Bittern Reservoir sediment during spring 2017 (ANOVA, $F_{(7,28)} = 2.90$, $p < 0.05$ - Figure 21a), with amphipods exposed to Drain One sediment having reduced growth compared to those exposed to Bittern sediment. *Austrochiltonia subtenuis* exposed to Toomuc Creek, Deep Creek and Yallock Cut sediment also had reduced growth compared to Bittern-exposed *A. subtenuis*, but the difference was not significant. This reflects the survival response in spring collected sediment, in that *A. subtenuis* exposed to Toomuc Creek and Yallock Cut sediment had reduced survival compared to controls, but this was not significant. Although survival was reduced in amphipods exposed to Bunyip River sediment, the *A. subtenuis* that survived were bigger than those exposed to Bittern sediment, although the difference was not significant.

There were no significant differences in growth of *A. subtenuis* exposed to sediment collected from any of the sites in autumn 2018 (ANOVA, $F_{(6,25)} = 2.26$, $p > 0.05$ – Figure 21b). As there was less than 50% survival of *A. subtenuis* collected from Toomuc Creek growth was not measured in the surviving *A. subtenuis*. *Austrochiltonia subtenuis* exposed to Lower Gum Scrub Creek and Drain One sediment were smaller than *A. subtenuis* from the other sites, although the differences were not significant compared to the controls (Figure 19b). Interestingly, although there was 100% survival of *A. subtenuis* exposed to Drain One sediment, they were smaller than those exposed to Bittern sediment (where there was 86% mean survival).

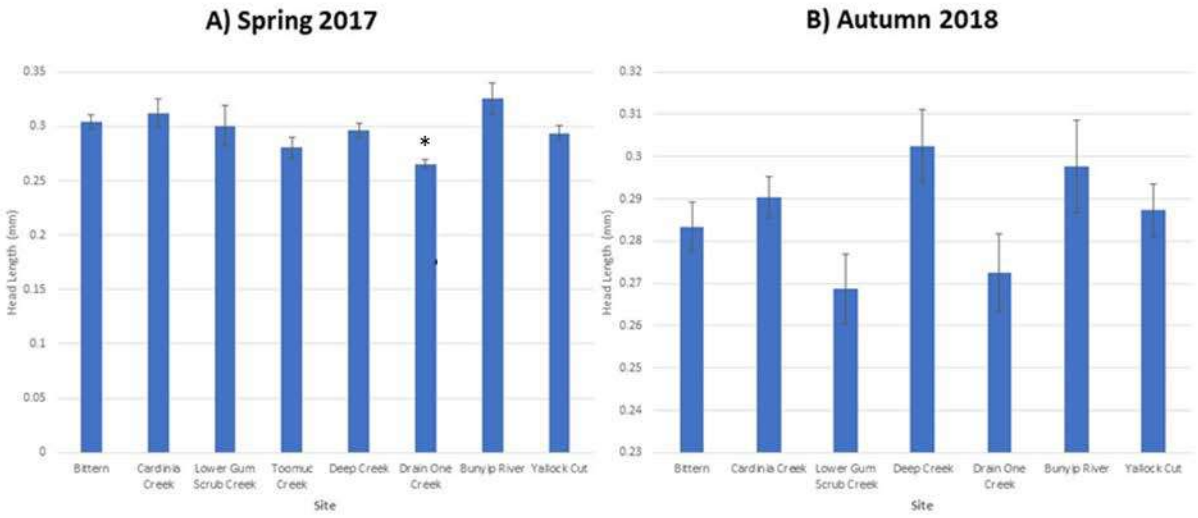

Figure 21: Mean (± SEM) growth (as measured by head lengths) of *Austrochiltonia subtenuis* following laboratory exposure to site sediments collected during A) spring 2017 and B) autumn 2018. N = 4 for sites, N = 8 for controls. * denotes significant differences between study site and control site growth, *P* <0.05. Toomuc Creek was not included in growth analysis for autumn as there was <50% survival.

Weight of Evidence

The chemical and biological lines of evidence applied in this study are presented in Table 7. The Cardinia Creek reference site contained low levels of pesticides and nutrients and physico-chemistry was predominantly in line with SEPP guidelines. Of the biological endpoints measured, stimulation of algal growth was observed, however there were no impacts to survival of amphipods or shrimp (Table 7).

Assessment of all lines of evidence for sites Yallock Cut, Drain One, Toomuc Creek and Lower Gum Scrub Creek show these sites have a combination of high numbers and concentrations of pesticides, elevated nutrient levels and/or physico-chemistry measures which exceed SEPP guidelines. Furthermore, these sites show signs of impairment in multiple biological endpoints (Table 7).

Bunyip River contained low levels of herbicides and nutrients in surface waters and physico-chemistry in line with SEPP guidelines. Biological endpoints showed inhibition of algal growth, however there were no affects to amphipods or shrimp (Table 7).

Moderate levels of pesticides were detected in Cardinia Creek together with elevated nutrients and physico-chemical measures, consistently exceeding SEPP guidelines. Stimulation of algal growth was observed, however there were no impacts to other biological endpoints assessed (Table 7).

Table 7: Summary of Weight of evidence approach – based on the sediment quality triad (modified from Chapman et al 2002 and Burton et al 2002).

Site	**Reference Cardinia Creek**	**Bunyip River**	**Yallock Cut Creek**	**Drain One Creek**	**Deep Creek**	**Toomuc Creek**	**Lower Gum Scrub Creek**	**Cardinia Creek**
Chemistry								
Physico-chemistry	Low DO	Good	High EC, turbidity, low DO	High turbidity, EC, elevated and low DO	High EC, turbidity	High EC, low DO	High EC, turbidity	High turbidity, low DO
Nutrients	Low	Low	Very High TN	Very High TN, High TP	High TP, TN	High TP, TN	High TP, TN	Very High TN, TP
Pesticides surface waters	Low fungicide	Low herbicides	High herbicides, fungicides. Moderate insecticides	High herbicides, fungicides. Low insecticides	High herbicides, fungicides. Low insecticides	Moderate herbicides, fungicides	High herbicides, fungicides. Low insecticides	Moderate Herbicides, fungicides
Pesticides sediments	not detected	Not detected	Low - fungicides, insecticides	Low - fungicides, herbicides	Not detected	Low - insecticide	Low - herbicide	Not detected
Summary of chemistry effects	-	-	++	++	++	+	**++**	+
Biological endpoints								
Amphipod Sediment toxicity	no impact	no impact	significant mortality	significantly reduced growth	no impact	significant mortality	no impact	no impact
In situ Shrimp	no impact	no impact	no impact	100% mortality	significant mortality	100% mortality	100% mortality	no impact
In situ Algal growth	stimulated	inhibited	stimulated	inhibited	inhibited	stimulated	inhibited	stimulated
Summary of effects	-	+	**++**	++	**++**	++	++	+
Overall Assessment	Adverse effects not predicted as chemistry indicates low contamination of pesticides and no toxicological endpoints showed signs of impacts.	Potential adverse effects predicted due to: low levels of herbicides and inhibition of algal growth	Significant adverse effects predicted due to: elevated chemistry; two toxicological endpoints impacted	Significant adverse effects predicted due to: elevated chemistry; all three toxicological endpoints impacted	Significant adverse effects predicted due to: elevated chemistry; two toxicological endpoints impacted	Significant adverse effects predicted due to: elevated chemistry; two toxicological endpoints impacted	Significant adverse effects predicted due to: elevated chemistry; two toxicological endpoints impacted	Potential adverse effects predicted due to: elevated chemistry

++ Significant adverse effects predicted; + potential adverse effects predicted; - No adverse effects predicted.

Discussion

Results from the current program suggest that land-based pesticide chemicals are entering creeks and rivers that flow into the northern segments of Western Port and at times represent a water quality issue. Chemical assessments indicated pesticide contamination occurred on both a spatial and temporal scale, with detections recorded in surface waters and /or sediments of freshwater sections at all seven sites across the 2017-2018 sampling period. There was a commonality of mixtures at all sites, made up of pesticides from different classes and at concentrations which may pose risk to resident flora and fauna. Up to 10 different pesticides were detected at any one site, with an average of 7 per site and a total of 21 different pesticides detected overall, including herbicides, fungicides and insecticides. Pesticides were predominately detected in surface waters, rather than sediments, with 20 different pesticides detected in surface waters (grab and passive samplers), while only four in sediments. Further, of the pesticides detected in surface waters 85% were in a dissolved phase, as shown by detection in passive samplers, indicating most are available for biota uptake. Herbicides contributed the most to pesticide contamination, detected in up to 91% of samples, followed by the fungicides and insecticides (in up to 65% and 25% of samples, respectively). Biological assessments indicated that flora and fauna are under stress and/or being exposed to pollutants in both surface waters and sediments. Impairment, including mortality of shrimp and amphipods and inhibition and stimulation of algal growth was observed at several sites. While concentrations of several pesticides were at levels which could be responsible for the observed toxicity at some sites, it is likely that the complex pesticide mixtures and general poor water quality (low dissolved oxygen, elevated EC) observed created poor environmental conditions resulting in the observed biological impairment. The current study was unable to isolate the precise chemical causes of biological impairment, however further studies, such as Toxicity Identification and Evaluation (TIE), could assist with this. These results are consistent with monitoring conducted in Watsons Creek and Western Contour Drain catchments from 2012 to 2016 (Myers et al 2018). Together, the chemical and biological data provides valuable information on the extent of pesticide contamination in the northern catchments of Western Port and the potential threats this poses to biota.

Spatial and Temporal Pesticide Patterns

Investigation of the distribution of pesticides in north-eastern catchments demonstrated that the creeks with the greatest pesticide contamination, in terms of numbers of pesticides, highest frequency of detection and highest concentrations were Yallock Cut, Lower Gum Scrub Creek, Drain One and Deep Creek. Between 7 and 10 different pesticides were recorded in these systems, including the herbicides simazine, diuron, atrazine, metolachlor, metribuzin; the fungicides tebuconazole, iprodione, metalaxyl, boscalid, pyrimethanil, propiconazole, procymidone; and the insecticides pirimicarb, DEET, malathion, p,p-DDE, p,p-DDD and p,p-DDT. The least contaminated creeks were the Bunyip River and the reference site in Cardinia Creek, where 3 and 2 different pesticides were detected, respectively, including the herbicides simazine, metolachlor and prometryn and the fungicide tebuconazole. The presence and distribution of pesticides will be a consequence of factors including pesticide usage, physicochemical properties of the compounds and environmental factors (e.g. hydrology, geology) (Kennedy et al 2012). The lower levels of pesticide contamination in Bunyip River and at the Cardinia Creek reference site is likely in part due to the hydrology and geology of these sites. For instance, Bunyip River drains a significantly greater catchment area in comparison to the other six catchments examined and as such has greater water flows. Similarly, the reference site in Cardinia Creek site is situated higher in the catchment in a region where the creek is wider and deeper and has greater flows in comparison to downstream. This results in greater dilution of any pesticides entering the surface waters and transport through these sites. Further, the geology of Bunyip River differs from all the other sites in that the sediments are predominantly sand. Pesticides typically partition to finer grained sediments rather than large grain size particles such as sand (Long et al 2010). Therefore, in the Bunyip River there are fewer pesticides retained in the system due to both geology and hydrology.

Pesticides can enter waterways via various pathways including surface runoff during irrigation and/or rainfall, aerial deposition during application (spray drift), and infiltration via groundwater (Whitehall et al 2010). Assessment of temporal trends in pesticide detections indicate that all these pathways are likely to play a role in the transport of pesticides to the catchments examined during this study. The greatest numbers of chemicals from different pesticide classes in surface waters were observed during winter and spring based on results from grab and passive samplers, respectively. This finding isn't surprising as periods of high rainfall and consequently higher flows, are expected to result in elevated concentrations and/or greater numbers of pesticides in streams as compounds are transported from sites of application into nearby waterways (Shaw et al 2010). Rainfall during winter and spring was 1.4 times greater than that over the summer/autumn months and likely a factor in increased pesticide detections during these seasons. This is further supported by results from previous monitoring in Western Contour Drain and Watsons Creek catchments where greater concentrations of pesticides were observed during storm events and during periods of high rainfall (Myers et al 2016).

While in general, pesticide contamination of the creeks was greater during the winter/spring seasons, examination of the passive sampler results indicated a high incidence of pesticides during summer and autumn. This was predominantly due to the detection of several herbicides, fungicides and insecticide in Yallock Cut, Drain One and Deep Creek. These results suggest that irrigation, groundwater and/or spray drift transport pathways contribute to pesticide contamination in these waterways. Previous monitoring in the catchments of Western Contour Drain and Watsons Creek in northwest Western Port, showed that surrounding land-use involving irrigated agriculture results in greater occurrence and concentrations of pesticides in creeks during low rainfall periods such as summer and autumn (Myers et al 2016). Further, groundwater was suggested as a potential source of pesticides to Western Contour Drain from previous monitoring programs (Myers et al 2016). Scientific data on the contributions from different pathways is needed to feed back into wider policy/regulatory frameworks around pesticide use near waterways and to enable effective management of pesticide inputs.

It is well-established that land-use influences the types and concentrations of pesticides that are transported to waterways (U.S. Geological Society 1999). Monitoring conducted across catchments in the north west of Western Port from 2012-2016 showed that waterways draining from highly productive agricultural land for vegetable and herb crops were a significant source for pesticides, particularly herbicides and fungicides, while urban land-uses were a low source of pesticides (Myers et al 2016; Myers et al 2018). While the current study did not specifically set out to determine sources of pesticides, investigation of the registered uses and land-use in the study area can give some insight into potential sources of the pesticides detected. The pesticides detected during the current monitoring program are registered for use in various agricultural situations, but also in many non-agricultural situations (Table 8). The most frequently detected pesticides occurring at all sites, except for in the Bunyip River, and during all seasons were the herbicides diuron and simazine. This result is not surprising as these two herbicides were in the top 4 most frequently detected pesticides in previous studies of Watsons Creek and Western Contour Drain catchments between 2012-2016 (Myers et al 2018). Both simazine and diuron are registered for agricultural applications on asparagus and pulse crops, as well as various grass crops grown in the areas abounding the study sites (Table 8). In addition, simazine is registered for many fruit and nut crops and various non-crop agricultural applications such as forestry, nurseries, flowers and vineyards which could be sources to the creeks (Table 8). Both compounds are also registered for non-agricultural applications such as factory sites, commercial and industrial areas, rights of way, toughs, tanks and dams for simazine;

while for diuron include bore drains, drainage ditches and irrigation channels[1] (see Table 8). The widespread detection of these compounds both spatially and temporally is likely due to use in both agricultural and non-agricultural applications across the catchments.

The triazine herbicide atrazine was also widely detected during the current study, detected at six sites Drain one, Deep Creek, Toomuc Creek, Lower Gum Scrub, Cardinia Creek and Yallock Cut. Contamination by atrazine in many sites is likely related to seasonal applications during winter, as this was when most of the detections occurred. The detection frequency of atrazine was nearly half that of detections during monitoring in north western catchments from 2012-2016 (17.5% this study; 36% 2012-16 study), potentially indicating lower use in the catchments of the current study. Registered uses for atrazine in agriculture are predominantly for grass crops. However, it is also the active ingredient in several products registered for use in non-crop and non-agricultural applications e.g. fallow areas, forestry and rights of way (Table 8). In 1997, industrial and several non-agricultural uses of atrazine (home garden uses, and all commercial turf uses) were banned due to concerns over environmental impacts including the potential for contamination of ground and surface water, and residue and efficacy uncertainties (APVMA 2008). It is likely that detections of atrazine are due to its application in a range of non-agricultural and agricultural situations, and not related to vegetable crops.

Atrazine, simazine and diuron have been widely detected in previous studies of Western Port streams, but also more broadly across Victoria in both urban and agricultural areas. Although these herbicides have been widely detected in waterways in the Melbourne Water region, little is known about their impacts on the health of rivers and the bays. Due to their physicochemical properties and aqueous half-lives they are considered relatively mobile, persistent and prone to off-site movement in runoff (Kennedy et al 2012). Their widespread detection in the current study supports the need to better understand the transport pathways and environmental impacts of these compounds to better understand the need for increased regulation, mitigation and management.

Herbicides detected during the current study included metribuzin, metolachlor and prometryn. Metribuzin was detected at 5 sites in winter; Metolachlor at 3 sites across various seasons, while prometryn was detected in the Bunyip River during spring. Detections for metolachlor and prometryn during this study (12.5% and 3% respectively) were lower than has been previously observed for these herbicides in Watsons Creek and Western Contour Drain over the period 2012-2016 (64% for both compounds), while metribuzin was detected at a greater frequency (22% this study compared to 3%). This result likely reflects differences in agricultural land uses between the catchments in the current and previous studies. Over 40 products are registered for use with these pesticides as the active ingredient. These are predominantly for agricultural purposes, with little to no non-agricultural registered uses. Sources are likely to include applications in various vegetable, grasses, sunflower and tomato crops across the catchments (Table 8).

The second most frequently detected pesticides in creeks during this study were the fungicides iprodione and tebuconazole. Both were widespread, detected at all sites (except Bunyip River and Cardinia Creek reference site for iprodione) and during all seasons, although most consistently during winter/spring for iprodione and winter/autumn for tebuconazole. In contrast to previous monitoring, iprodione was detected less frequently (31% samples in this study compared to 51% samples from 2012-2016), while tebuconazole was detected in nearly double the samples (40% this study; 19% 2012-2016). Further, this is the first time tebuconazole has been detected in sediments from sites in Western Port catchments. Iprodione has previously been reported in samples from Lower Gum Scrub Creek at sites higher in the catchment than investigated during the current study

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[1] Applications of diuron in irrigation channels and drainage ditches is only allowed if all water can be contained on farm (APVMA 2012).

(Pettigrove 2018). Iprodione is registered in over 50 products, while tebuconazole in over 100, including both agricultural and non-agricultural applications (Table 8). Sources in the current catchments are more likely related to agricultural applications than non-agricultural and may include turf, grasses, fruits, flowers, vegetables (Table 8).

Several other fungicides were also detected including metalaxyl, boscalid, propiconazole I and II, procymidone and pyrimethanil. Metalaxyl was detected across all seasons in Drain One and Yallock Cut creeks (15% of samples). It has been detected in previous monitoring across Western Port catchments, at a similar frequency (Myers et al 2018). It is registered in over 22 products, mostly for use in agricultural applications, rather than non-agricultural (Table 8). Sources in the current catchments could include vegetable crops such as brassica and root and tuber vegetables such as carrots and celeriac (Table 8). Boscalid, propiconazole, procymidone and pyrimethanil were detected in <6% of samples, occurring at single sites on one or more sampling occasions. These fungicides were detected less frequently than in monitoring conducted across Watsons Creek and Western Contour Drain from 2012-2016 were they occurred in 10-14% of samples (Myers et al 2018). Interestingly, pyrimethanil was detected for the first time in Western Port creeks in surface waters. Pervious monitoring has only detected this fungicide in sediments. This suggests that the occurrence may have been related to a recent application and transport into the Yallock Cut. These fungicides are registered for use in a range of agricultural and non-agricultural situations (Table 8). Potential sources in the catchments where they were detected include vegetable crops, turf, fruit, grasses and timber production (Table 8).

Several insecticides were detected in creeks monitored during the current study. The most frequently detected insecticide was pirimicarb, occurring in two sites (Yallock Cut and Lower Gum Scrub Creeks) during multiple seasons. This insecticide is registered for use in 17 products, predominantly in agricultural situations (Table 8). Other insecticides detected include the synthetic pyrethroid bifenthrin on one occasion in sediments at Toomuc Creek. Bifenthrin is registered in over 280 products for use in agricultural and non-agricultural situations (Table 8) and has been previously detected in Western Port creeks and more widely in urban creeks across greater Melbourne area (Pettigrove 2018). The insecticides malathion, DEET, DDT, DDD and DDE were detected on single occasions in Yallock Cut, Deep Creek and Drain One, respectively. Both insecticides are registered for use in products for non-agricultural applications only (Table 8), sources are therefore likely related to home garden or personal insect protection uses. The organochlorine insecticides DDD, DDT, DDE were detected on a single occasion in Drain One. These insecticides were widely used to control insects in agriculture, however due to their widespread toxicity and risks to human health their use has been banned in Australia since the 1980s (Australian Government 2013) and as such their detections are likely related to historic use across the catchments.

Understanding the sources of pesticides, transport pathways and residence times in Western Port catchments is crucial to reducing inputs and subsequent risks to aquatic biota. Assessment of land-uses and pesticides detected indicates that agricultural applications, notably vegetable crops and pastures/grasses are likely to play a significant role in pesticide contamination of creeks. However, it is likely that non-agricultural applications are also sources. Research moving forward needs to focus on narrowing down the sources, investigating the role of different transport pathways and assessing options to reduce inputs.

Table 8: Pesticides detected during monitoring of seven creeks in catchments of North-east Westernport and associated registered agricultural and non-agricultural uses in Victoria (APVMA 2018).

Pesticide (a.i)	Group	Number of registered products	Agricultural applications				Non-agricultural applications
			Crop Type			non-crop-agricultural	
			Grasses	Vegetables	Fruit & Seed		
Diuron	Herbicide	78	barley cereal rye oats triticale wheat	asparagus Pulses		fallow areas	bore drain weed control drainage ditches irrigation channels
Simazine	Herbicide	93	lucerne pasture subclover canola	asparagus Pulses	nuts Berries Citrus Pome currents	forestry hops non-crop general nursery flowers vineyards	commercial Industrial premises public utilities rights of way troughs/tanks/dams impervious surfaces (carpark, footpaths) aerodromes
metribuzin	Herbicide	48		asparagus beans pulses potato vetch	tomato		
Metolachlor	Herbicide	42	pasture clover	beans brassica sweet corn sweet potato	sunflower		
Atrazine	Herbicide	83	sorghum lucerne grass crops general	sweet corn		fallow areas forestry	roadside and rights of way
Prometryn	Herbicide	12	currie cocksfeet seed crop demeter fescue crop pasture ryegrass sirocco phalaris	carrot celery chickpea potato	sunflower peanut		
Iprodione	Fungicide	57	lucerne canola	celery lettuce potato soybeans	nuts berries grapes kiwi citrus passionfruit stone		ornamentals recreational turf
Tebuconazole	Fungicide	119	ryegrass fescue seed crop	green bean pea onion lettuce	grapes	flowers	lawn
Metalaxyl	Fungicide	22		berries brassica curcurbit lettuce onion pulses root and tuber rhuburb soybean sweet corn capsicum	avocado stone fruit tomato	grapevine flowers	ornamental
Boscalid	Fungicide	7		curcurbit brassica capsicum eggplant lettuce onion root & tuber	pome fruit tomato	grapevine	turf

Table 8 Continued: Pesticides detected during monitoring of seven creeks in catchments of North-east Westernport and associated registered agricultural and non-agricultural uses in Victoria (APVMA 2018).

Pesticide (a.i)	Group	Number of registered products	Agricultural applications				Non-agricultural applications
			Crop Type			non-crop-agricultural	
			Grasses	Vegetables	Fruit & Seed		
Propiconazole	Fungicide	99	oats barley ryegrass wheat		stone fruit peanut	spearmint/peppermint boronia	sporting fields parks soft/hard wood timber for building
Procymidone	Fungicide	20	canola	pulses onion potato garlic	stone fruit		ornamental
Pyrimethanil	Fungicide	6		potato	berries tomato citrus	grapevine	ornamental home garden
DEET	Insecticide	4					pest-control personal use
Pirimicarb	Insecticide	17	lucerne	asparagus brassica pulses root and tuber capsicum curcurbit endive artichoke kale leek lettuce shallot spinach watercress	stone fruit pome Fruit berries citrus tomato	flowers	ornamental
Malathion	Insecticide	2					home fruit trees ornamentals home vegetables
Bifenthrin	Insecticide	280	barley canola clover lucerne wheat	legumes pulses	stone fruit citrus grape tomato	flowers	commercial/industrial premises domestic pest control public utilities turf barrier control ornamentals

Risks to Aquatic Biota from Pesticides

While pesticides are used in agricultural and non-agricultural settings to protect crops, animals and infrastructure from diseases, pests and weeds, residues that end up in waterways may negatively affect non-target sensitive species of plants and animals. Further, where pesticides are partitioned once they end up in a waterway, dissolved or particulate bound, can have implications for their bioavailability and ecotoxicological impact (Oliver et al 2012). Pesticides in a dissolved phase are generally expected to be more readily available to aquatic organisms compared with those that are attached to colloidal material (Oliver et al 2012). Subsequently pesticides occurring in the dissolved phase would be expected to pose a greater risk as they are more bioavailable for direct uptake by aquatic biota. Of the twenty pesticides detected in surface waters, seventeen were detected in both grab and passive samplers, indicating that pesticides were predominantly in the dissolved phase.

Risk to aquatic biota from individual pesticides was assessed through comparison of concentration data (grab and sediment samples only) to the Australian and New Zealand trigger values (TVs) (ANZECC and ARMCANZ, 2000). Concentrations of individual pesticides that exceed the TV for a chemical at a site indicate potential for adverse biological effects to occur and that further investigation is warranted to determine the potential risk of that chemical to the resident biota. In the current study diuron and *p’p’*-DDT exceeded trigger values. That said, TVs only exist for 5 of the 14 pesticides detected in surface waters and for none of the pesticides detected in sediments. This is a crucial knowledge gap that needs addressing to allow a more comprehensive and reliable estimate of the risk posed by pesticides. Based on the results from the current research and that undertaken in Western Port catchments over the last seven years risks from fungicides and herbicides need to be addressed through determining toxicity thresholds for local flora and fauna species.

ANZECC and ARMCANZ trigger values are an indication of the risks posed from single pesticide concentrations, however in reality flora and fauna are exposed to mixtures of pesticides. These mixtures are not only restricted to one group of pesticides, rather consist of pesticides from multiple classes (herbicides, fungicides and insecticides) with different modes of action. These complex mixtures would provide opportunity for interactive effects (including synergism and antagonism) on biota. The potential risks to biota may be further exacerbated by the nature of the pesticide exposure patterns and the occurrence of multiple stressors. Pesticide exposures may be highly variable, pulsed exposures or low level, and of a chronic nature. Across the seven sites, both exposure characteristics were observed. For instance, in Bunyip River, Cardinia Creek and Toomuc Creek pesticide detections were of a variable nature, occurring sporadically across sampling events. While in Yallock Cut, Drain One and Lower Gum Scrub Creeks pulses of elevated concentrations together with consistent detection of low levels of pesticides, in particular simazine, diuron, tebuconazole and iprodione, across all sampling events was observed. These results show that biota may be exposed to elevated pesticide concentrations and/or low-level mixtures for periods of days to months.

In addition to complex mixtures of pesticides, other factors may also be exerting stress on the local biota, for example high concentrations of suspended solids, nutrients and poor water quality often occur in conjunction with pesticide contamination. Elevated nutrients and suspended solids have been identified as a water quality issue in creeks draining catchments in northern sections of Western Port (Myers et al 2014; Myers et al 2016; Morris et al 2007; Keough et al 2011). Results from the current study indicate that nutrients and suspended solids remain a water quality issue, with concentrations of both parameters exceeding water quality guidelines detected at all sites on numerous sampling occasions. Nutrient concentrations were elevated throughout the sampling period across many sites, however highest concentrations were generally measured during summer. This corresponded with low oxygen levels in Cardinia, Toomuc and Deep creeks, the occurrence of surface water algal blooms in Yallock Cut and Deep Creek and excessive growth of benthic and filamentous algae in Cardinia and Lower Gum Scrub Creeks, respectively. The elevated levels during summer suggest that nutrients are being transported into the system via fertiliser overspread or wind drift, runoff during irrigation or through groundwater. Elevated nutrient levels during summer periods are of concern due to increased risks of excessive algal growth and eutrophication. Elevated nutrients and suspended solids have been implicated in declines in seagrass in Western Port (Morris et al 2007). Increased suspended solids reduces light attenuation in the water column, while increased nutrients and warmer water temperatures during summer can lead to increased epiphytic and/or filamentous algal growth which results in smothering of leaves, choking of mangrove seedlings and established trees (Morris et al 2007). These stressors in conjunction with elevated pesticides have potential for increased risk of biological impairment. That said, the understanding of interactive effects of pesticides, nutrients and suspended solids is limited, especially for local fauna and flora and warrants further investigation.

Biological Impairment in the Catchments

Evidence of biological impairment, including mortality of shrimp and amphipods and inhibition and stimulation of algal growth was apparent at several sites indicating that flora and fauna is under stress and/or being exposed to pollutants. It is likely that multiple stressors including elevated concentrations and mixtures of pesticides, elevated nutrients, and poor water quality played a role in the observed biological impacts. Inhibition of algal growth was observed at several sites during both Spring and Autumn *in situ* assessments, while severe impacts to survival of Australian Glass Shrimp were observed during Autumn assessments.

During Spring and Autumn exposure to surface waters in Drain One, Deep Creek and the Bunyip River caused inhibition of algal growth. Additionally, in Autumn *in situ* exposure in Lower Gum Scrub Creek, and Yallock Cut also resulted in algal growth inhibition. Multiple herbicides were detected across these sites together with several fungicides and often an insecticide at concentrations which could result in toxicity and may account for the observed inhibition. Herbicides and fungicides pose a real threat to non-target aquatic phototrophs as their primary function is to control unwanted plants, algae and fungi. Aquatic phototrophs play a key role in aquatic ecosystems, providing vital services such as nutrient cycling, food resources and habitat (Stockner 1988; Smith et al 2012). Understanding risks posed by herbicides and fungicides to these groups is crucial to their protection.

Stimulation of algal growth was observed during Spring at four sites; Cardinia Creek, Toomuc Creek, Cardinia Creek reference site and Yallock Cut. High concentrations of nutrients were present at these sites, which are likely to be the cause of stimulated growth, together with optimal growth conditions for algae e.g. warm surface water temperatures, high light attenuation. Elevated nutrient concentrations appeared to play a role in alleviating expected toxicity of herbicides across several sites, particularly during Spring. For example, in both Yallock Cut and Drain One creeks highest concentrations of herbicides, fungicides and insecticides were detected during Spring, however greatest inhibition of algal growth occurred during Autumn assessments. During Spring, nutrient concentrations, particularly nitrogen, at these sites were up to 25 times those measured in Autumn. Nutrients are well known to stimulate productivity of aquatic algae and plants and limited research has demonstrated elevated nutrients can alleviate inhibitory impacts of pesticides (DeLorenzo et al 2001). However, further research is needed to improve our understanding of nutrient and pesticide interactions, notably in local species.

Severe impacts to Australian Glass shrimp were observed in Lower Gum Scrub Creek, Toomuc Creek, Drain One and Deep Creek during Autumn deployments. While a few pesticides were detected at these sites, it is unlikely they were the primary cause for mortality, as similar types and concentrations were observed during both Spring and Autumn assessments. Assessment of physicochemical data indicated that there was a significant change in electrical conductivity between Spring and Autumn assessments. During spring, electrical conductivity in the four sites averaged 1602 µS/cm, while in Autumn 23061 µS/cm. This could be the cause of the observed mortality.

Assessment of the toxicity of sediments showed reduced survival of amphipods at two sites, Toomuc Creek and Yallock Cut, indicating that fauna is being exposed to stressors and/or pollutants not only through surface waters, but also through sediments. Chemistry data showed the presence of the insecticide bifenthrin in sediments from Toomuc Creek at levels which may cause toxicity to aquatic fauna and may account for the reduced survival of amphipods. While in Yallock Cut there were no insecticides detected during the Autumn assessment round, the fungicide tebuconazole was detected and could account for the observed toxicity. Similar concentrations of tebuconazole were observed in Drain One, with no associated toxicity observed in amphipods. This difference can be explained based on assessment of sediment particle size at the two sites. Yallock Cut sediments were composed of nearly double the fine clay fraction present in Drain One sediments. As pesticides most commonly bind to the finer sediment fractions, Yallock Cut would be expected to have had a greater amount of tebuconazole present compared to that in Drain One.

Evidence from biological assessments indicates that surface waters and sediments from these creeks is at times a risk for flora and fauna health. Biological assessments play a vital role in understanding risks posed by pollutants and water quality in these systems. To manage these systems to protect environmental values moving forward, we need to ensure appropriate tests using local species are available for assessment of pollutants being detected.

Conclusions and Recommendations

The findings of the current study show frequent and widespread contamination by pesticides across the north-east catchments investigated which discharge into Western Port. Pesticides are present in surface waters and sediments in complex mixtures and often at concentrations likely to impact on resident flora and fauna. Herbicides and fungicides are the most frequently detected pesticide groups, also occurring at the highest concentrations. Key pesticides detected, based on frequency of occurrence and concentration, include:

Herbicides
- Diuron
- Simazine
- Atrazine
- Metolachlor

Fungicides
- Tebuconazole
- Iprodione

Insecticides
- Bifenthrin
- Pirimicarb

Periods of poor water quality (low dissolved oxygen, elevated turbidity) and elevated nutrients were also detected across the sites creating a multiple stressor environment. Sources of pesticides and nutrients are likely to include both agricultural and non-agricultural applications in the catchments, further investigation is needed to determine these. Catchments presenting the greatest risks to aquatic health, based on pesticide levels and detections, nutrient concentrations and poor water quality are Yallock Cut, Deep Creek, Drain One and Lower Gum Scrub Creeks. It is recommended that sources of pesticides and nutrients in these systems be further investigated and research into the transport pathways and persistence of the key pesticides detected be undertaken in order to determine and assess appropriate management actions to reduce inputs.

Biological impairment in flora and fauna, following exposure to surfaces waters or sediments, was observed across several sites. Concentrations of several pesticides were at levels likely to have resulted in the observed toxicity alone, however the complex pesticide mixtures and poor water quality created a multiple stressor environment which would cause biological impairment. Elevated nutrients were likely the cause of excessive algal growth across a few sites, notably during summer sampling. It is possible that elevated nutrients also alleviated expected toxicity of some pesticides, however further research into pesticide nutrient interactions is needed to better understand this relationship. It is recommended that research is instigated to investigate nutrient and pesticide interactions, and to assess the impacts of single compounds and pesticide mixtures on local flora and fauna species in order to undertake more comprehensive risk assessment. It is proposed that initial focus be around key herbicides and fungicides detected across Western Port catchments. For fungicides there may need to be an initial focus on development and validation of toxicity bioassays and assessment methods specific for this group of pesticides, as currently these are lacking.

Recommendations:

Several recommendations for further monitoring and research are proposed based on the outcomes from the current study. In priority order, they include:

1. Sourcing of Pesticides

- Determine the major sources of pesticides in Lower Gum Scrub Creek, Yallock Cut Creek, Drain One Creek and Deep Creek.

2. Transport pathways, pesticide persistence and management actions

- Determine the dominant transport pathways for pesticides to Western Port creeks (e.g. groundwater, surface water runoff, aerial deposition, dissolved or sediment bound). Key pesticides for initial focus: diuron, simazine, atrazine, metolachlor, tebuconazole, iprodione.
- Determine if pesticide detections are related to recent application and subsequent runoff or due to persistence?
- Determine and assess management actions to reduce pesticide inputs?

3. Herbicide and Fungicide Threats to Western Port

- Determine concentrations of herbicides, singly and in mixtures, that present a concern for local flora and fauna e.g. plants, frogs, fish. Initial focus on key herbicides detected, e.g. diuron, simazine, atrazine, metolachlor
- Determine whether concentrations of fungicides are a concern for local flora and fauna. Initial focus on development of assessment methods with local species and key fungicides detected, e.g. tebuconazole, iprodione.
- Investigate the effect of nutrient enrichment on pesticide toxicity. Initially, focus should be on herbicide and nutrient interactions on local flora.

4. Nutrient threats to Western Port

- Determine the source and transport pathways and total loads of nutrients throughout the year and the risk they pose for increased nuisance algal growth and eutrophication.
- Assess the impacts of nutrients on fresh and estuarine biota. Initial focus on characterisation of soft sediment chemistry and microfauna/flora using eDNA techniques.
- Determine and assess management actions to reduce nutrient inputs?

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Appendices

Appendix 1: Pesticides screened for in grab surface waters, passive sampler disks and sediments during the current study and their detection limits. H = herbicide, I = Insecticide, F = Fungicide, MISC = miscellaneous.

Pesticide	Type	Detection Limit	Pesticide	Type	Detection Limit ug/disk
Simazine	H	0.01	pp DDD	I	0.01
Diuron	H	0.01	pp DDT	I	0.01
Iprodione	F	0.01	Endrin	I	0.01
Metolachlor	H	0.01	Endrin aldehyde	I	0.01
Prometryn	H	0.01	Endrin Ketone	I	0.01
Linuron	H	0.01	alpha Endosulfa	I	0.01
Metalaxyl	F	0.01	beta Endosulfan	I	0.01
Atrazine	H	0.01	Endosulfan sulf	I	0.01
Procymidone	F	0.01	Methoxychlor	I	0.01
Chlorothalonil	F	0.01	Dicofol	I	0.01
Dimethomorph	F	0.01	Demeton S met	I	0.01
Tebuconazole	F	0.01	Dichlorvos	I	0.01
Diazinon	I	0.01	Chlorpyrifos met	I	0.01
Dimethoate	I	0.01	Fenthion	I	0.01
Propiconazole	F	0.01	Ethion	I	0.01
Boscalid	F	0.01	Chlorfenvinphos	I	0.01
Fenamiphos	F	0.01	Chlorfenvinphos	I	0.01
Difenoconazole	F	0.01	Parathion ethyl	I	0.01
Propiconazole	F	0.01	Parathion methy	I	0.01
Cyprodinil	F	0.01	Pirimiphos meth	I	0.01
Carbaryl	I	0.01	Pirimiphos ethyl	I	0.01
Pirimicarb	I	0.01	Bromophos ethy	I	0.01
Buprofezin	I	0.01	Carbophenothio	I	0.01
Metribuzine	H	0.01	Coumaphos	I	0.01
Propiconazole	F	0.01	Dioxathion	I	0.01
Prochloraz	F	0.01	Formothion	I	0.01
Pendimethalin	H	0.01	Methacrifos	I	0.01
Methoprene	I	0.01	Methidathion	I	0.01
Azinphos ethyl	I	0.01	Mevinphos	I	0.01
Phorate	I	0.01	Phosalone	I	0.01
Thiometon	I	0.01	Profenophos	I	0.01
Triazophos	I	0.01	Prothiofos	I	0.01
Permethrin	I	0.01	Bifenthrin	I	0.01
Bupirimate	F	0.01	Bioresmethrin	I	0.01
Chlorpyrifos	I	0.01	Cyfluthrin	I	0.01
Malathion	I	0.01	Cyhalothrin	I	0.01
Fenitrothion	I	0.01	Cypermethrin	I	0.01
Azinphos meth	I	0.01	Fenvalerate	I	0.01
Fenchlorphos	I	0.01	Phenothrin	I	0.01
Deltamethrin	I	0.01	Dichlofluanid	F	0.01
Diphenylamine	F	0.01	Dicloran	F	0.01
Imazalil	F	0.01	Fenarimol	F	0.01
Hexazinone	H	0.01	Flusilazole	F	0.01
Naphthol1	MISC	0.01	Hexaconazole	F	0.01
HCB	F	0.01	Penconazole	F	0.01
Heptachlor	I	0.01	Pyrimethanil	I	0.01
Heptachlor ep	I	0.01	Vinclozolin	F	0.01
Aldrin	I	0.01	o Phenylphenol	F	0.01
gamma BHCLi	I	0.01	Fenoxycarb	I	0.01
alpha BHC	I	0.01	Molinate	H	0.01
beta BHC	I	0.01	Oxyfluorfen	H	0.01
delta BHC	I	0.01	Trifluralin	H	0.01
trans Chlordan	I		Piperonyl Butoxi	SYN	
cis Chlordane	I	0.01	Propargite	I	0.01

Oxychlordane	I	0.01	Tebufenpyrad	I	0.01
Dieldrin	I	0.01	Tetradifon	I	0.01

Appendix 2: Nutrient methods and detection limits for water samples.

Analytical Parameter	Method Code	Analytical Method	Method Reference	Limit of Reporting (mg/L)
Ammonia as N	EK055G	Determined by direct colorimetry by Discrete Analyser	APHA 4500-NH3 G	<0.01
Nitrite as N	EK057G	Determined by direct colorimetry by Discrete Analyser	APHA 4500-NO2- B	<0.01
Nitrate as N	EK058G	Nitrate is reduced to nitrite by way of a chemical reduction followed by quantification by Discrete Analyser. Nitrite is determined separately by direct colourimetry and result for Nitrate calculated as the difference between the two results	APHA 4500-NO3- F	<0.01
Nitrite and Nitrate as N (NOx)	EK059G	Combined oxidised Nitrogen (NO2+NO3) is determined by Chemical Reduction and direct colourimetry by Discrete Analyser.	APHA 4500-NO3- F	<0.01
Total Kjeldahl Nitrogen as N	EK061G	An aliquot of sample is digested using a high temperature Kjeldahl digestion to convert nitrogenous compounds to ammonia. Ammonia is determined colorimetrically by discrete analyser.	APHA 4500-Norg D	<0.1
Total Phosphorus as P By Discrete Analyser	EK067G	This procedure involves sulphuric acid digestion of a sample aliquot to break phosphorus down to orthophosphate. The orthophosphate reacts with ammonium molybdate and antimony potassium tartrate to form a complex which is then reduced and its concentration measured at 880nm using discrete analyser.	APHA 4500-P H, Jirka et al (1976), Zhang et al (2006).	<0.01