Coliban Water Monitoring Program

Monitoring Program for Assessing the Benefits of Environmental Offsets on the Condition of the Campaspe River: Year 4 (2021)

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The Aquatic Environmental Stress Research Group aims to identify and address the ecological impacts of pollution in aquatic environments.

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Glossary

AQUEST	Aquatic Environmental Stress Research Group based at RMIT University
Autotrophic	Relating to an organism that manufactures its own food from inorganic substances, such as carbon dioxide and nitrogen, using light or its own reserves (ATP) for energy. All green plants and algae, and some bacteria and protists, are autotrophs.
Autotrophic Index or AI	A measure of the autotrophic-heterotrophic balance of the community present. It is calculated as the ratio of AFDM (Ash-Free Dry Mass) to chlorophyll-a.
Ash free dry mass (AFDM)	The weight of the organic material in a sample.
Bacteroides	Rod-shaped, anaerobic bacteria of the genus *Bacteroides*, occurring in the alimentary and genitourinary tracts of humans and other mammals.
Chlorophyll-a	A green pigment present in all green plants and in cyanobacteria, which is responsible for the absorption of light to provide energy for photosynthesis. It is often used as a surrogate for algal biomass.
E. coli	*Escherichia coli*, also known as *E. coli*, is a coliform bacterium of the genus *Escherichia* that is commonly found in the lower intestine of warm-blooded organisms.
Ecotoxicology	A scientific discipline combining the methods of ecology and toxicology in studying the effects of toxic substances and especially pollutants on the environment.
Heterotrophic	Relating to an organism that cannot manufacture its own food and instead obtains its food and energy by taking in organic substances,

	usually plant or animal matter. All animals, protozoans, fungi, and most bacteria are heterotrophic.
Macroinvertebrate	Aquatic macroinvertebrates are small animals that live for all, or part, of their lives in water. There are many different types of macroinvertebrates such as dragonfly larvae, mosquito larvae, water fleas, beetles and snails.
Passive Sampler	An environmental monitoring technique involving the use of a collecting medium, such as a man-made device or biological organism, to accumulate chemical pollutants in the environment over time.
POCIS	Polar organic integrated sampler – a type of passive sampler.
SFMW	Stream frontage management works
SFMP	Stream frontage management program
WRP	Water reclamation plant
Wastewater	Wastewater is any water that has been affected by human use. It is used water from any combination of domestic, industrial, commercial or agricultural activities, and any sewer inflow or sewer infiltration. Therefore, wastewater is a by-product of domestic, industrial, commercial and/ or agricultural activities. Types of wastewaters include domestic wastewater from households, municipal wastewater from communities (also called sewage), industrial wastewater, and agricultural wastewater.

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Executive Summary

Stream frontage management (SFM), including rehabilitation measures, such as the removal of exotic vegetation, riparian revegetation, and improved stock management, have become increasingly common methods applied to attempt to improve water quality and reduce the transport of pollutants to waterways (Hughes and Quinn, 2014; McKergow et al., 2016). To understand the effectiveness of SFM actions in improving water quality and biodiversity, the monitoring of river conditions during and following works is needed, but is rarely undertaken. The current program is unique as it is assessing the effects of stream frontage management works (SFMW) on ambient water quality and biodiversity, while also trying to understand whether other factors may be influencing water quality and instream health, over a 13 km stretch of the Upper Campaspe River, Snipes Creek and Post Office Creek around Kyneton over a 5-year period. As of December 2020, woody weed control, planting, fencing and the installation of off stream watering had been completed across the four SFM sites included in the Caring for the Campaspe project. These sites are now largely in a management phase until June 2023.

In Year 4, water quality, aquatic ecology, nutrient bioavailability, and ecotoxicology were surveyed at eight sites along the Campaspe River, and in two associated tributaries, between August and December 2021. While the full benefits of SFM are not likely to be observed in four years of monitoring, results to date show evidence of differences in river condition emerging between sites based on riparian condition e.g., between SFMW sites, native vegetation sites and sites where no interventions have occurred, which remain willow dominated with stock access.

At SFMW sites, there is evidence that the works initially led to increases in nutrient and sediment inputs, but once vegetation has become more established, and banks more stabilised, abiotic conditions appear to have stabilised. Similar abiotic conditions are observed at sites surrounded by established native vegetation. Since monitoring started in 2018 across the SFMW and established native vegetation sites, macrophyte cover has become less temporally variable and has substantially increased. In contrast, filamentous algal cover, particularly medium and long filamentous algae, has simultaneously declined, with nuisance levels not observed since Year 1 monitoring. With this stabilisation of macrophyte communities we have also seen an increase in biofilm communities and improvements in dissolved oxygen levels, particularly at SFMW sites. Highest macroinvertebrate diversity and taxon richness occurs at the SFM influenced sites, followed by the native vegetation sites, which is likely related to better habitat structure, food resources and the presence of relatively stable water levels during dry periods (notably at Campaspe River Sites 3-6) at these sites. Increases in macroinvertebrate diversity were observed in Year 4 at the native vegetation sites (Campaspe Sites 9 and 10), which was likely due to increased rainfall during 2021.

In contrast, sites where no interventions have occurred are in the poorest abiotic and biotic condition. Sites are characterised by elevated concentrations of dissolved nutrients, which were often found to exceed guideline values, had lower dissolved oxygen levels and water temperatures, and had poorer water clarity. These sites are also dominated by opportunistic macrophyte species, including *Azolla* and *Lemna*, that blanket the water surface, reducing light and oxygen to the water column. While declines in macrophyte cover were observed in Year 4 at these sites, likely due to reduced availability of nutrients and high rainfall, the presence of good quality macrophytes and benthic algal supply remains poor. These factors continue to result in reduced instream processing and greater export of nutrients from these sites. Poorest macroinvertebrate diversity and taxon richness was observed at these sites, likely a result of poor habitat, lack of quality food resources and elevated nutrients. Without intervention, these sites are likely to remain in poor abiotic and biotic condition.

Several additional pressures continue to be observed across the study area, including the presence of toxicity and a range of pollutants associated with urban, industrial and agricultural runoff, and wastewater inputs. Numerous pesticides, pharmaceuticals, heavy metals and hydrocarbons have been detected at levels which could pose a risk to river ecological health.

Continued improvements to the ecological health of the river are expected at sites influenced by SFMW in Year 5. However, improvements in the condition of many of these sites is complicated by the surrounding residential, industrial and agricultural land-uses which create additional challenges for stream management.

Introduction

In 2012, the North Central Catchment Management Authority (NCCMA) established the "Caring for the Campaspe" project, a stream frontage management program (SFMP) along the Campaspe River aimed at enhancing the health of the waterway and improving the biodiversity of the river. In 2018, Coliban Water provided additional funding to the NCCMA to undertake a further 14.3 km of environmental improvement work along the river from Carlsruhe to below Kyneton. Works included the removal of willow trees, blackberry, hawthorn and other weeds and revegetating with native trees and shrubs, installing 13.1 km of fencing to keep livestock out of the waterway, installing off-stream watering systems as an alternative water source for livestock and supplementary replanting, weed control and revegetation watering. These works are expected to improve the health of the riparian habitat and reduce instream faecal contamination and nutrient pollution. Longer term benefits are expected as the riparian vegetation becomes more established and provides shade, habitat, and food for aquatic animals. Further details on the program and its location are provided in Myers *et al*. 2019.

The Aquatic Environmental Stress Research Group (AQUEST), from RMIT University, was commissioned by Coliban Water to undertake a 5-year monitoring program to assess the benefits of the SFMP on the ecological condition of the Campaspe River, with a particular focus on the Kyneton area. The program incorporates water quality assessments, together with aquatic ecology surveys and toxicology techniques to investigate improvements to water quality and biodiversity in the Campaspe River, from Carlsruhe to Redesdale, and in two associated tributaries. Results from Years 1 to 3 of monitoring are provided in Myers *et al*. 2019, 2020 and 2021. This report presents the outcomes from Year 4, and makes comparisons with the first three years of the monitoring and assessment program.

Study Objectives

The five-year monitoring program aims to assess changes to the health of the Campaspe River as a result of the stream frontage management works. The program will assess, over five years, whether environmental offsets (riparian revegetation and stock exclusion fencing) will:

- Reduce nutrient concentrations during base flows, and whether these works lead to reduced nutrient enrichment in the river (indicated by direct measurements of nutrient concentrations in water, and assessments of algal and plant growth).
- Reduce faecal contamination from cattle during base flows (determined by *E. coli* and a specific biomarker of cattle faeces).
- Improve the ecological health of the river (assessed through aquatic macroinvertebrate assemblages and *in situ* toxicology assessments).

The objectives of Year 4 monitoring were to:

- Collect monitoring data which contribute to the study objectives.
- Compare the results of Year 4 with the results from Years 1, 2 and 3 of monitoring.
- Assess and report on the short-term outcomes of the SFMP on the ecological health of the Campaspe River, and associated tributaries, and evaluate the potential to achieve the desired longer-term outcomes.
- Understand whether other factors may be influencing water quality and instream health through assessments of water and sediment chemistry, and ecotoxicology.

Methods

Study Area

The 5-year monitoring and assessment program is focused on the Upper Campaspe River from Carlsruhe to Redesdale which runs through agricultural, residential and industrial areas. Stream Frontage Management Works were targeted at four locations along the Campaspe River (Figure 1). These works include initial woody weed control, revegetation, fencing and off stream watering installations undertaken from February 2019 to June 2020. Weeding, supplementary watering and revegetation maintenance programs will be undertaken from Spring/Summer 2020 to Spring/Summer 2023. Ten sites (Table 1 and Figure 1), including eight along the Campaspe River and one in both Post Office Creek and Snipes Creek, were selected to assess the benefits of the SFMW and understand other factors influencing stream health. Detailed descriptions of each sampling site are provided in the Year 1 Monitoring Report (Myers *et al.* 2019).

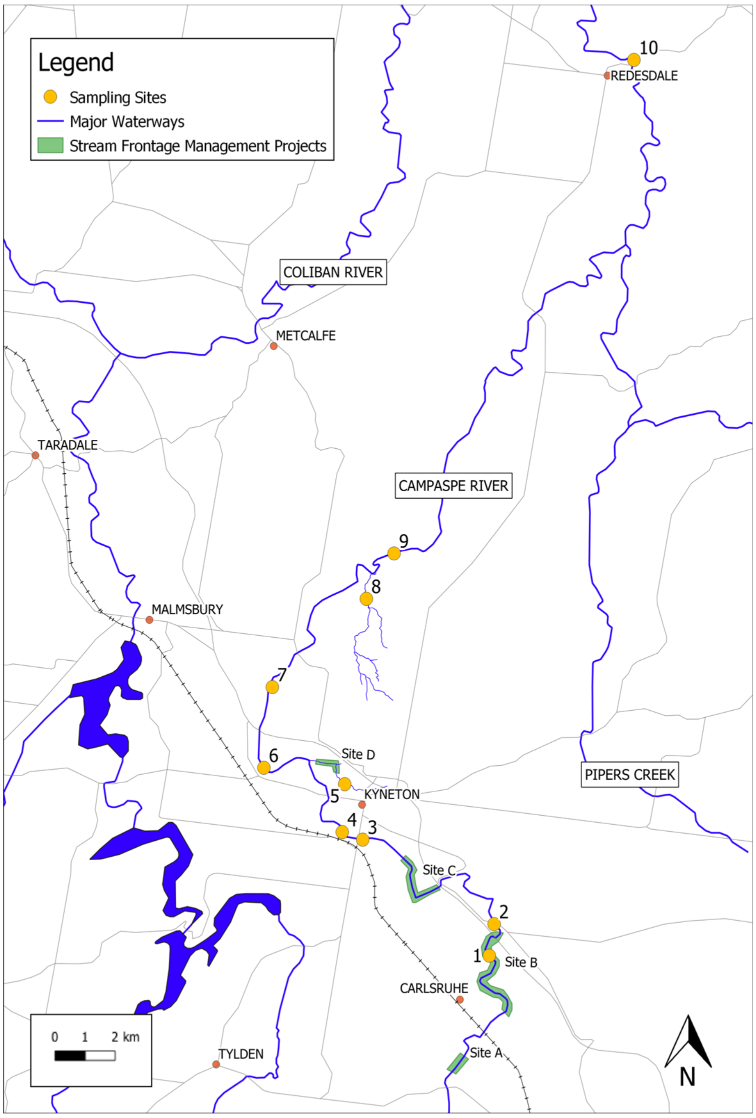

Figure 1: Locations of monitoring sites within the Campaspe River system and SFMW. Water flows from Site 1 downstream to site 10

Table 1: Locations of the ten sampling sites within the Campaspe River and associated tributaries

Site #	GPS coordinates	River/Stream	Location
1	37°17’31.6” S, 144°29’38.4” E	Campaspe River	Cheveley Rd, Carlsruhe
2	37°16’57.1” S, 144°29’43.2” E	Campaspe River	Cobb & Co Rd, Carlsruhe
3	37°15’21.4” S, 144°27’10.2” E	Campaspe River	Mollison St, Kyneton
4	37°15’13.7” S, 144°26’49.1” E	Campaspe River	Botanic Gardens, Kyneton
5	37°14’21.1” S, 144°26’51.0” E	Post Office Creek	Wedge St, Kyneton
6*	37°14’11.7” S, 144°25’13.6” E	Campaspe River	Burton Ave, Kyneton
7*	37°12’31.9” S, 144°25’25.7” E	Campaspe River	Old Station Rd, Kyneton
8	37°10’57.4” S, 144°27’16.7” E	Snipes Creek	Barbower Rd, Edgecombe
9	37°10’07.2” S, 144°27’49.6” E	Campaspe River	Boundary Rd, Langley
10	37°00’57.4” S, 144°32’28.2” E	Campaspe River	Heathcote-Redesdale Rd, Redesdale

*Kyneton WRP discharge point to the Campaspe River is situated between sites 6 and 7.

Monitoring

To assess the benefits of the SFM works to river health and understand other factors that may influence river condition, a variety of indicators, including water and sediment chemistry, aquatic ecology, physical habitat condition and ecotoxicology are being monitored. Samples are collected annually from all ten sites at five time points, targeted when the river is flowing, to assess seasonal trends. During Year 1, monitoring was conducted from September through December 2018 and in July 2019, Year 2 monitoring was conducted from August to December 2019, while Year 3 monitoring was disrupted due to covid-19 and conducted in August, November and December 2020, and January to February of 2021. Year 4 monitoring was conducted from August through December 2021 (Table 2).

The monitoring methods applied are summarized in Table 3. Detailed information on the methodologies used is provided in the Year 1 Monitoring Report (Myers *et al.* 2019).

Table 2. Monitoring schedule to date. Samples have been collected for Years 1, 2, 3 and 4 of the 5-year monitoring program. Grey cells indicate future monitoring periods.

Year	Month											
	J	F	M	A	M	J	J	A	S	O	N	D
2018									Year 1	Year 1	Year 1	Year 1
2019							Year 1	Year 2	Year 2	Year 2	Year 2	Year 2
2020*								Year 3			Year 3	Year 3
2021	Year 3	Year 3						Year 4	Year 4	Year 4	Year 4	Year4
2022												

* Sampling was affected due to restrictions introduced to address the COVID-19 pandemic.

Table 3. Summary of monitoring methods. Grey cells indicate when samples were collected for each monitoring parameter in Year 4.

Monitoring	Aug	Sep	Oct	Nov	Dec	Description
Surface Water Chemistry						
Physico-chemistry	▨	▨	▨	▨	▨	Parameters included water temperature, dissolved oxygen (% saturation), pH, electrical conductivity, and turbidity measured monthly.
Nutrients	▨	▨	▨	▨	▨	Water samples analysed for ammonia as N (NH_4-N), total nitrogen (TN), total Kjeldahl Nitrogen (TKN), nitrate and nitrite (NOx), orthophosphate (OP) and total phosphorus (TP) monthly.
Faecal monitoring*		▨		▨		*E. coli* was used as the key indicator of faecal contamination and a Bacteroides assay was used to determine the origin of faecal pollution (e.g., human, bovine). Samples are collected on 3 occasions each year.
Passive samplers				▨		Polar Organic Chemical Integrated Samplers (POCIS) were deployed at each site for a 4-week period to detect pollutants including personal care products, pharmaceuticals, herbicides, insecticides and fungicides present in surface waters annually. (see Appendix 2 for full list).
Sediment Chemistry						
Sediment chemistry				▨		Heavy metals, petroleum hydrocarbons and multi-residue pesticides were analysed from fine (<4µm) sediment samples collected annually.
Aquatic Ecology						
Macroinvertebrate survey				▨		Rapid Bioassessment (RBA) method applied annually. Collection and identification took place according to EPA Victoria guidelines (EPA Victoria, 2003). Biological indices (number of families, SIGNAL and EPT indices) determined.
Benthic algal production			▨		▨	Thin discs were suspended, in triplicate, in the water column for a 4-week period bi-annually. These artificial substrates were analysed for biofilm biomass (measured as AFDM and chlorophyll-a).
Physical Habitat						
Instream habitat assessment	▨	▨	▨	▨	▨	Percentage cover of aquatic macrophytes and filamentous algae. The length of filamentous algae was also noted (short <2cm, medium 2-10cm, long >10cm).
Surface Water Toxicity						
Floral Toxicity			▨		▨	The growth of algae immobilised in alginate beads, was used to assess the toxicity of surface waters and availability of nutrients to floral species. Algal beads were deployed in cages for 10 days, thereafter biomass determined bi-annually.
Faunal toxicity				▨		The survival and reproductive ability of the mud snail, *Potamopyrgus antipodarum* was used to assess surface water toxicity. Snails were deployed in cages for 4 weeks annually.

* Undertaken at six sites (sites 2, 4, 5, 6, 7, and 8)

Results

Stream Frontage Management Program – Works and Maintenance Phases

The stream frontage management works began in February 2019. Initial woody weed control (56 ha) and revegetation (15 ha) was undertaken from April to October, and September to November 2019, respectively. Livestock exclusion fencing (12 km), and offstream watering works began in August 2019. Works across all SFM sites were completed at the end of 2020 and sites are now in the maintenance phase of the project.

Rainfall

In Year 4 monthly rainfall was highest in October (80.0 mm), and lowest in December (25.0 mm) (Figure 2). Below average monthly rainfall was reported for August and December 2021, and above average in September, October, and November of 2021. Mean monthly rainfall in Year 4 (57.7 mm) was greater than in monitoring Years 1, 2 and 3 (44.7, 34.5, and 44.3 mm, respectively).

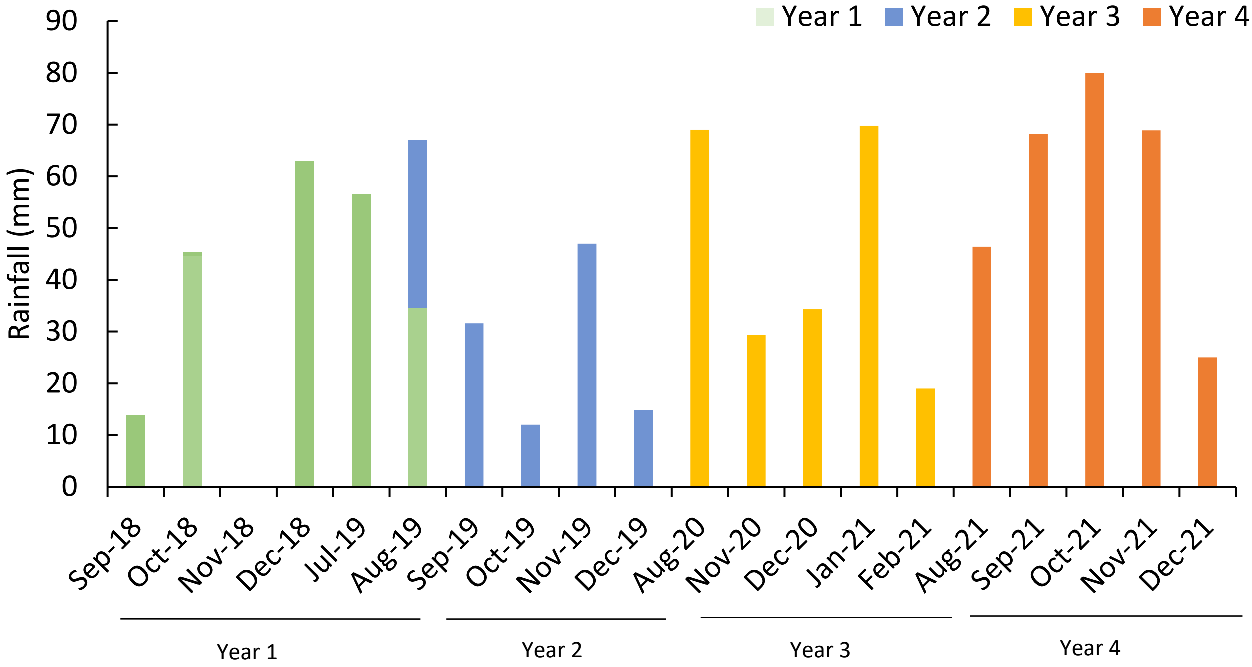

Figure 2: Monthly rainfall during sampling at Kyneton from year 1 (2018) to year 4 (2021) (weather observation station: 088036, Kyneton Post Office) (BOM, 2021). No data was available for November 2018.

Surface Water Chemistry

Physico-chemistry

Surface water temperatures were temporally variable. Highest water temperatures (23.7°C) were recorded at Site 1, while lowest water temperature (10.2°C), occurred at Campaspe Site 7 and tributary Site 8 (Table 4); however, mean water temperatures were highest at Campaspe Site 3 (16.6°C), and lowest at Campaspe Site 7 and tributary Site 8 (14.4°C). Average water temperatures generally tended to increase over the first 3 years of monitoring then declined in Year 4 for Campaspe Sites 1-6 and 10 and tributary Site 5 (Table 5). While at Campaspe Sites 7 and 9 average water temperature has been fairly stable over the 4 years and at tributary Site 8 tended to decline over Years 1-3, then increased in Year 4 (Table 5).

Mean dissolved oxygen concentrations were highest at Campaspe Site 3 (110.9% saturation) and lowest at Campaspe Site 7 (84.4%) (Table 4). Comparing dissolved oxygen annually, Year 4 sampling concentrations were generally higher than in the previous 3 years. Further, Campaspe River Site 7 tends to have lower dissolved oxygen concentrations (<66%), than all other all sites (Table 5). Dissolved oxygen concentrations at all sites were below the Environmental Reference Standards (ERS) trigger value of 130% (Table 4 and 5).

Mean pH levels were within the recommended ERS range of 6.8 to 8.0 units during all sampling months, except at Campaspe Sites 1 and 2, which were below the ERS guidance range (6.5 and 6.7pH units respectively; Table 4). Minimum pH units were below the ERS guidance range at all sites, except at Campaspe Sites 3 and 7, during Year 4 monitoring (Table 4). An annual comparison of pH indicates a general decrease from Year 1 to Year 4 sampling across sites (Table 5).

Average electrical conductivity increased at all Campaspe sites as you traversed downstream, ranging from 237.6 to 397.0 µS/cm (Table 4). Electrical conductivity was noticeably greater in the tributary Sites 5 and 8, being 711.6 and 910.8 µS/cm, respectively (Table 4). Tributary sites 5 and 8 have consistently exhibited high electrical conductivity throughout the four years of monitoring. All the sites show a decrease in mean electrical conductivity in Year 4 compared to the previous years (Table 5).

Mean turbidity measures exceeded the ERS trigger value of 15 NTU at Campaspe Site 10, while maximum measured turbidity exceeded at Campaspe Sites 1, 3, 7, and 10 and tributary Site 8 in Year 4 (Table 4). Annually turbidity varies across sites, with no observable trends (Table 5).

Table 4. Mean, minimum and maximum temperatures, dissolved oxygen, pH, electrical conductivity and turbidity measured across sites during Year 4 monitoring. Values highlighted in orange exceed Environmental Reference Standards trigger value.

Site #	Temperature (°C)			Dissolved Oxygen (% saturation)			pH (pH units)			Electrical conductivity (µs/cm)			Turbidity (NTU)		
	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
Campaspe River															
1	15.5	10.4	23.7	90.9	73.2	107.7	6.5	5.7	7.3	237.6	195.3	277.0	11.2	8.1	18.3
2	15.5	10.3	23.4	82.5	75.4	92.3	6.7	5.6	7.5	247.8	218.0	272.0	9.1	8.1	10.3
3	16.6	10.8	23.6	91.3	69.9	110.9	7.4	7.2	7.6	282.4	211.0	332.0	9.9	5.6	19.5
4	16.4	11.5	22.6	85.8	57.7	98.4	6.9	6.4	7.2	302.8	284.0	321.0	8.4	5.7	12.7
6	16.4	11.5	21.5	85.2	52.9	106.2	7.0	6.7	7.3	333.8	286.0	376.0	8.8	6.3	14.0
Kyneton WRP discharge between Sites 6 and 7															
7	14.4	10.2	19.9	66.0	35.5	84.4	7.1	6.9	7.6	329.4	226.8	404.0	14.9	7.5	29.1
9	15.3	10.7	20.1	83.9	60.2	97.1	7.0	6.0	7.8	384.8	339.0	436.0	8.6	6.4	10.0
10	14.8	10.4	21.6	88.7	66.4	98.9	7.2	6.6	7.9	397.0	275.0	472.0	32.0	6.4	96.4
Tributaries															
5	15.0	10.3	22.0	69.0	54.4	83.7	7.0	6.7	7.3	711.6	498.0	884.0	7.3	4.1	10.2
8	14.4	10.2	21.8	62.6	38.4	93.4	6.9	6.7	7.1	910.8	755.0	1206.0	11.5	9.1	15.2
Trigger Values				max 130			6.8 - 8.0			≥2000			≥15		

Table 5. Mean temperatures, dissolved oxygen, pH, electrical conductivity and turbidity measured across sites during Years 1, 2, 3 and 4 monitoring. Values in highlighted in orange exceed Environmental Reference Standards trigger value.

Site #	Temperature (°C)				Dissolved oxygen (% saturation)				pH (pH units)				Electrical conductivity (µs/cm)				Turbidity (NTU)			
Sampling Year	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Campaspe River																				
1	13.9	15.9	18.0	15.5	60.1	79.0	58.0	90.9	7.6	7.8	7.5	6.5	400.0	380.8	399.0	237.6	6.8	8.8	23.4	11.2
2	13.5	16.1	16.2	15.5	55.5	85.5	62.1	82.5	7.6	8.1	7.5	6.7	383.8	410.0	406.6	247.8	5.4	14.3	11.5	9.1
3	14.2	18.4	19.3	16.6	85.3	86.1	77.5	91.3	7.7	7.7	7.8	7.4	459.6	428.5	461.8	282.4	5.0	9.0	5.8	9.9
4	13.2	18.0	19.5	16.4	87.0	86.6	84.4	85.8	7.8	7.8	8.2	6.9	433.6	413.3	419.4	302.8	4.2	9.7	5.5	8.4
6	15.5	18.4	17.8	16.4	71.8	78.9	65.1	85.2	7.6	7.2	7.3	7.0	412.4	450.0	482.8	333.8	8.9	9.1	7.4	8.8
Kyneton WRP discharge between Sites 6 and 7																				
7	13.9	13.0	14.3	14.4	66.1	56.3	30.3	66.0	8.0	7.3	7.1	7.1	430.8	473.6	478.2	329.4	9.2	9.0	22.2	14.9
9	15.1	14.6	15.6	15.3	79.8	78.0	60.0	83.9	8.2	7.4	7.3	7.0	491.6	498.2	557.2	384.8	6.7	7.9	9.5	8.6
10	15.8	15.6	17.2	14.8	82.3	81.2	69.2	88.7	7.7	7.3	7.3	7.2	591.0	570.2	579.6	397.0	6.0	8.7	11.4	32.0
Tributaries																				
5	13.6	16.7	18.5	15.0	67.0	81.8	61.7	69.0	7.7	7.5	7.3	7.0	556.8	666.4	823.2	711.6	10.9	15.4	8.5	7.3
8	14.9	13.9	13.2	14.4	66.3	49.6	58.7	62.6	7.7	7.0	7.0	6.9	1173.0	1493.0	1081.0	910.8	14.8	34.8	11.3	11.5
Trigger Values					**max 130**				**6.8 - 8.0**				**≥2000**				**≥15**			

Nutrients

Total nitrogen concentrations ranged from 0.1 mg/L to 2.2 mg/L (Figure 3) in Year 4 of monitoring, and exceeded the ERS guideline value (≤1.05 mg/L) at Campaspe River sites on:

- One occasion for Sites 3, 6, 9 (August 2021), Site 2 (September 2021), Site 4 (November 2021) and Site 7 (December 2021)
- Two occasions for Site 10 (August and September 2021)

In the tributary sites, exceedances were observed on:

- One occasion at Site 8 (August 2021)
- Three occasions at Site 5 (August, September and October 2021)

Annual changes in mean total nitrogen concentrations have been variable across sites. At Campaspe Sites 3, 4 and 6 total nitrogen concentrations have generally been stable across sampling years, and for Campaspe Sites 9 and 10, after an increase in concentrations from Year 2 to Year 3, total nitrogen appears to have stabilised in Year 4 (Figure 4). At Campaspe Site 1 and tributary Site 5 and Campaspe Sites 2 and 7 total nitrogen concentrations tended to increase over the first two to three years, and thereafter declined, while mean total nitrogen has been consistently decreasing at Tributary Site 8 (Figure 4).

Ammonia concentrations met the ERS guideline value (≤0.9 mg/L) on all sampling occasions in Year 4 of monitoring, and ranged from <0.01 to 0.39 mg/L (Figure 3). Mean ammonia concentrations are generally stable at Campaspe Sites 2, 4 and 10, and tributary Site 5 across monitoring years, while gradual declines are observed for Campaspe Sites 6 and 7 and tributary site 8 (Figure 5). At Campaspe Sites 1, 9 and 3 there was an increase in mean ammonia during Year 4 sampling, from previous stable concentrations over Years 1-3 (Figure 5).

Monthly mean total phosphorus ranged from 0.02 to 0.33 mg/L over the Year 4 monitoring period (Figure 6) and exceeded the ERS guideline value (≤0.055 mg/L) at Campaspe River sites on:

- One occasion at Site 1 (November 2021)
- Two occasions at Site 2 and 4 (September and November 2021, October and November 2021, respectively)
- Four occasions at Sites 6 (August, September, November, December 2021), 7 and 10 (September, October, November, December 2021), and
- All occasions at Site 9

In the Tributary sites, exceedances were observed on:

- Three occasions for Site 8 (August, November, December 2021)

Mean total phosphorus has fluctuated annually at Campaspe Sites 1, 2, 3, 4 and 6 (Figure 7), while at Sites 9 and 10, and the tributary Sites 5 and 8, it has tended to decrease (Figure 7). At Campaspe River Site 7 there has been a steady increase in mean total phosphorus up to Year 3 and a decrease in Year 4 (Figure 7). Across all monitoring years, mean total phosphorus generally exceeds ERS guideline values (Figure 7).

Monthly orthophosphate concentrations are shown in Figure 6. Concentrations ranged <0.01 to 0.35 mg/L and were consistently lowest in the upper reaches of the Campaspe River (Sites 1-6) and at Site 10 and tributary Sites 5 and 8, and highest at Campaspe River Sites 7 and 9 (Figure 6).

On an annual basis, orthophosphate concentrations have generally remained stable across most Campaspe River sites and the two tributary sites (Figure 8). The upper reaches of the Campaspe River (Sites 1 to 6), Site 10 and tributary Site 5 have consistently had lower concentrations of orthophosphate throughout the 4 years of monitoring, while Campaspe Sites 7 and 9 and tributary Site 8 have consistently had the highest concentrations (Figure 8).

The ratio of orthophosphate to total phosphorus was highly variable across the Year 4 sampling period at all sites (Figure 9). Orthophosphate ratios have generally increased annually at Campaspe Sites 1, 3, 4 6 and 10, as well as at the two tributary sites (Sites 5 and 8), while they have tended to decrease at Campaspe Sites 2, 7 and 9, up to Year 3, before increasing in Year 4 (Figure 10).

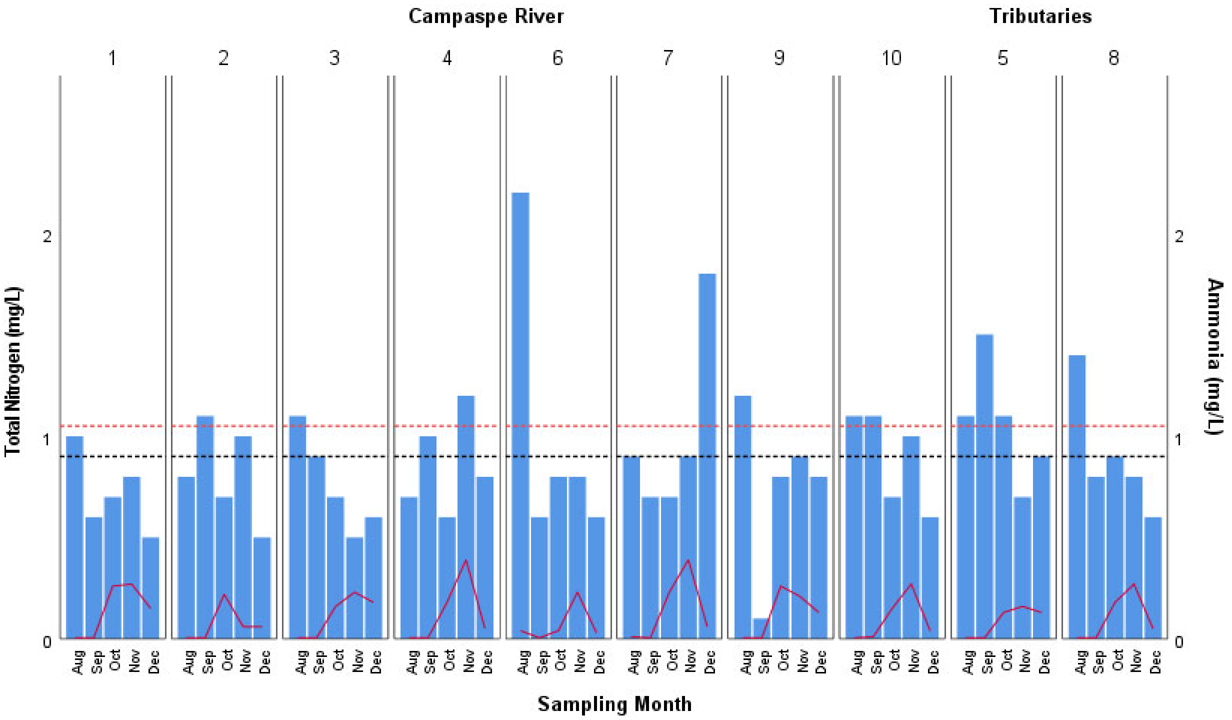

Figure 3. Monthly total nitrogen (blue bars) and ammonia (red line) concentrations in surface waters during Year 4 monitoring. The horizontal red and black dashed lines indicate ERS water quality guideline values for Total Nitrogen (1.05mg/L) and Ammonia (0.9mg/L) respectively. The Kyneton WRP discharge is between Sites 6 and 7.

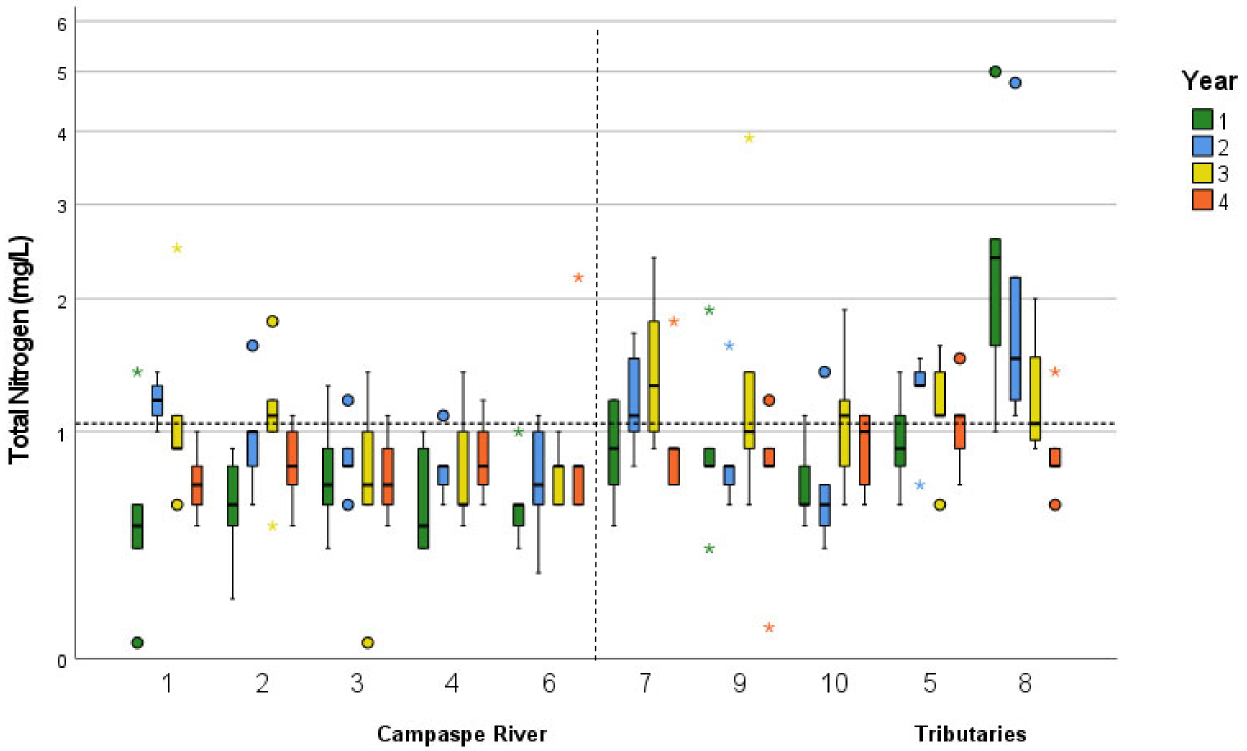

Figure 4. Total nitrogen concentrations in surface waters during Years 1 to 4 monitoring. The vertical dashed line indicates Kyneton WRP discharge between Sites 6 and 7. The horizontal dotted line indicates ERS water quality guideline value (1.05 mg/L). N = 5.

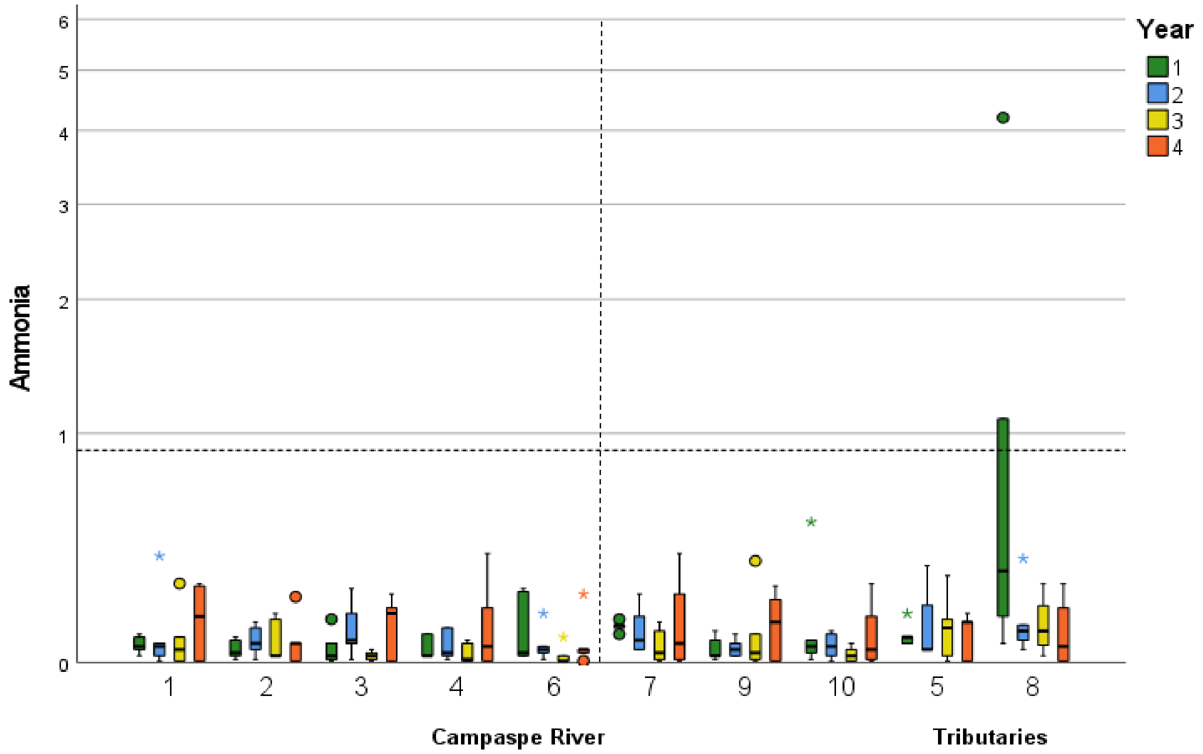

Figure 5. Ammonia concentrations in surface waters during Years 1 to 4 monitoring. The vertical dashed line indicates Kyneton WRP discharge between Sites 6 and 7. The horizontal dotted line indicates ERS water quality guideline value (0.9 mg/L). N = 5.

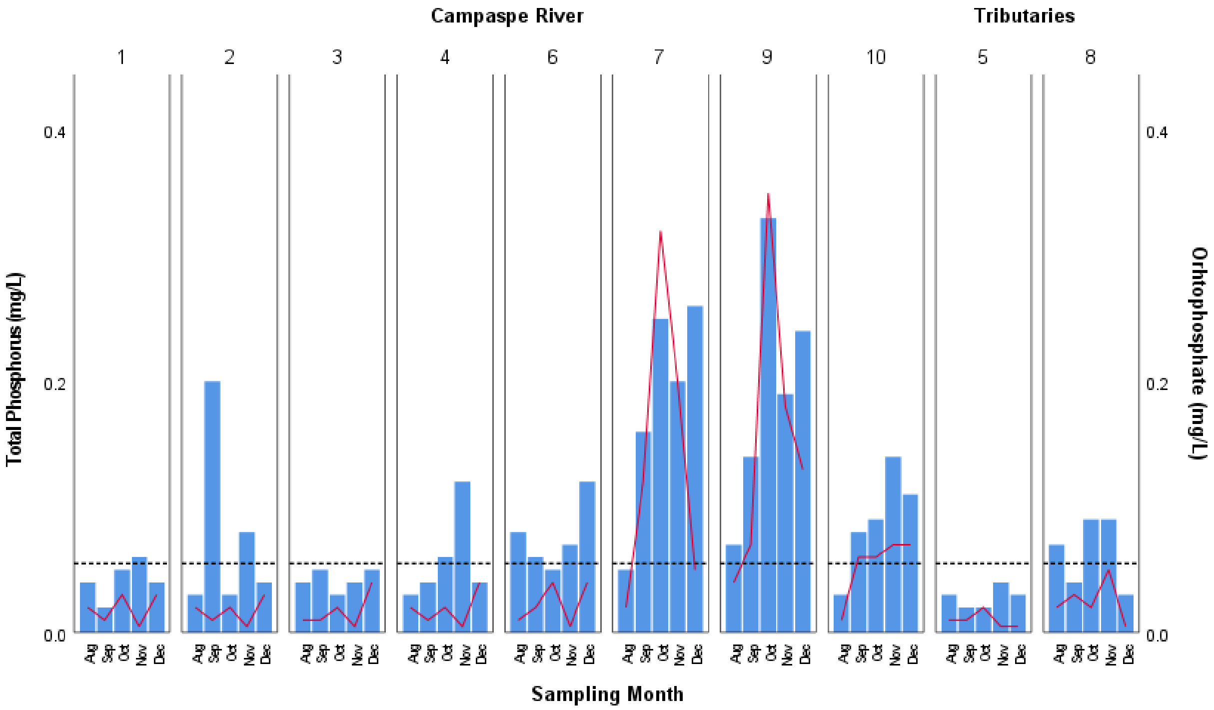

Figure 6. Monthly total phosphorous (blue bars) and orthophosphate (red line) concentrations in surface waters during Year 4 of monitoring. The horizontal black dashed line indicates ERS water quality guideline value for Total Phosphorus (0.055mg/L). The Kyneton WRP discharge is between Sites 6 and 7.

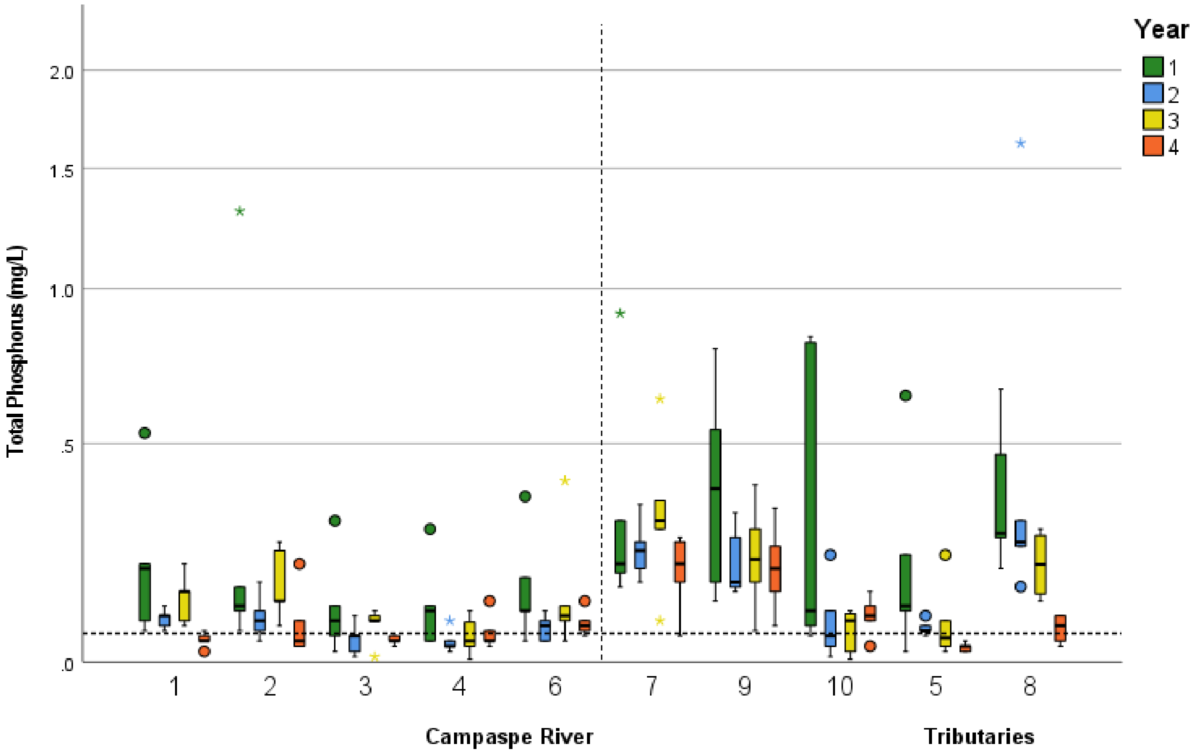

Figure 7. Total phosphorus concentrations in surface waters during Years 1 to 4 of monitoring. The vertical dotted line indicates Kyneton WRP discharge between Sites 6 and 7. The horizontal dotted line for indicates ERS water quality guideline value (0.055 mg/L). N = 5.

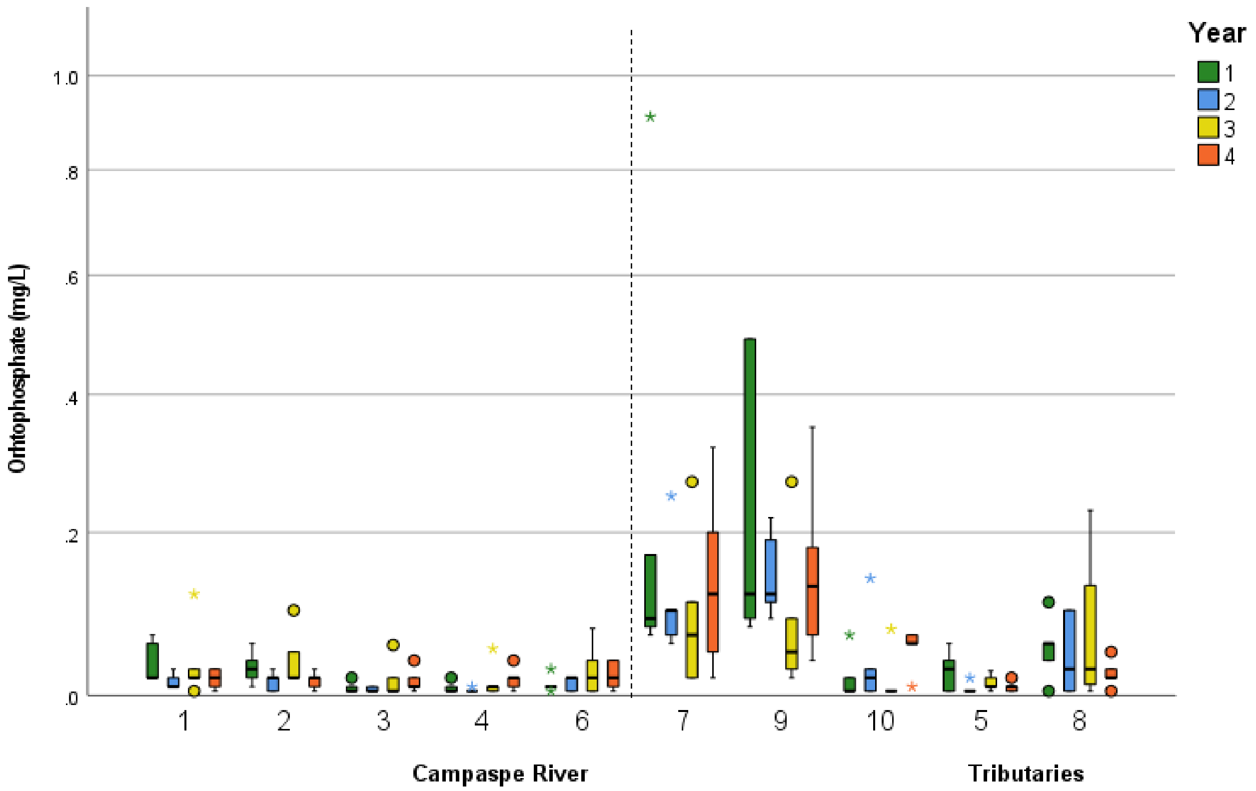

Figure 8. Orthophosphate concentrations in surface waters during Years 1 to 4 of monitoring. The vertical dotted line indicates Kyneton WRP discharge between Sites 6 and 7. N = 5.

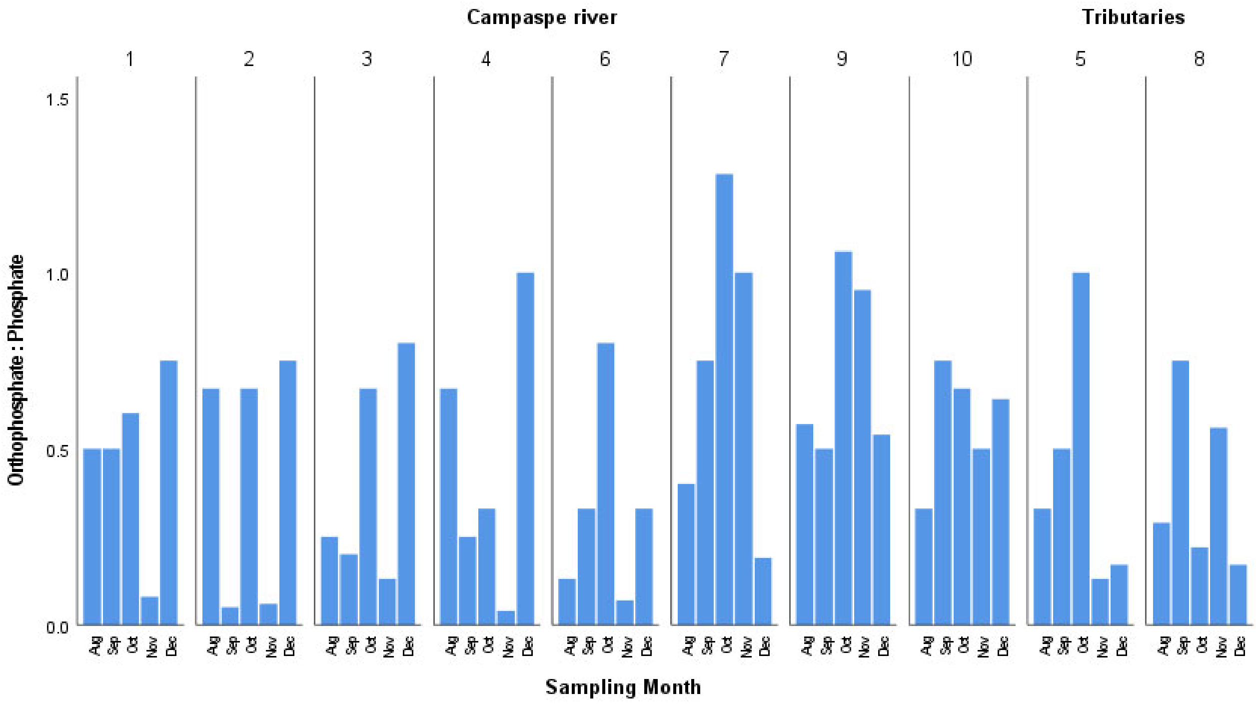

Figure 9. Ratio of orthophosphate to total phosphorus in surface waters at study sites from August 2021 to December 2021. The Kyneton WRP discharge is between Sites 6 and 7.

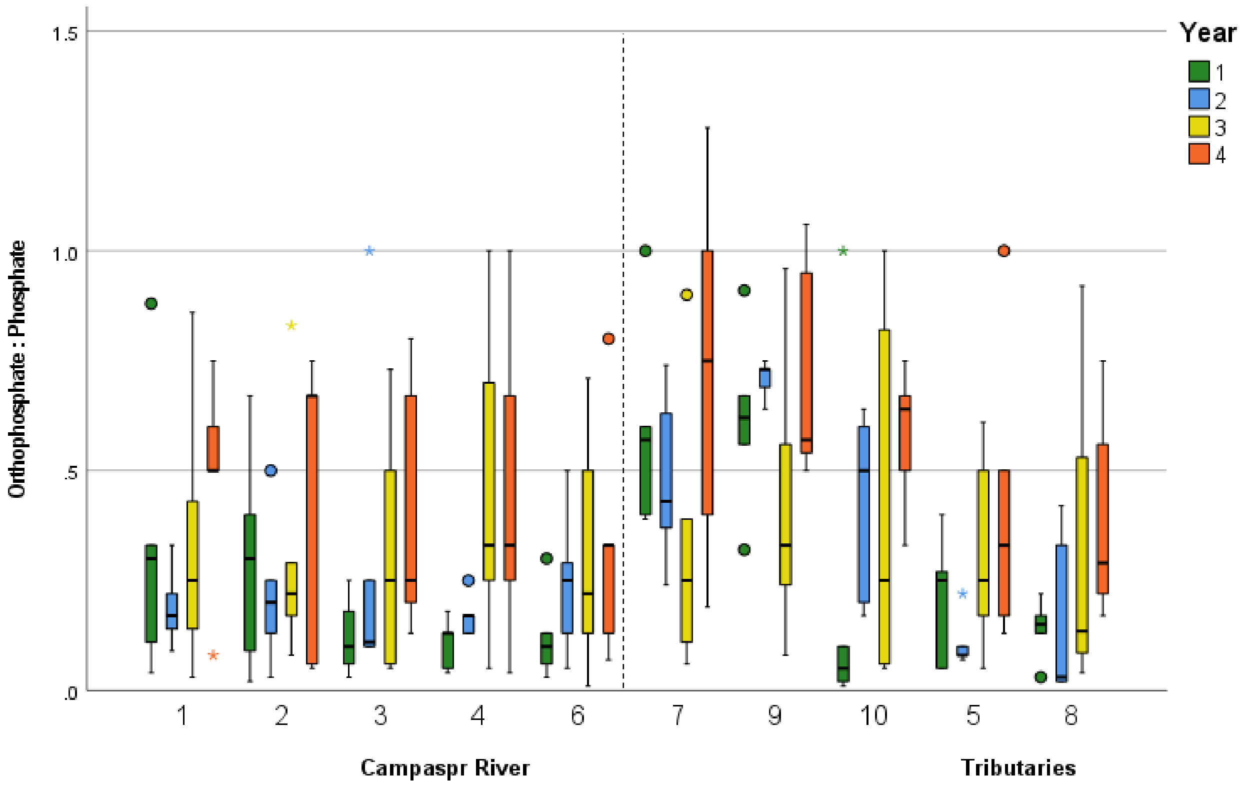

Figure 10. Ratio of orthophosphate to phosphorous in years 1 to 4 of monitoring. The vertical dotted line indicates Kyneton WRP discharge between Sites 6 and 7.

Faecal Monitoring

Escherichia coli (*E. coli*) levels in Year 4 were mostly below the ERS trigger values for a Class A/B rating (<260 organisms/100mL), indicating the water quality was suitable for primary contact and secondary recreation, and suitable for livestock drinking and application to pasture (with conditions)[1](Figure 11). The exception to this was at Campaspe Site 7 and tributary Site 8 in November 2021, which exceeded 260 organisms per 100 mL and would be considered equivalent quality to Class C recycled water (not suitable for primary contact or livestock drinking; suitable for secondary contact recreation; 261-550 organisms/100mL) (Figure 11).

Mean *E. coli* levels fluctuated at Campaspe Sites 4, 6 and 7 across years 1 to 4 of monitoring (Figure 12). While at Campaspe Site 2 there has been a decline annually in *E. coli* levels (Figure 12). Tributary sites 5 and 8 and Campaspe Site 9 have tended to show increases in *E. coli* levels annually (Figure 12). Campaspe Sites 4 and 7 and Tributary site 8 have exceeded the ERS guidelines for Class A/B water in one or more years, while Campaspe Site 7 and tributary Site 8 have also exceeded ERS guidelines for Class C water (Figure 12).

Analysis for the presence of *Bacteroides* was used to determine the source of faecal contamination at sites during November 2020 and January 2021 (Table 5). Three *Bacteroides* markers were applied, two, HF183 and Lachno3, are indicators of human faecal contamination, while the third marker is an indicator of ruminant faecal contamination (cattle, sheep). Faecal contamination of human origin based on the detection of either the HF183 marker gene, Lachno3 marker gene, or both marker genes, was observed at Campaspe Sites 6, 7, and 9 and tributary Site 5, in November 2020, and at Campaspe Sites 2, 6 and 9, and tributary Site 5, in January 2021 (Table 5). The concentrations of the HF183 and Lachno3 marker genes were above the

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[1] ERS objectives are a guideline only, as required sample numbers for comparison not met.

gastrointestinal (GI) risk benchmark of 4.50 $\log_{10}$ GC/L (HF183) and 5.14 log10 GC/L (Ahmed *et al.* 2019; Zhang *et al.* 2019) at all sites where they were detected and quantifiable (Table 5).

The ruminant marker, BacR, was detected at four sites in November 2020, Campaspe Sites 6, 7 and 9, and tributary Site 8, and was quantifiable at three sites (Campaspe Sites 7 and 9, and tributary Site 8) (Table 5). During January 2021, BacR was detected at three sites (Campaspe Sites 6, 7 and 9) and quantifiable at two sites (Campaspe Sites 6 and 7) (Table 5).

Faecal contamination of human origin has been detected annually at Campaspe Site 7 and tributary Site 5 (Table 5), while at Campaspe Sites 2, 6 and 9, and tributary Site 8, human faecal contamination has been detected in at least two years of monitoring (Table 5). Stock faecal contamination has been detected annually at tributary Site 8, while in multiple years at Campaspe Sites 6, 7 and 9 (Table 5). At Campaspe Sites 2 and 4 stock contamination was detected during Year 2 monitoring (Table 5).

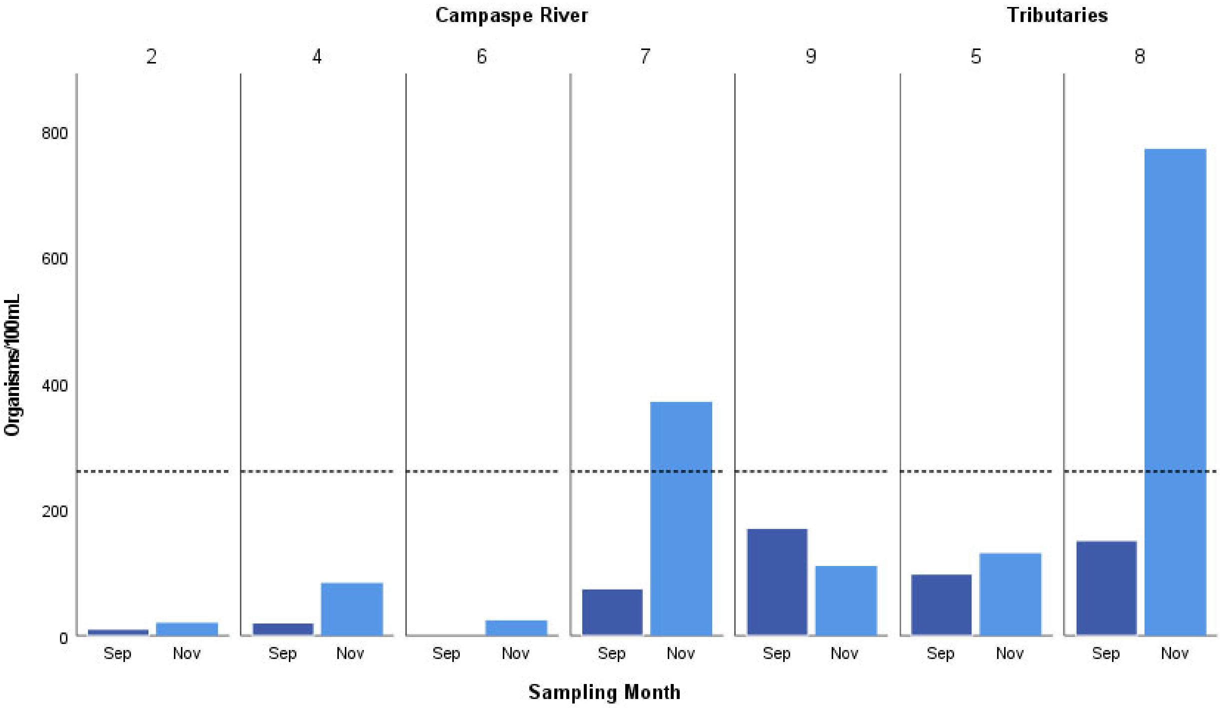


Figure 11: ***E. coli*** **measured at select sites during year 4 monitoring. The horizontal dashed line indicates ERS guideline value for Class A/B water (<260 organisms/100mL). The Kyneton WRP discharge point is between Sites 6 and 7.**

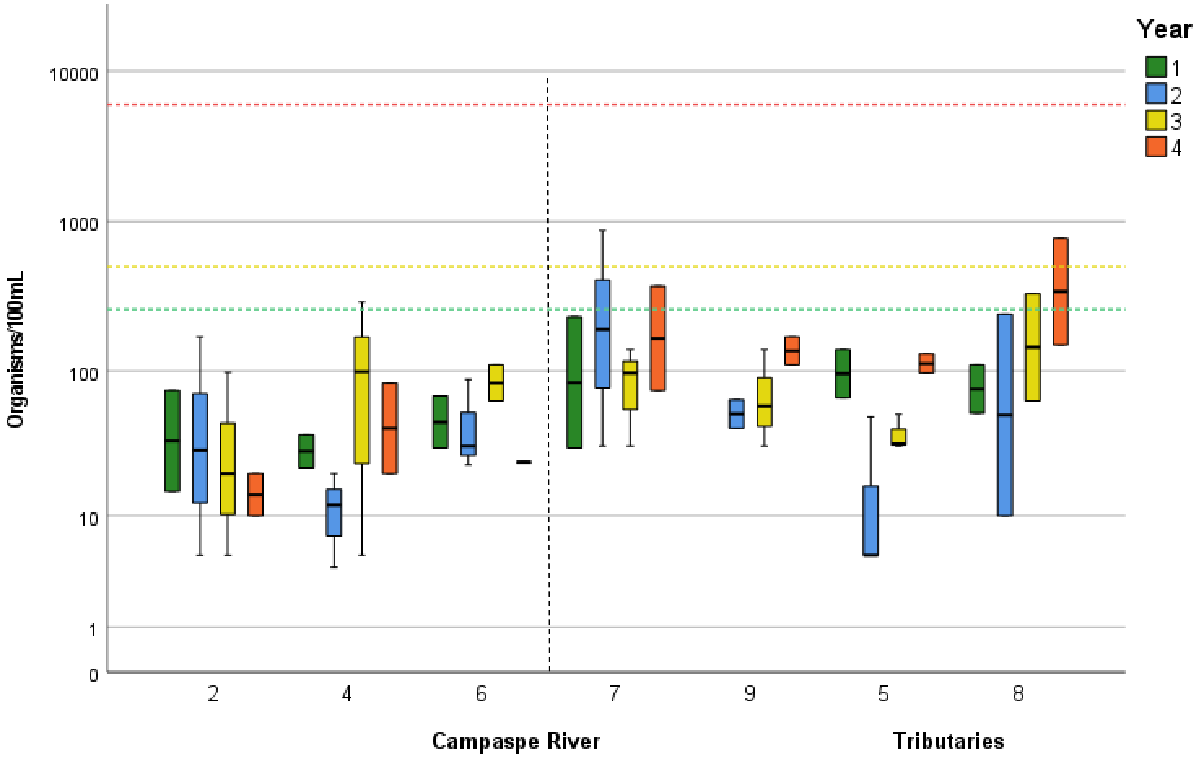


Figure 12. Mean *E. coli* concentrations during years 1, 2, 3 and 4 of monitoring. The green, yellow and red horizontal dashed lines indicate ERS guideline values for Class A/B, Class C, and Class D water, respectively. The black vertical dashed line indicates Kyneton WRP discharge point between Sites 6 and 7.

Table 5. Concentrations (gene copies/L) of human and ruminant associated microbial source tracking MST marker genes in water samples collected between December 2018 and January 2021.

Year	Date	Site		Human markers (GC/L)		Ruminant marker (GC/L)
				HF183	Lachno3	BacR
1	Dec-18	Campaspe River	2	ND	ND	ND
			4	ND	ND	ND
			6	ND	ND	ND
			7	ND	ND	ND
		Tributary	5	ND	ND	ND
			8	ND	4.64 ± 0.11	ND
	Aug-19	Campaspe River	2	ND	ND	ND
			4	ND	ND	ND
			6	ND	ND	ND
			7	**4.86 ± 0.02**	ND	ND
		Tributary	5	ND	+	ND
			8	ND	3.55 ± 0.24	+
2	Sep-19	Campaspe River	2	3.87 ± 0.31	**5.76 ± 0.04**	**6.21 ± 0.04**
			4	3.96 ± 0.17	**5.62 ± 0.04**	3.24 ± 0.17
			6	**5.18 ± 0.01**	**6.19 ± 0.01**	ND
			9	4.04 ± 0.11	5.49 ± 0.01	**5.26 ± 0.02**
		Tributary	5	4.18 ± 0.10	5.83 ± 0.02	**ND**
			8	3.60 ± 0.09	**5.62 ± 0.03**	3.24 ± 0.14
	Nov-19	Campaspe River	2	3.46 ± 0.40	**6.17 ± 0.03**	ND
			4	4.23 ± 0.07	4.93 ± 0.02	ND
			6	ND	ND	ND
			7	+	ND	3.72 ± 0.13
		Tributary	5	+	3.70 ± 0.10	**ND**
			8	+	4.95 ± 0.12	ND
3	Nov-20	Campaspe River	2	ND	ND	ND
			4	ND	ND	ND
			6	**72.23 ± 18.15**	**33.93 ± 20.95**	+
			7	**261.92 ± 86.22**	**315.19 ± 76.28**	**26.97 ± 5.77**
			9	**23.42 ± 7.39**	+	**182.69 ± 32.55**
		Tributary	5	**15.92 ± 1.87**	ND	ND
			8	ND	ND	**305.97 ± 8.42**
	Jan-21	Campaspe River	2	**8.97 ± 2.59**	+	ND
			4	ND	ND	ND
			6	**465.02 ± 59.66**	**217.18 ± 50.54**	0.97 ± 0.97
			7	**ND**	ND	**455.74 ± 181.20**
			9	**ND**	**103.61 ± 52.44**	+
		Tributary	5	**9.48 ± 2.71**	+	ND

Bold indicates values that exceed the GI risk benchmark of 4.50 $\log_{10}$ GC/L (HF183) and 5.14 $\log_{10}$ GC/L (BacR) (Ahmed et al. 2019)
ND = not detected
+ = detected but not quantifiable

Passive Samplers – Water toxicants

Concentrations of pharmaceuticals, personal care products (PPCP) and pesticides detected in surface waters are shown in Tables 6 and 7. Seven PPCPs and ten pesticides were detected in Year 4 monitoring. This included the pharmaceuticals carbamazepine at all sites, excluding tributary Site 5; venlafaxine at six Campaspe sites; sulphapyridine and sulfamethoxazole at Campaspe Sites 3 and 2, respectively; paracetamol at Campaspe Site 7 and tributary site 8; and trimethoprim and erythromycin at Campaspe Site 7 (Table 6). Over the 4 years of sampling there have been nine different PPCPs detected within the study area (Table 6), those detected in previous years, but not detected in Year 4 include oleandomycin and cholestrol (Table 6).

Of the pesticides detected in Year 4, five were herbicides, four fungicides and one an insecticide (Table 8). The herbicide atrazine and insecticide imidacloprid were detected at all sites in Year 4, while the herbicide hexazinone was detected for the first time at all sites, except for the sites in the two tributaries. The fungicide carbendazim was also detected at all sites, excluding Campaspe Site 10 and tributary Site 8. Simazine, an herbicide, was detected at seven sites, while ametryn was detected for the first time at three sites. The herbicide diuron and fungicide thiabendazole (first time detected) were detected at two sites each, while tebuconazole and cypronil (first time detected), both fungicides, were each detected at a single site (Table 7). Fifteen pesticides have been detected during the 4 years of monitoring. In previous years several other herbicides (MCPA, triclopyr and 2,4-D) and the insecticides carbaryl and propiconazole have been detected in surface waters (Table 7).

Sediment Chemistry

Concentrations of heavy metals, petroleum hydrocarbons and pesticides found in the sediments of sites are presented in Table 8. ANZECC/ARMCANZ Sediment Quality Guidelines (2000) were exceeded for chromium, lead, nickel, zinc and total petroleum hydrocarbons (TPH) during Year 4 monitoring.

Nickel exceeded the guideline value at all sites in Year 4, which is consistent with previous years monitoring (Table 8), while lead, zinc and chromium each exceed at a single site (tributary Site 5 for lead and zinc, and Campaspe Site 3 for chromium) during Year 4 (Table 8). Concentrations of lead and zinc in sediments collected from tributary Site 5 have consistently exceeded the guideline value over the four years of monitoring. Chromium concentrations at Campaspe Site 3 were generally stable over Years 1-3, however increased to exceed the guideline value in Year 4 (Table 8).

Petroleum hydrocarbons were above ANZECC/ARMCANZ Sediment Quality Guidelines (2000) at all sites, except Campaspe Sites 4 and 10, in Year 4 (Table 8). While concentrations have consistently exceeded guideline values across all monitoring years, overall, concentrations were generally lower in Year 4 compared to previous years, except for Campaspe Site 6 (Table 8).

In Year 4 monitoring, four pesticides were detected in sediments, being propiconazole, tebuconazole, bifenthrin and permethrin (Table 8). All pesticides were detected at tributary Site 5. There were no pesticides detected in sediments during Years 1 and 3, however, two insecticides, bifenthrin and permethrin, were detected at tributary site 5 in Year 2 (Table 8).

Table 6. Pharmaceuticals and personal care products detected in surface waters using POCIS passive samplers (µg/0.2 g sorbent) across sites for Year 1 to Year 4.

Site Number	Pharmaceuticals and Personal Care Products (µg/L)																
	Venlafaxine			Oleandomycin	Sulphapyridine		Sulfamethoxazole		Trimethoprim		Paracetamol		Carbamazepine			Cholestrol	Erythromycin
	Yr1	Yr3	Yr4	Yr3	Yr3	Yr4	Yr3	Yr4	Yr3	Yr4	Yr3	Yr4	Yr2	Yr3	Yr4	Yr2	Yr4
Campaspe River																	
1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	1.67	2.2	3.8	<10	<1
2	<1	<1	1.0	<1	<1	<1	<1	<1	<1	<1	<1	<1	2.38	4.9	2.3	<10	<1
3	<1	<1	1.0	<1	<1	<1	<1	<1	<1	<1	<1	<1	1.71	3.3	2.3	<10	<1
4	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<0.5	3.0	1.7	<10	<1
6	<1	<1	1.0	2.8	<1	<1	<1	<1	<1	<1	<1	<1	<0.5	5.4	1.7	19.8	<1
Kyneton WRP discharge between sites 6 and 7																	
7	<1	40.0	37.5	21.5	34.0	10.0	4.8	2.25	9.45	4.65	4.15	<1	38.33	43.0	19.0	84.6	1.25
9	4.6	16.0	24.0	9.1	13.0	6.8	1.4	1.4	<1	<1	<1	1.2	14.25	46.0	15.0	<10	<1
10	<1	1.1	5	1.1	<1	1.0	<1	<1	<1	<1	<1	<1	4.42	9.0	6.4	<10	<1
Tributaries																	
5	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	7.7	<1	<0.5	<1	<1	<10	<1
8	3.7	<1	<1	<1	<1	<1	<1	<1	<1	<1	1.4	2.2	<0.5	2.9	1.0	<10	<1

Table 7. Pesticides detected in surface waters using POCIS passive samplers (µg/0.2 g sorbent) across sites for Years 1 to 4.

Site Number	Herbicides														Insecticides				Fungicides						
	Simazine			Atrazine		Diuron			MCPA	Triclopyr		2,4-D	Ametryn	Hexazinone	Imidacloprid			Carbaryl	Propiconazole	Tebuconazole		Carbendazim		Thiabendazole	Cyprodinil
	Yr1	Yr3	Yr4	Yr1	Yr4	Yr1	Yr3	Yr4	Yr1	Yr1	Yr2	Yr2	Yr4	Yr4	Yr1	Yr3	Yr4	Yr1	Yr3	Yr3	Yr4	Yr3	Yr4	Yr4	Yr4
Campaspe River																									
1	<1	12.0	21.0	<1	4.0	<1	<1	<1	<1	8.6	16.8	<5	<1	11.0	1.3	<1	1.0	<1	<1	<1	<1	<1	4.0	<1	1.0
2	<1	18.0	12.0	<1	3.0	<1	<1	<1	<1	9.5	6.8	<5	1.0	9.0	0.8	<1	1.0	<1	<1	<1	<1	<1	2.0	<1	<1
3	<1	22.0	<1	<1	4.0	<1	<1	<1	<1	8.0	14.2	9.6	<1	19.0	1.1	<1	1.0	<1	<1	<1	<1	<1	2.0	<1	<1
4	1.8	58.0	3.0	<1	3.0	<1	<1	<1	<1	7.3	9.3	11.0	1.0	13.0	<1	<1	1.0	1.8	<1	<1	<1	<1	2.0	<1	<1
6	1.8	56.0	<1	<1	5.0	1.8	<1	29.0	12.6	11.2	28.9	8.4	<1	25.0	1.3	3.8	1.0	<1	<1	<1	<1	<1	2.0	<1	<1
Kyneton WRP discharge between sites 6 and 7																									
7	<1	42.0	2.0	<1	2.0	1.7	3.2	<1	<1	7.5	14.5	<5	<1	12.5	3.2	6.4	1.0	1.6	<1	<1	<1	2.3	2.0	2.0	<1
9	<1	22.0	<1	<1	3.0	1.9	1.0	<1	7.5	7.5	17.8	<5	<1	22.0	6.0	4.5	2.0	<1	<1	<1	<1	1.2	2.0	2.0	<1
10	3.5	18.0	5.0	1.5	5.0	1.7	<1	<1	<1	6.1	<2.5	<5	<1	25.0	1.9	2.2	1.0	3.5	<1	<1	<1	<1	<1	<1	<1
Tributaries																									
5	1.7	58.0	7.0	<1	5.0	4.8	4.6	14.0	<1	5.7	86.7	44.4	1.0	<1	3.2	7.6	1.0	1.7	4.9	4.7	5	2.9	2.0	<1	<1
8	<1	<1	1.0	<1	1.0	1.7	<1	<1	<1	15.1	10.3	6.7	<1	<1	<1	<1	1.0	<1	<1	<1	<1	<1	<1	<1	<1

Table 8. Heavy metals, petroleum hydrocarbons, and pesticides detected in sediments across sites from Years 1 to 3.

Site Number	Heavy metals (mg/kg)																			
	Arsenic				Barium				Beryllium				Cadmium				Chromium			
	Yr1	Yr2	Yr3	Yr4	Yr1	Yr2	Yr3	Yr4	Yr1	Yr2	Yr3	Yr4	Yr1	Yr2	Yr3	Yr4	Yr1	Yr2	Yr3	Yr4
Campaspe River																				
1				5		170	150	210		1	1	1						40	53	44
2						180	200	210		1	1	1						45	49	63
3	5				210	180	210	170	1	1	1	1					73	69	75	**92**
4	6	7		5	200	250	260	250	1	1	1	1					62	59	71	67
6			6		170	170	160	200	1	1	1	1					64	66	73	63
Kyneton WRP discharge between Sites 6 and 7																				
7					150	190	230	190		1	1						19	65	69	65
9	11	16	5	8	220	280	320	210	1	1	1	1					17	66	**87**	70
10	11	8		6	190	180	160	160	1	1	1	1					31	41	37	40
Tributaries																				
5	10	11	11	12	260	280	310	340	1			1					56	55	57	60
8		19			310	840	240	240	1		1	1		1			54	37	66	53
Trigger Value*	20				-				-				1.5				80			

* Trigger value from ANZECC/ARMCANZ Sediment Quality Guidelines (2000)

Values **bold** and highlighted in orange exceed trigger value

Site Number	Heavy metals (mg/kg)																			
	Cobalt				Copper				Lead				Manganese				Mercury			
	Yr1	Yr2	Yr3	Yr4	Yr1	Yr2	Yr3	Yr4	Yr1	Yr2	Yr3	Yr4	Yr1	Yr2	Yr3	Yr4	Yr1	Yr2	Yr3	Yr4
Campaspe River																				
1		10	11	19		16	16	15		18	17	18		249	148	708				
2		18	12	22		30	20	17		37	23	21		460	155	728				
3	26	20	26	26	28	27	30	26	19	19	28	21	777	470	498	548				
4	28	35	24	29	29	27	28	22	35	29	29	26	986	1980	767	972				
6	25	27	23	22	34	37	39	25	20	22	31	22	402	508	114	713			0.1	
Kyneton WRP discharge between Sites 6 and 7																				
7	12	14	19	14	18	21	18	22	19	22	19	20	252	192	302	234				
9	31	42	18	27	25	27	29	25	17	28	20	31	1460	1990	1550	1040	**0.7**	**1.2**	**0.2**	
10	19	16	28	17	23	21	18	16	31	25	18	13	674	479	253	560				
Tributaries																				
5	24	25	42	26	51	53	48	55	**87**	**83**	**86**	**81**	451	427	548	554	0.1	0.1	**0.2**	0.1
8	43	206	13	20	24	17	19	19	12	8	12	12	1300	13400	554	231				
Trigger Value*	-				65				50				-				0.15			

[*] Trigger value from ANZECC/ARMCANZ Sediment Quality Guidelines (2000)

Values **bold** and highlighted in orange exceed trigger value

Site Number	Heavy metals (mg/kg)												Petroleum Hydrocarbons (mg/kg)				Pesticides (mg/kg)					
	Nickel				Vanadium				Zinc				C10 - C36 Fraction (sum)				Bifenthrin		Permethrin		Propiconazole	Tebuconazole
	Yr1	Yr2	Yr3	Yr4	Yr1	Yr2	Yr3	Yr4	Yr1	Yr2	Yr3	Yr4	Yr1	Yr2	Yr3	Yr4	Yr2	Yr4	Yr2	Yr4	Yr4	Yr4
Campaspe River																						
1		**23**	**26**	**26**		30	42	36		100	73	109		**2190**	**540**	**520**						
2		**27**	**28**	**31**		38	39	46		135	86	90		**1300**	**970**	**670**						
3	**48**	**44**	**54**	**51**	62	54	72	69	171	146	124	97	**770**	**1020**	**830**	**510**						
4	**46**	**43**	**45**	**40**	55	55	57	54	183	166	170	147	**550**	**1270**	**1120**	130						
6	**38**	**38**	**40**	**33**	53	57	56	48	173	169	172	149	**690**	**1480**	**1360**	**1790**						
Kyneton WRP discharge between Sites 6 and 7																						
7	**24**	**28**	**30**	**30**	39	46	50	41	120	132	125	150	210	**560**	**420**	**350**						
9	**48**	**48**	**64**	**43**	61	61	80	53	81	86	102	101	**330**	**1010**	**670**	**620**						
10	**36**	**33**	**28**	**30**	40	38	31	34	111	96	70	77	**490**	**3060**	**420**	140						
Tributaries																						
5	**44**	**45**	**42**	**48**	50	49	53	55	**849**	**836**	**722**	**817**	**960**	**2050**	**1900**	**590**	0.016	0.011	0.011	0.02	0.01	0.01
8	**35**	**45**	**36**	**31**	67	67	68	57	95	65	78	89	210	**3730**	**600**	**580**						
Trigger Value*	21				-				200				280				-		-		-	-

* Trigger value from ANZECC/ARMCANZ Sediment Quality Guidelines (2000)

Values in **bold** and highlighted in orange exceed trigger value

Aquatic Ecology

Macroinvertebrate Survey

Macroinvertebrate indices (SIGNAL2, number of families and ETP families) are indicators of waterway condition and are used to assess if waterways meet the biological standards outlined in the Environmental Reference Standards (ERS) objectives. SIGNAL2 scores were below the ERS objectives in Year 4 for all sites, except Site 9 (which had a score of 4.09) (Table 9). Sites 6 and 7 along the Campaspe River and tributary Site 5 have consistently had SIGNAL2 scores below 3.4 across the last four years of monitoring. SIGNAL2 scores at Campaspe Sites 4 (3.18) and 10 (3.20) have been variable and show a reduction from the first and second years of monitoring despite supporting a high number of families. There is no clear spatial or temporal trend in SIGNAL2 scores across the greater study area.

The number of families at Campaspe Sites 1, 3 and 10, and tributary Site 5, were below the ERS objectives, with Site 5 consistently below the ERS objectives over the four years of monitoring (Table 9). The number of families was lowest at tributary Site 8 (16 families) and at the Campaspe Site 1 (16 families) and greatest at Campaspe Sites 2 (23 families), 4 (24 families) and 9 (23 families). The number of families present at tributary Site 8 (19-20 families) and in the Campaspe River at Sites 6 (22-27 families) and 9 (23-24 families) have been consistently improving over the last few years. Also in the Campaspe River, Site 1 has decreased this year compared to previous years, Sites 2, 3, 7 and 10 have been variable throughout the years, and Site 4 has had a consistently high numbers of families across the 4 years.

The total number of families in the insect orders Ephemeroptera (mayflies), Plecoptera (stoneflies) and Trichoptera (caddisflies) (EPT) are used to indicate signs of aquatic pollution and poor habitat. Although there are no ERS objectives for EPT taxa in edge habitats, the number of EPT families has been calculated for general guidance. EPT family diversity was greatest at Campaspe Sites 4, 6 and 9 (5-6 families). In contrast, only 1 EPT taxa was found at Campaspe Site 1 and tributary Site 5. Improvements to the number of EPT taxa at tributary Site 8 has occurred over the last two years (Year 3 and 4) and in Year 4 at Site 7 (Table 9).

All sites showed variation in the taxonomic composition of macroinvertebrate assemblages across the four years (Figure 13). The degree of change was marginal for most sites, except for Sites 8 and 5, the tributaries, which showed a moderate change in species composition (Figure 13). A moderate change was also seen at Campaspe Site 10 across the years. Overall, there is a separation between Years, with Years 1 and 2, and Years 3 and 4 are more like each other.

The most abundant taxa varied between sites and years (Figure 14). Taxa found at most sites across all years include Physidae (bladder snails), Oligochaeta (worms), Dytiscidae larvae (diving beetles), Orthocladiinae (non-biting midge Chironomidae), Micronectiidae (true bugs) and Chiltoniidae (sideswimmers) (Figure 14 a-f). Like Year 3, in Year 4, Leptoceridae (cased caddisflies), Coenagrioniidae (damselflies) and Chiltoniidae (sideswimmers) were more abundant across most sites compared to the previous years (Figure 14 f, g, l). In tributary Site 8, several of the most common taxa were recorded for the first time in Year 3, including Chiltoniiidae, Leptophlebiidae (mayflys) and Physidae (bladder snails), and they have continued to be found in Year 4 (Figure 14 a, f, h). Leptoceridae (cased caddisflies) have been found for the first time at Campaspe Site 7 and continue to be present at Site 9 from Year 3 (Figure 14 g). Chironominae (non-biting midge Chironomidae) were present at most sites across all years, except Year 2, while Gripopterygidae (stoneflies) were abundant at most sites in Year 2 only, and generally absent or low in abundance in Years 1, 3 and 4 (Figure 14 i, k). Atyidae (shrimp) were present across most sites and found for the first time in Year 4 at the Campaspe Sites 1 and 2 and in the tributary Site 8 (Figure 14 j).

Table 9. Macroinvertebrate biological indices at each site sampled during Years 1 to 4.

	SIGNAL2				Number of Families				Number of ETP Families			
Site	**2018**	**2019**	**2020**	**2021**	**2018**	**2019**	**2020**	**2021**	**2018**	**2019**	**2020**	**2021**
Campaspe River												
1	**3**	**3.17**	N/A	2.8	20	**19**	N/A	16	2	2	N/A	1
2	3.4	3.8	N/A	3.09	20	**15**	N/A	23	3	3	N/A	2
3	4.05	3.73	N/A	3.11	20	27	N/A	19	6	5	N/A	4
4	3.54	3.77	**3.18**	3.27	24	24	23	24	6	5	5	5
6	**3.13**	**3.38**	**3.18**	**3.95**	**15**	23	27	22	2	4	5	6
Kyneton WRP discharge between sites 6 and 7												
7	**2.94**	**3.31**	**2.67**	**2.55**	**18**	**16**	**12**	20	0	1	0	3
9	3.6	**3.23**	3.43	**4.09**	**16**	**14**	24	23	3	1	4	5
10	3.6	4.8	**3.2**	**3.89**	**19**	**10**	20	19	5	3	5	2
Tributaries												
5	**3.13**	**2.85**	**2.87**	**2.73**	**16**	**13**	**16**	16	3	1	0	1
8	**2.89**	3.67	**3.26**	**3.16**	**10**	**12**	**19**	20	1	1	3	4
ERS Objectives^												
Edge	3.4				20				N/A			

^ Environmental Reference Standards Objectives are used as a guideline only as only one season was sampled (Spring)
Values presented in orange and **bold** are below State Environment Protection Policy (Waters) biological objectives
Samples were not assessed for sites 1, 2 and 3 in Year 3 of monitoring as samples were damaged.

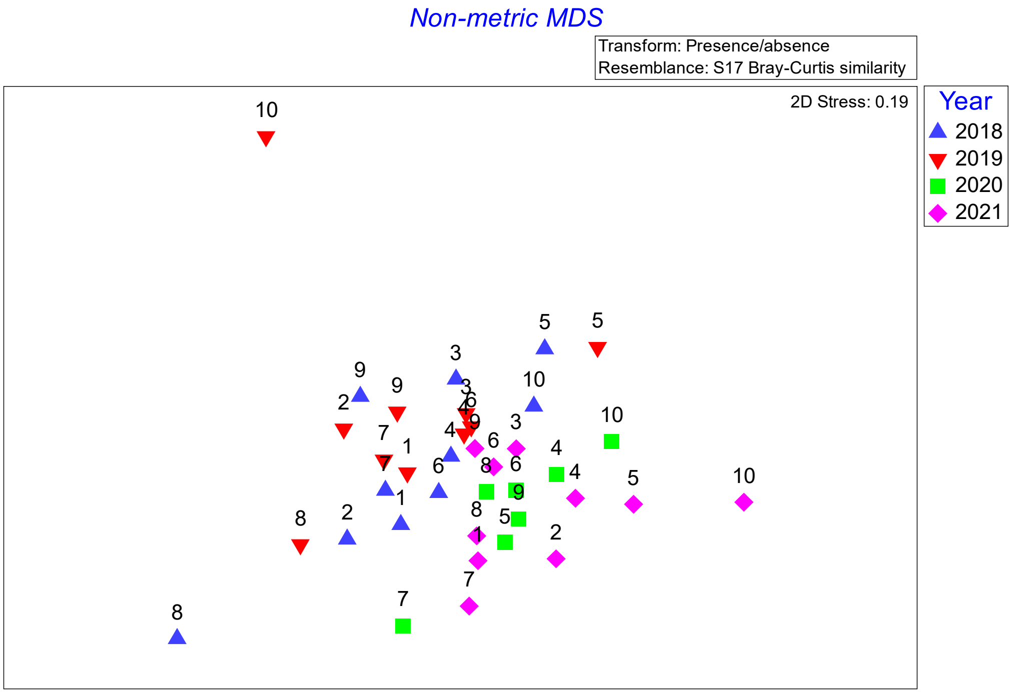
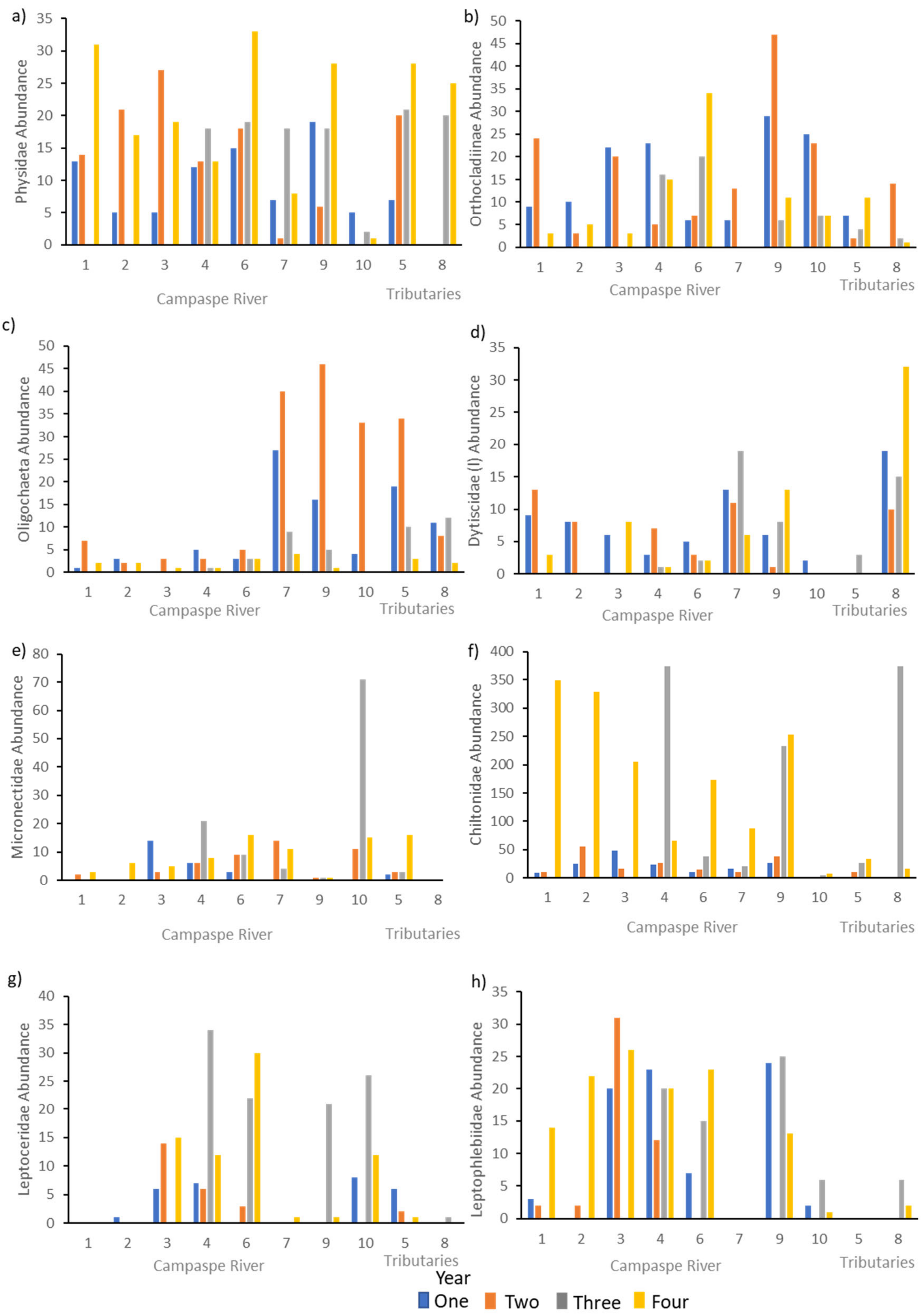


Figure 13. Non-metric multidimensional scaling ordination based on the presence or absence of macroinvertebrate taxa and the Bray-Curtis dissimilarity index. Points close together are similar in composition whereas points further apart have greater variation in their taxonomic composition.


a)
Physidae Abundance
0
5
10
15
20
25
30
35
1
2
3
4
6
7
9
10
5
8
Campaspe River
Tributaries
b)
Orthocladiinae Abundance
0
5
10
15
20
25
30
35
40
45
50
c)
Oligochaeta Abundance
d)
Dytiscidae (l) Abundance
e)
Micronectidae Abundance
0
10
20
30
40
50
60
70
80
f)
Chiltonidae Abundance
0
50
100
150
200
250
300
350
400
g)
Leptoceridae Abundance
h)
Leptophlebiidae Abundance
Year
One
Two
Three
Four

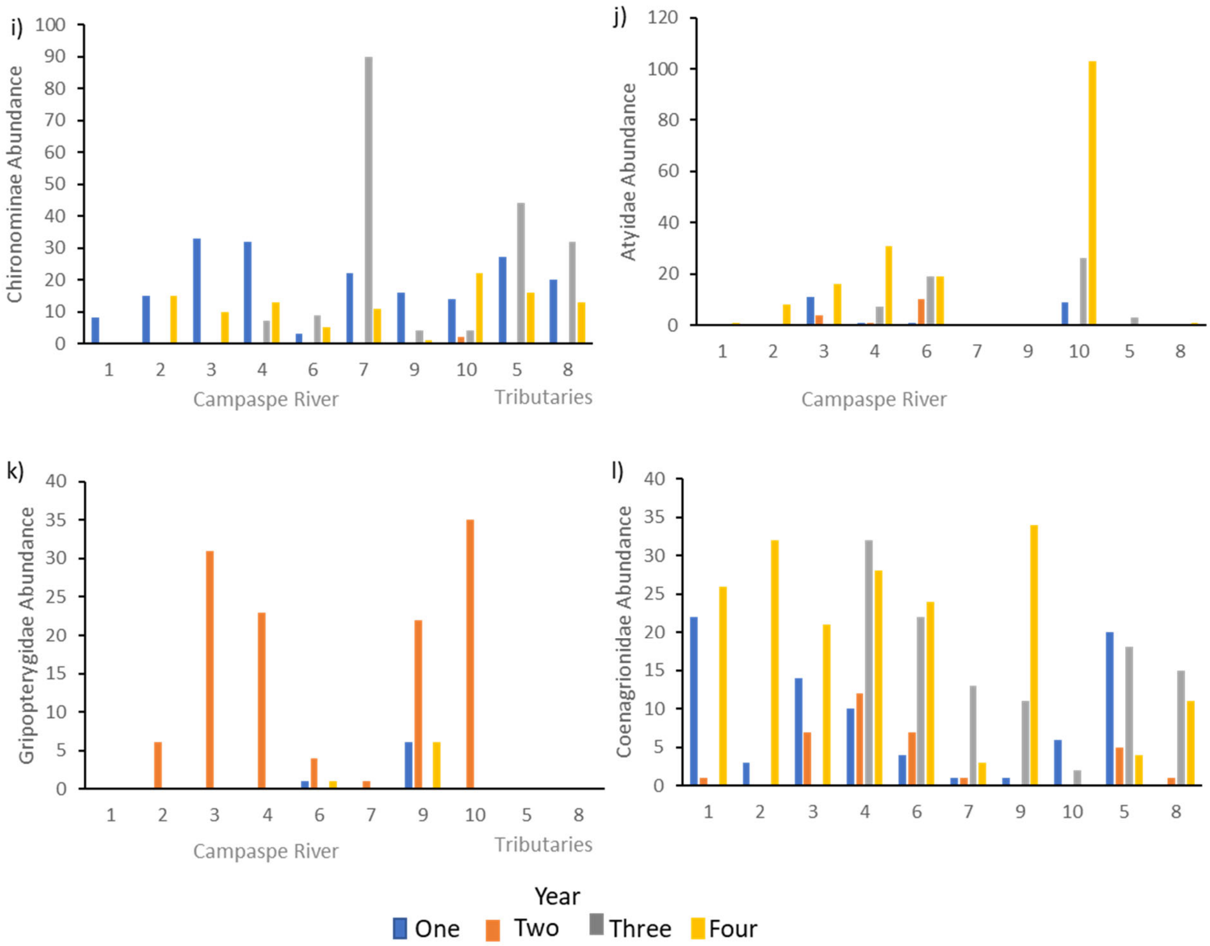


Figure 14: Abundance of common macroinvertebrate taxa at sites sampled during Years 1 to 4. a) Physidae b) Orthocladiinae c) Oligochaeta d) Dytiscdae larvae e) Micronectidae f) Chiltoniidae g) Leptoceridae, h) Leptophlebiidae, i) Chironominae, j) Atyidae, k) Gripopetergydae, l) Coenagrionidae. Samples were not assessed for Sites 1, 2 and 3 in Year 3 of monitoring as samples were damaged.

Benthic Algal Production

Benthic algal production, measured as chlorophyll-a and ash free dry mass (AFDM), were assessed in October and December 2021 (Figures 15 and 17). Chlorophyll-a, an indicator of the autotrophic component of algal biofilms, significantly differed between sites during the two sampling months (October 2021: Kruskal-Wallis, H=21.88, df=9, p=0.009 and December 2021: Kruskal-Wallis, H=23.34, df=9, p=0.005) (Figure 15). In October 2021 higher chlorophyll-a concentrations were observed at Campaspe Site 6 and tributary Site 5 compared to most other sites. While in December 2021, chlorophyll-a concentrations at Campaspe Sites 1, 4, 6, 9, and tributary Site 5, were generally greater than those at all other sites (Figure 15). Seasonal variation within a site was observed, with higher chlorophyll-a generally measured in October 2021 at all sites compared to December 2021 (Figure 15).

Mean chlorophyll-a concentrations in Year 4 were generally comparable to those observed in Year 3 (Figure 16). At Campaspe Site 4 and the two tributary sites there was a sharp decline in chlorophyll-a from Years 1-2 to Years 3-4, while most other sites have fluctuated across years or remained stable (Figure 16).

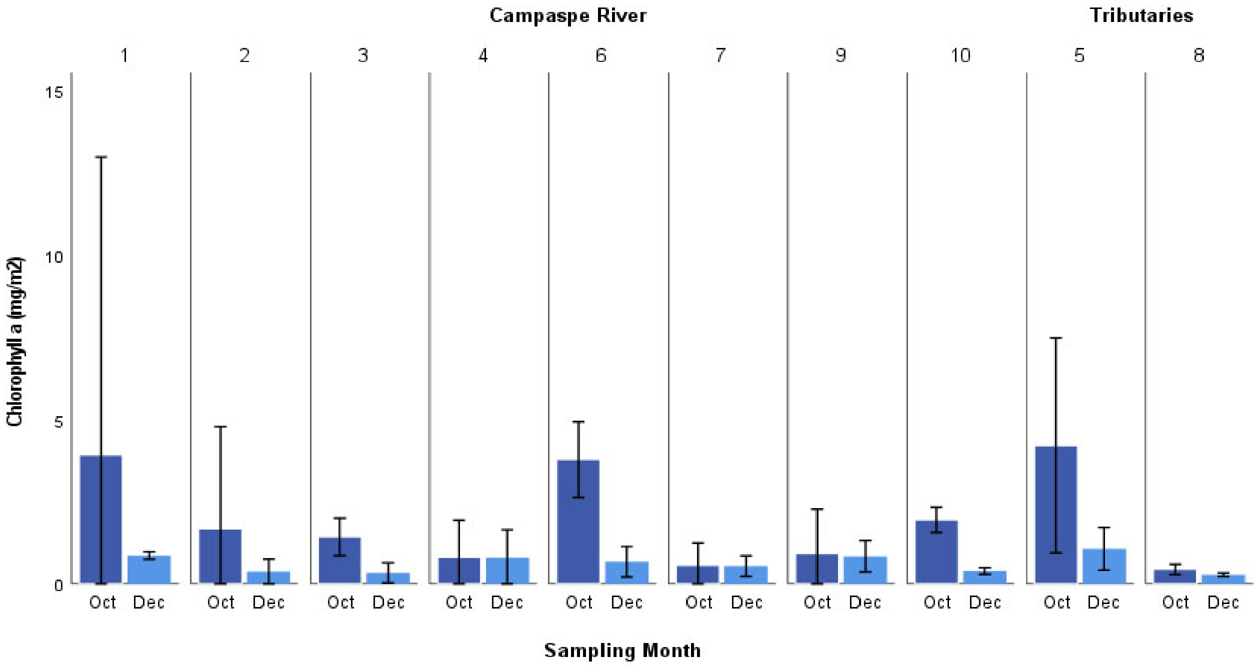


Figure 14: Chlorophyll-a (±SE) of biofilms on artificial substrates deployed across sites during October and December 2021. The Kyneton WRP discharge point is between Sites 6 and 7.

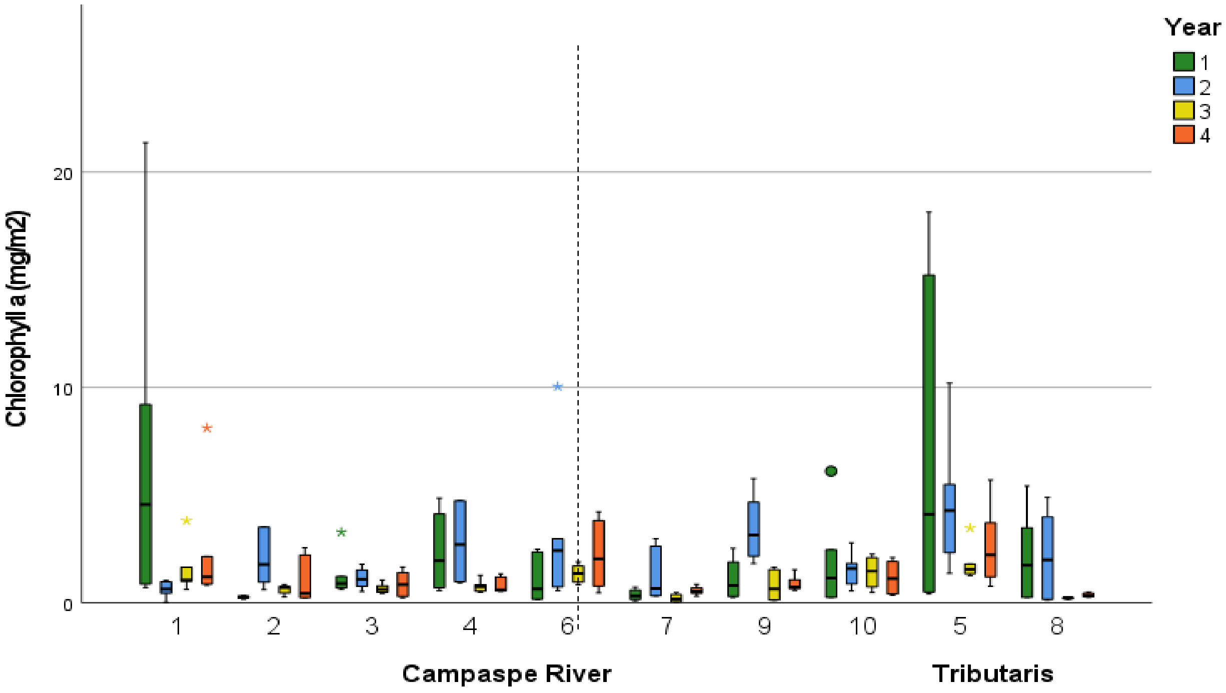


Figure 15. Mean chlorophyll-a of biofilms on artificial substrates deployed across sites during Years 1 to 4 of monitoring. The vertical dashed line indicates Kyneton WRP discharge point between Sites 6 and 7.

AFDM, a measure of the total amount of organic material, including autotrophic, heterotrophic and detrital carbon, significantly differed between sites in October 2021 (Kruskal-Wallis, H=19.01, df=9, p=0.024), and in December 2021 (Kruskal-Wallis, H=16.95, df=9, p=0.0.05). In October 2021, AFDM concentrations were generally higher than all other sites at Campaspe Site 6 and tributary Site 5, while in December 2021, AFDM was higher at tributary Site 5 compared to all other sites (Figure 17). Seasonal variation was observed at Campaspe Sites 1 and 6, and tributary Site 8, with higher AFDM in October 2021, and at tributary Site 5, where higher AFDM occurred in December 2021 (Figure 17). AFDM showed little seasonal variation at all other sites (Figure 16).

Annually, AFDM has generally declined at Campaspe Sites 1, 2, 3, 4 and 9, and tributary Site 8 (Figure 18). A similar pattern was observed at Campaspe Site 10, however, in Year 4, it increased. For Campaspe Site 6 and tributary Site 5 AFDM has fluctuated, while at Campaspe Site 7 it has been stable, but consistently low (Figure 18).

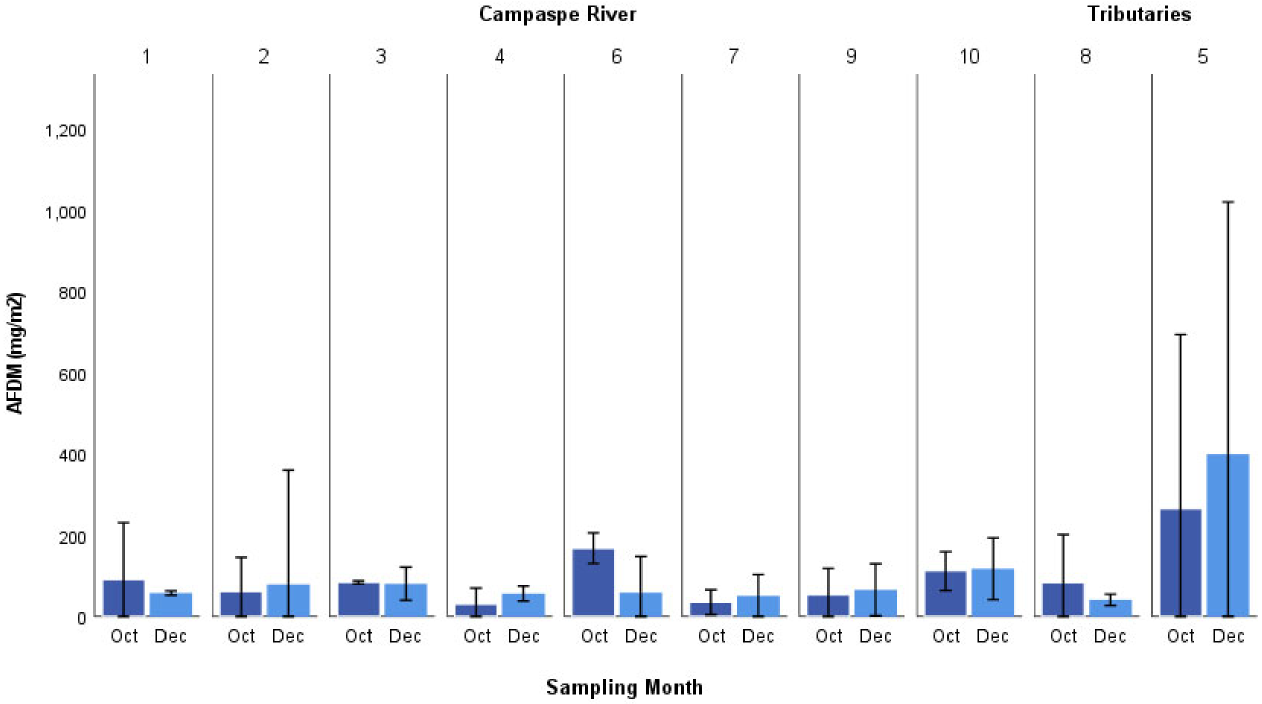


Figure 16: Mean ash-free dry mass (AFDM) (±SE) of biofilms on artificial substrates deployed across sites during October and December 2021. The Kyneton WRP discharge point is between Sites 6 and 7.

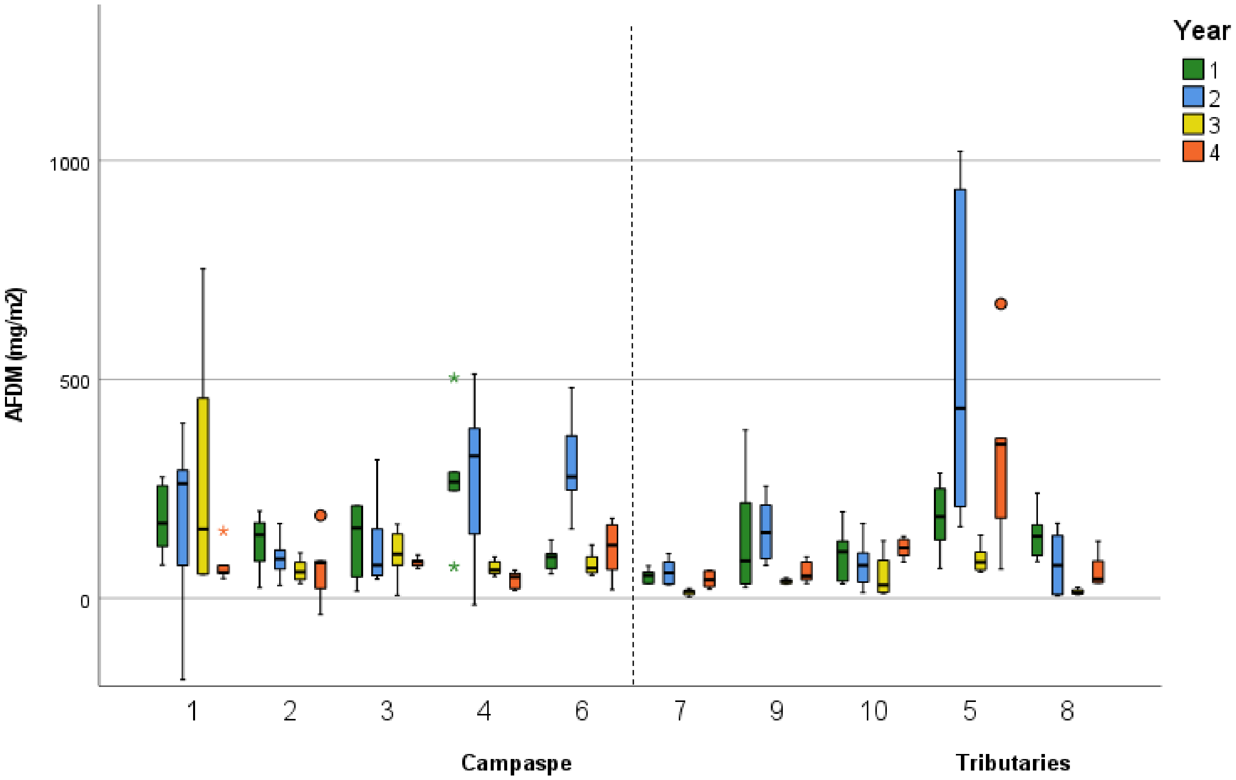


Figure 17. Annual change in ash-free dry mass (AFDM) (±SE) of biofilms on artificial substrates deployed across sites. Vertical dashed line indicates Kyneton WRP discharge point between Sites 6 and 7.

The Autotrophic Index (AI), calculated from the ratio of AFDM to chlorophyll-a, provides a measure of the autotrophic-heterotrophic balance of the biofilm community. Values up to 100 generally indicate a community dominated by viable algae, while values over 400 are indicative of a community dominated by heterotrophic organisms and/or organic detritus (Biggs and Close, 1989), which suggests biofilm communities are impacted by sources of organic pollution. Significant differences between sites were observed in October 2021 (Kruskal-Wallis, H=17.03, df=9, p=0.048), and in December 2021 (Kruskal-Wallis, H=17.87, df=9, p=0.037). During October 2021, benthic communities at all sites were dominated by viable algae, with mean AI values <100. The exception was tributary Site 8, where the mean AI approached 200, indicating the community was more heterotrophic dominated (Figure 19). In December 2021, AI values at Campaspe Sites 2, 3 and 10, and tributary Site 5, exceeded 400, indicating a greater dominance of heterotrophic organisms in these communities, compared to all other sites (Figure 19). Seasonal variation was observed, with AI values generally being greater in December than October (Figure 19).

AI values have generally declined annually at all sites, indicating an increase in viable algal communities, with Year 4 values being lower than previous years, except for Campaspe Site 10, and the two tributary sites, where there was an increase in AI values during Year 4, relative to Year 3 (Figure 19).

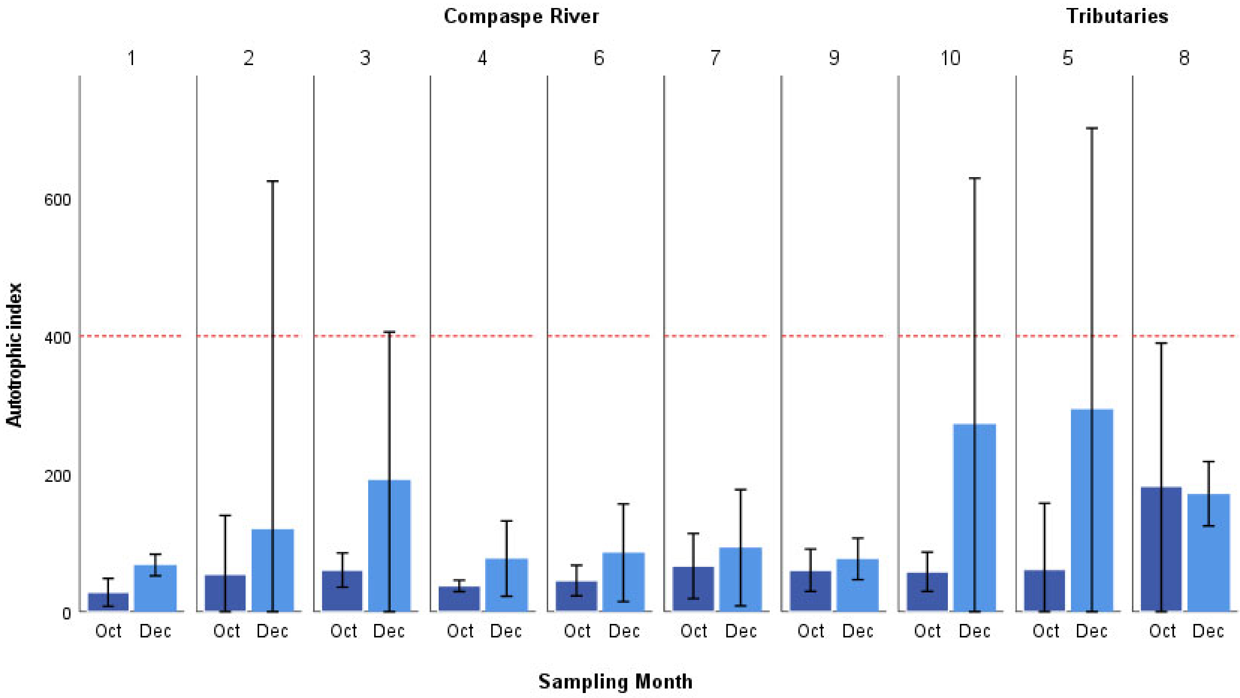


Figure 18: Autotropic index (±SE) of biofilms on artificial substrates deployed across sites during October and December 2021. The horizontal red dashed line indicates level above which communities are impacted by organic pollution. The Kyneton WRP discharge point is between Sites 6 and 7.

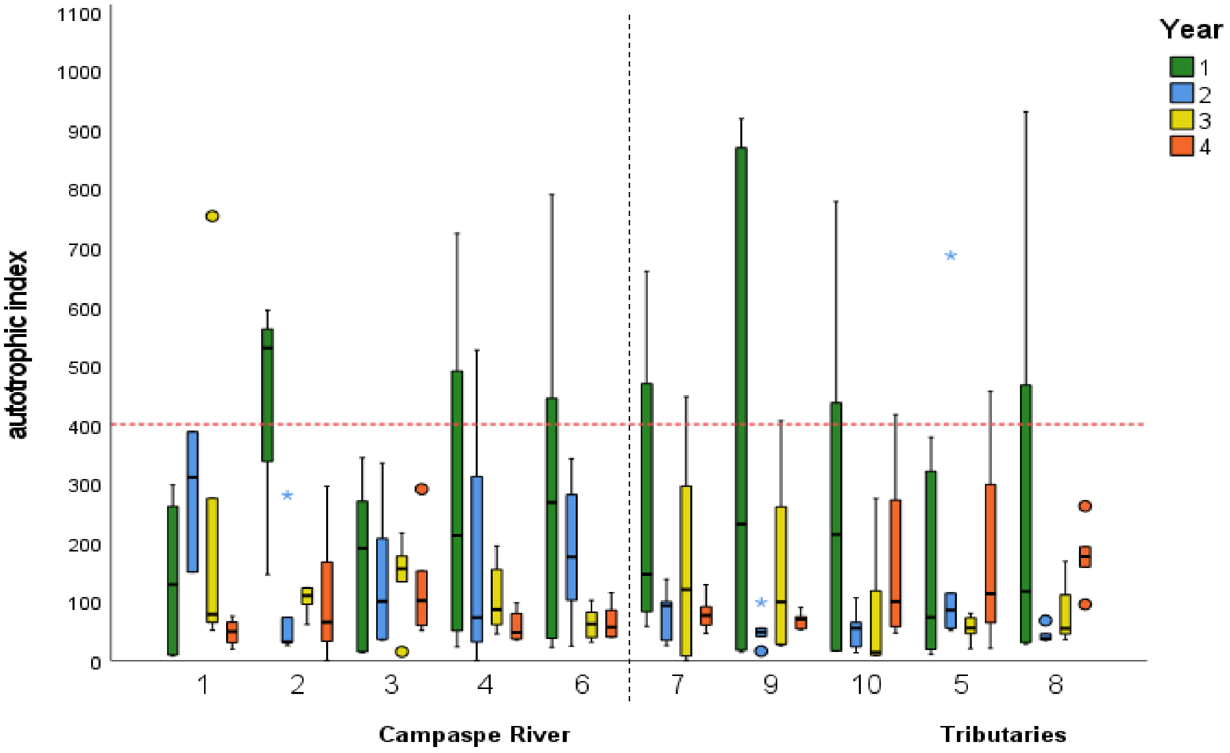


Figure 19: Annual autotropic index (±SE) of biofilms on artificial substrates at monitoring sites. The horizontal dashed line at 400 indicates level above which communities are impacted by organic pollution. Vertical dashed line indicates Kyneton WRP discharge point between Sites 6 and 7.

The community structure of cyanobacteria, chlorophytes (green algae) and diatoms provides an indicator of ecosystem health, with communities that are dominated by cyanobacteria and filamentous algae indicating nutrient enrichment of surface waters, whereas strong diatom communities are indicative of a healthy system, providing a high-quality food source for aquatic invertebrates. Communities in both October and December 2021 were comprised of both cyanobacteria and diatoms, with chlorophytes (green algae) only present at Campaspe Sites 2 and 3, and at tributary Site 8, in December 2021 (Figure 21). The composition was slightly more dominated by diatoms at Campaspe Sites 4, 7 and 10, and tributary site 5, while cyanobacteria were slightly more dominant at Campaspe Sites 1, 2, 3, 6 and 9, and tributary Site 5 (Figure 21).

The proportion of cyanobacteria to diatoms was relatively consistent between seasons at Campaspe Sites 3 and 7, and both tributary sites, while there was a higher contribution of cyanobacteria in December compared to October at all other sites (Figure 21).

Compared with previous years, there is a clear absence of chlorophytes in Year 4 (Figure 21). At most sites in Year 4 there has also been an increase in the proportion of diatoms making up the algal community, as compared to the previous two years (Figure 21).

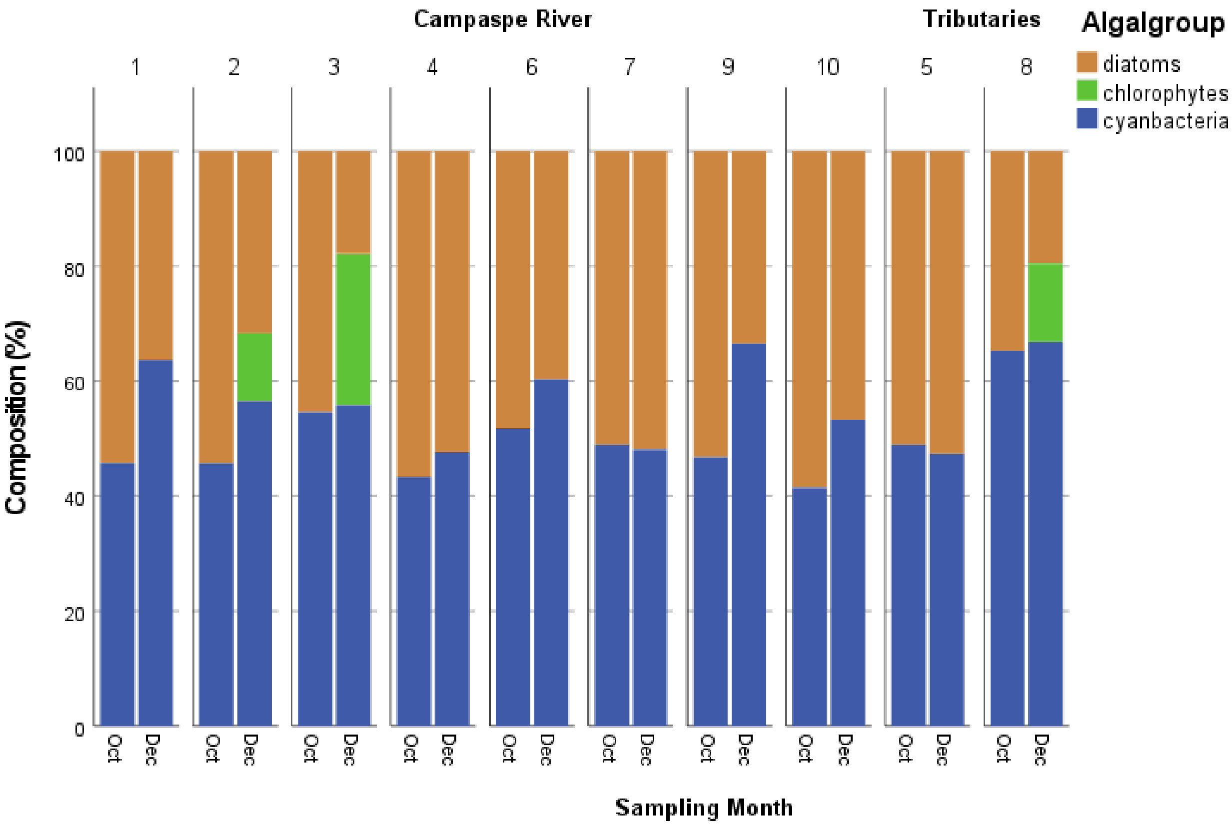


Figure 20: Relative composition of algal groups (Cyanobacteria, Chlorophytes and Diatoms) on artificial substrates deployed in October and December 2021. The Kyneton WRP discharge point is between Sites 6 and 7.

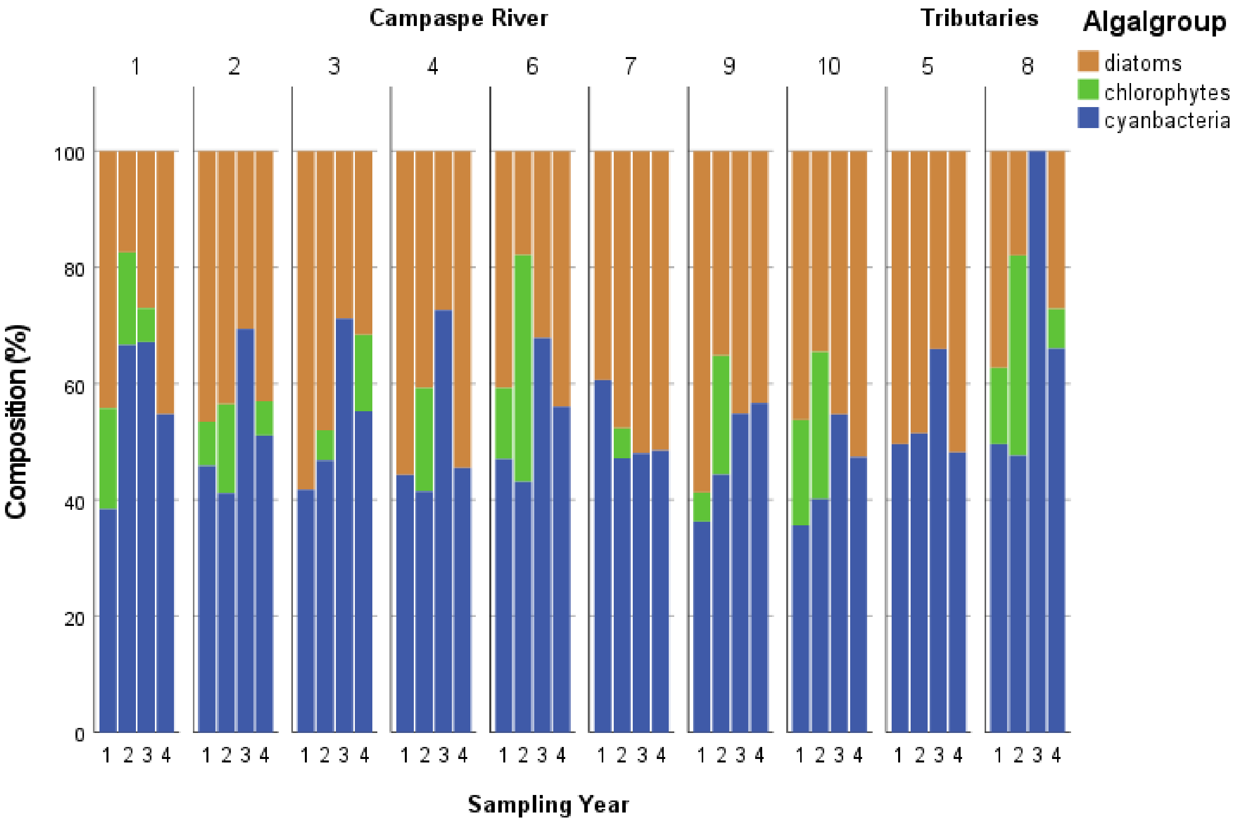


Figure 21: Annual relative composition of algal groups (Cyanobacteria, Chlorophytes and Diatoms), on artificial substrates. The Kyneton WRP discharge point is between Sites 6 and 7.

Physical Habitat

Instream habitat Assessment

Monthly mean macrophyte cover, an indicator of instream habitat availability, ranged from 0-100 % across sites during Year 4 monitoring, and is shown in Figure 23. Campaspe Sites 1 and 6 consistently had the greatest monthly mean macrophyte cover (range 83-100 %) (Figure 23), while at Campaspe Sites 7, 9 and 10 monthly mean cover ranged 0 to 100 % but was mostly below 69 % (Figure 23). At the tributary sites, mean cover ranged from 54 to 96 % for Site 5, and 4 to 11 % at Site 8 (Figure 23). Of note was a steady increase in monthly mean cover at Campaspe Sites 7 and 9 and tributary Site 5, while at Campaspe Site 10 it steadily decreased during monthly monitoring (Figure 23).

Mean macrophyte cover at most sites has generally increased over time, up to Year 3, except for Campaspe Site 10 where it has declined (Figure 24). In Year 4, mean cover appears to have stabilised at Campaspe Site 1-4 and 9, while marked increases in cover were observed at Campaspe Sites 6 and 10, and tributary Site 5, and decreases in cover were observed at Campaspe Site 7 and tributary Site 8 in Year 4 (Figure 24). Mean macrophyte cover remains consistently highest in the upper reaches of the study area, i.e. Campaspe Sites 1-4, and lowest at Campaspe Site 10 and tributary Site 8 (Figure 24).

Monthly filamentous algae (>2cm length) cover, an indicator of nutrient enrichment, ranged from 0 % to 53 % at sites during Year 4 monitoring, and is shown in Figure 22. The occurrence of filamentous algae >2cm was sporadic across sampling months at the different sites, mostly occurring only in 1 to 3 months (Figure 23). The exception was at Campaspe Sites 1, 3, 10, and tributary Site 8, where filaments >2cm were observed in four sampling months (Figure 23).

The occurrence of filamentous algae >2cm has generally decreased over time at all sites, except at Campaspe Site 10 and tributary Site 8, where it has tended to increase (Figure 24). At Campaspe Site 7, there has been no filamentous algal growth observed over the last 2 years of monitoring (Figure 24). Since sampling in Year 1, where mean cover reached nuisance levels at three sites (Sites 6, 9 and 5), levels have remained generally below 10% (Figure 24).

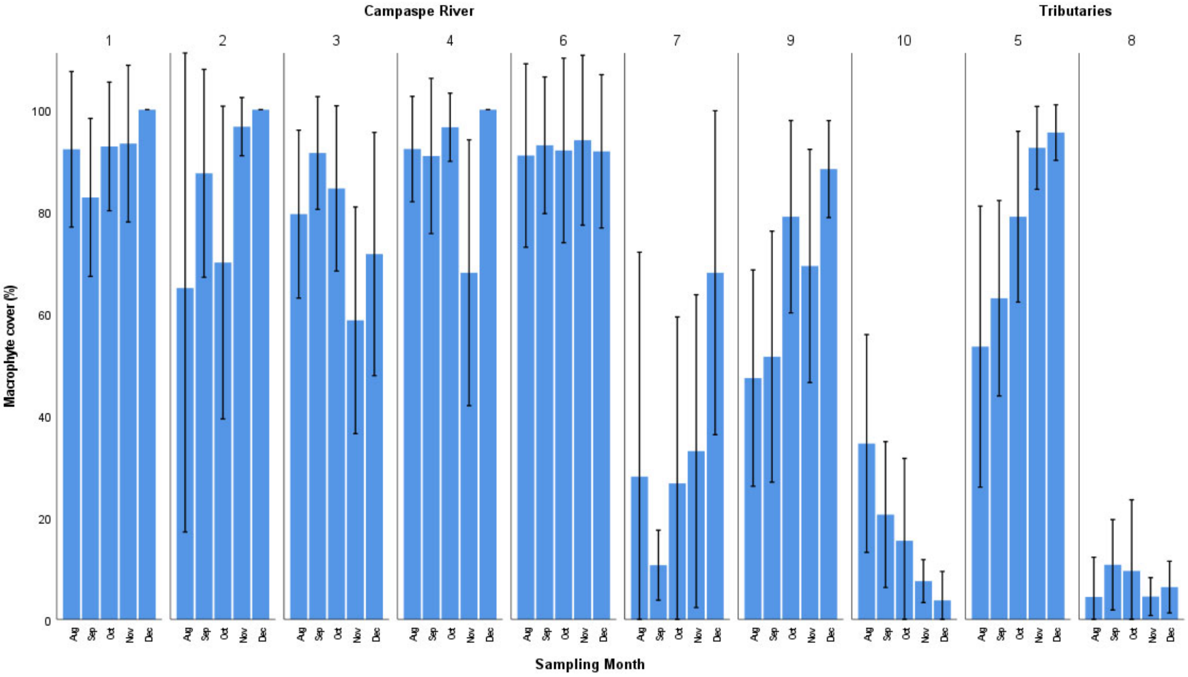


Figure 22. Monthly mean macrophyte cover at monitoring sites during Year 4. The Kyneton WRP discharge point is between Sites 6 and 7.

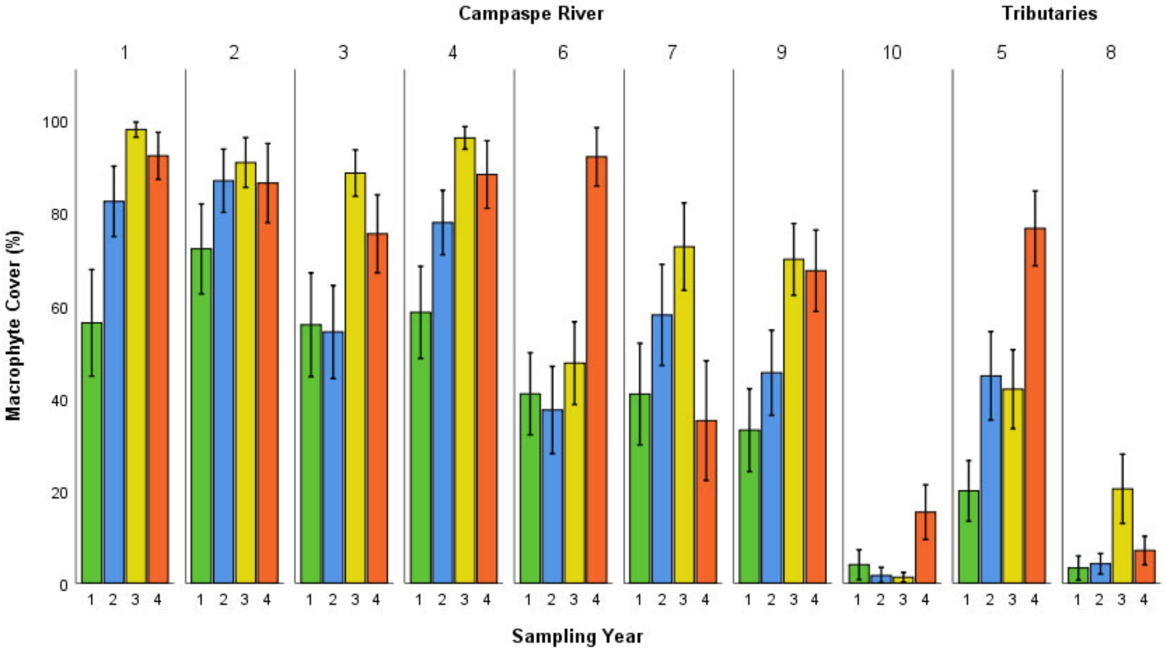


Figure 23. Mean macrophyte cover at sites in Years 1 to Years 4 of monitoring. The Kyneton WRP discharge point is between Sites 6 and 7.

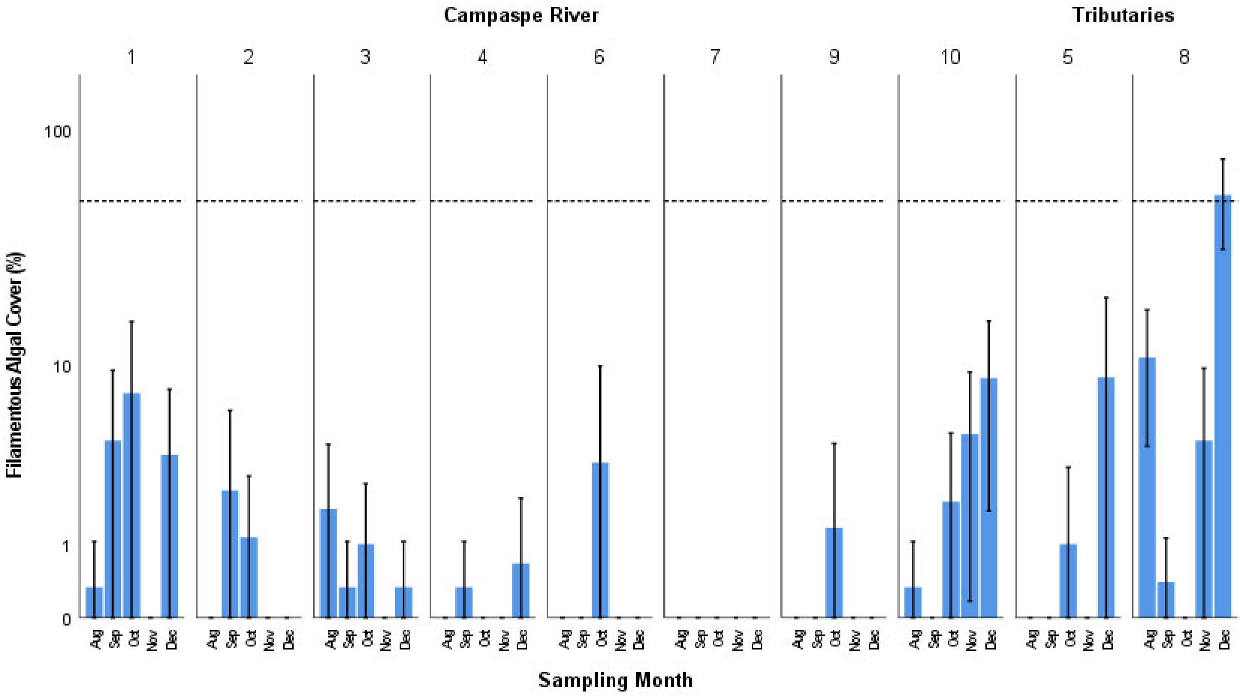


Figure 24: Monthly mean filamentous algal (>2cm length) cover at sites during Year 3. The Kyneton WRP discharge point is between Site 6 and 7. Horizontal dashed line represents nuisance algal cover threshold (30%).

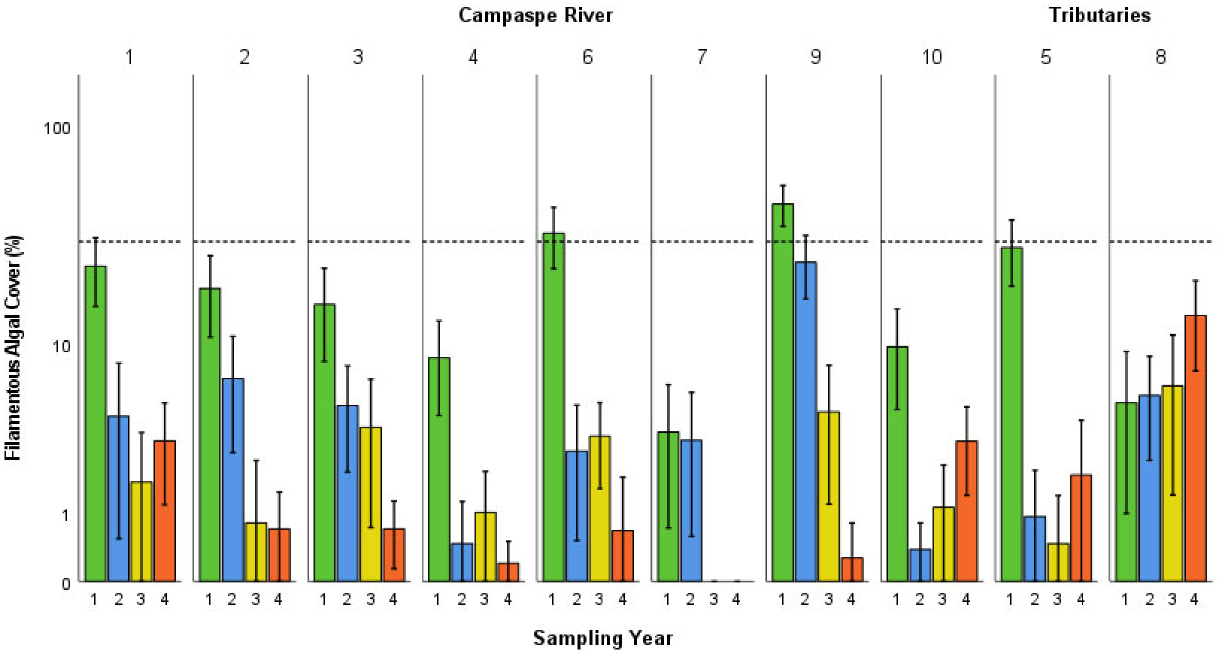


Figure 25. Mean filamentous algal (>2 cm length) cover in Years 1 to 4 of monitoring. The Kyneton WRP discharge point is between Sites 6 and 7. Horizontal dashed line represents nuisance algal threshold (30%).

Surface Water Toxicity

Faunal toxicity – Mud snails

Moderate impacts to mud snail survival were observed at Campaspe River Sites 1 and 3 (Figure 26). No impacts on mud snail survival were observed at any of the other study sites, where the average survival range was 88 to 100 %. (Figure 26). Average mud snail survival has varied annually (Table 10). At all sites, expect Campaspe Sites 2 and 9, poor survival has been observed in at least one of the four years of monitoring, while at Campaspe Site 3, the last 2 years have shown reduced mud snail survival and at Campaspe Site 1, Year 1 and 4 monitoring showed reduced survival (Table 10).

Embryonic production, an indicator of reproductive success and potential exposure to endocrine disrupting chemicals, ranged from 33 % to 54 % in Year 4 (Figure 27, Table 10). Lowest embryonic production was observed at Sites 2, 3 and 6 in the Campaspe, and tributary Site 5, where production was <37%, while the highest rates were observed at Campaspe Sites 7 and 10 (54 % and 51 %, respectively), and tributary Site 8 (52%) (Figure 27). Yearly assessments of embryonic production show varied results across sites (Table 10). General declines in embryonic production have been observed at Campaspe Sites 3 and 6. At all other sites, production rates have fluctuated between sampling years (Table 10), with increases observed at Campaspe Sites 4 and 7, and tributary Site 8, and declines observed at Campaspe Sites 1, 2, 9 and 10, and tributary Site 5 in Year 4, relative to Year 3 (Table 10).

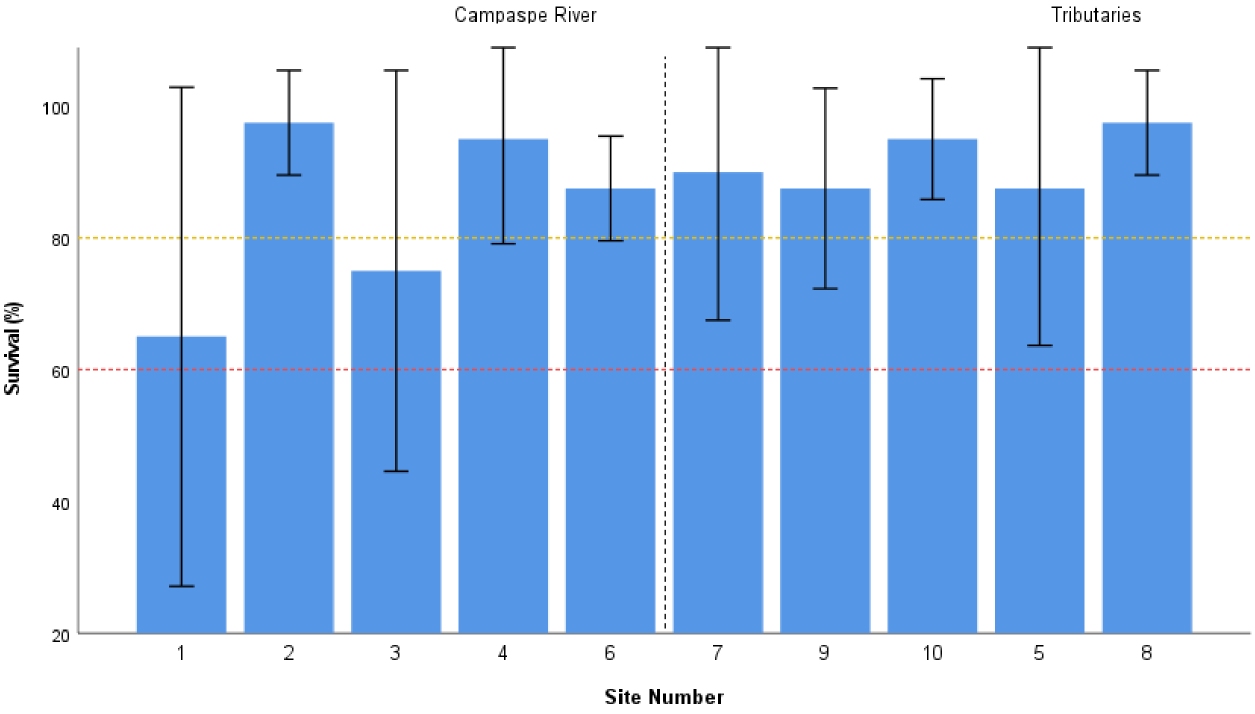


Figure 26: Mean survival (±SE) of mud snails, *P. antipodarum*, across sites during December 2021. Vertical dashed line indicates Kyneton WRP discharge point between Sites 6 and 7. Orange and red horizontal dashed lines represent the <80% and <60% thresholds below which moderate and high impacts are occurring, respectively.

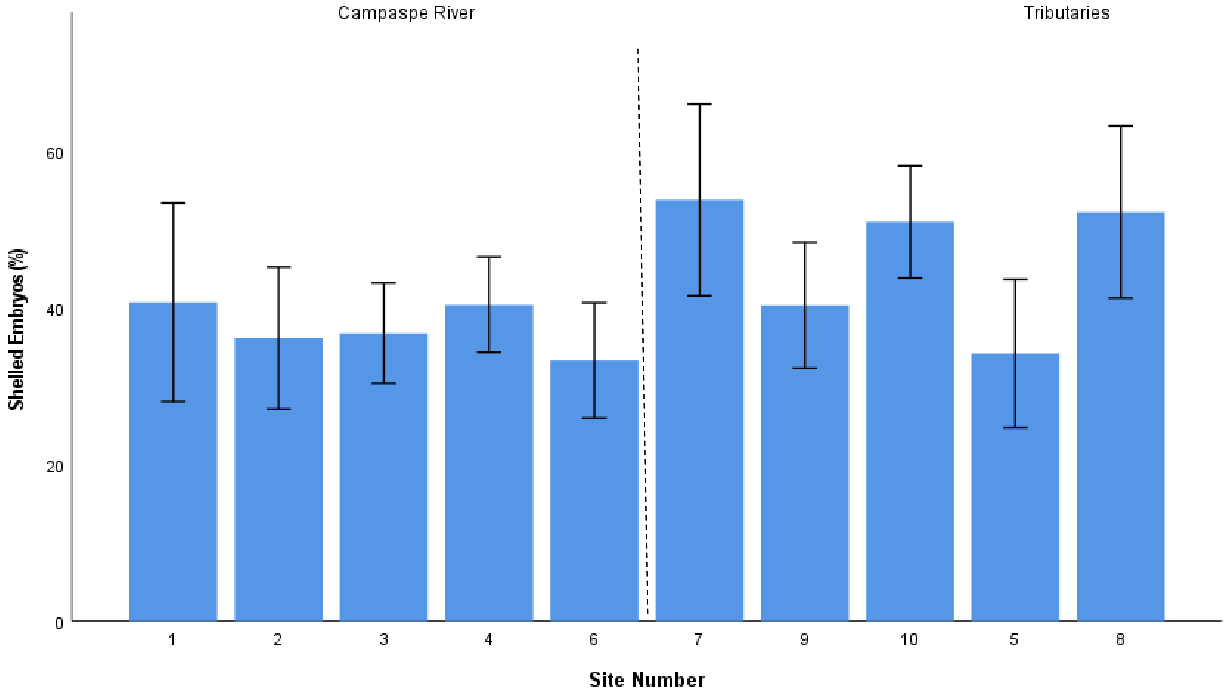


Figure 27: Mean percentage (±SE) of mud snails, *P. antipodarum*, with shelled embryos present across sites in 2021. The vertical dashed line indicates Kyneton WRP discharge point between Sites 6 and7.

Table 10: Mean survival of mud snails, *P. antipodarum*, and percentage of snails with shelled embryos present during Years 1 to 4 of monitoring.

Site	**Survival (%)**				**Shelled Embryos (%)**			
	2018	2019	2020	2021	2018	2019	2020	2021
Campaspe River								
1	78	98	100	65	61	45	48	41
2	88	96	98	98	48	43	48	36
3	90	96	60	75	52	40	40	37
4	95	94	73	95	51	47	29	40
6	60	98	100	88	54	50	38	33
7	83	68	100	90	52	44	32	54
9	88	98	83	88	56	50	52	40
10	98	86	63	95	49	41	55	51
Tributaries								
5	60	98	100	88	39	46	36	34
8	83	92	78	98	34	40	46	52
Average survival < 80% indicates a moderate impact (highlighted in orange) and < 60% indicates a high impact (highlighted in red).								

Floral Toxicity - Algae

During Year 4, algal toxicity was assessed in October and November 2021. No significant inhibition or stimulation of growth was observed during October 2021 (Table 11), however, high inhibition of algal growth was observed in November 2021 at Campaspe Sites 4 (50%) and 10 (54%) in the Campaspe, while moderate growth inhibition occurred at Campaspe Sites 3 (43%) and 9 (24%) (Table11). Substantial stimulation of algal growth, ranging 20 to 83% of site controls, was observed during November 2021 at Campaspe Sites 2 and 6, and tributary Site 8 (Table 11).

Yearly assessments of algal toxicity show varied results across sites (Table 11). Campaspe Sites 3, 4 and 6 show moderate to high growth inhibition in three of the four monitoring years, while Campaspe Site 1, and tributary Site 5, in two of the four years. Campaspe Site 7 and tributary Site 8 show moderate to high stimulation of growth in three of the four years, and Campaspe Sites 2, 3, 6 and 9, and tributary Site 5 in two of the four years. Interestingly, Campaspe Site 10 has generally shown very little inhibition or stimulation of algal growth until Year 4, where high inhibition was observed during November 2021 sampling (Table 11).

Table 11: Mean inhibition of algal growth (relative to site control) across sites during Years 1 to 4 of monitoring.

Site	Year/ Month							
	1		2		3		4	
	Nov-18	Jul-19	Sep-19	Nov-19	Nov-20	Jan-21	Oct-21	Nov-21
Campaspe River								
1	56	28	0	52	-242	0	-1	-20
2	39	ND	-6	14	-236	10	0	-83
3	53	16	-30	55	-43	18	0	43
4	36	0	29	37	26	8	-1	50
6	53	27	18	56	-110	52	7	-42
7	-142	-8	0	-43	-336	35	9	11
9	38	4	-31	21	-106	15	15	24
10	24	7	20	8	21	-11	16	54
Tributaries								
5	-121	-66	0	69	-155	66	-7	-10
8	-261	-14	-6	-9	-129	ND	0	-30

🟧 > 50% inhibition of algal growth

▨ >30% inhibition of algal growth

🟩 >-50% stimulation of algal growth

▧ >-30% stimulation of algal growth

Positive values indicate growth inhibition, values of 0-20% indicate minimal impact, 20-50% indicate moderate impact (highlighted as pale orange) and >50% indicate a high impact (highlighted in orange).
Negative values indicate increases in biomass, suggesting that site conditions are encouraging growth and thus indicate nutrient enrichment. ND = Not detected

Discussion

The principle focus of the SFM program is to reduce the delivery of sediments, nutrients, and pathogens (related to stock) to the Campaspe River, thus improving the ecological health of the river. While the benefits of SFM are not likely to be observed in four years of monitoring, results to date show that the abiotic and biotic conditions of the river are both spatially and temporally dynamic, and there is evidence of differences in river condition emerging between sites based on riparian condition e.g.: between SFMW works sites, native vegetation sites and sites where no interventions have occurred (i.e. remain willow dominated, with stock access).

What are the changes in abiotic conditions – nutrients, water quality and associated macrophyte and algal growth?

Sites where no SFM interventions have occurred, and which remain dominated by willows, with free stock access to waterways, continue to be characterised as being in the poorest abiotic condition. These include the Campaspe River site at Old School Rd and the tributary site in Snipes Creek. These sites are characterised by higher concentrations of dissolved nutrients (TN, TP, Orthophosphate), often exceeding guideline values, lower dissolved oxygen levels and water temperatures, and poorer water clarity. Unrestricted stock access to waterways and willow-dominated riparian zones are factors well known to contribute increased nutrients and sediments to waterways (Biggs 2000; Shearman and Wilcock 2011; Hughes and Quinn 2014; McKergow et al 2016). Stock graze pasture to the water's edge, damaging the banks, and with no physical barrier to runoff, faeces, urine and nutrients enter waterways more freely when it rains (Shearman and Wilcocks 2011). Willows, while historically used to control bank erosion, invade the entire riverbank and bed, shading the entire river and reducing water temperatures. Willows contribute large amounts of leaf litter to sites over a short period, which is very different to the behaviour of native species, which have a more continuous leaf fall (Lester et al., 1994). Willows also increase the retention of sediments, as well as organic material, which reduces aquatic habitat and stimulates bacterial activity. All these factors lead to increases in instream nutrient concentrations (Bobbi 1999). Without intervention, these sites are likely to remain in poor abiotic condition.

In contrast, sites dominated by established native vegetation, and those influenced by SFMW, are generally in a healthier and more stable abiotic condition. Elevated levels of nutrients (TN, TP and orthophosphate) occur, as well as some variations in water quality parameters; however, this is likely related to nutrient and sediment transport from upstream reaches dominated by poor riparian habitat and/or that have unrestricted stock access, or due to recent, and less established, SFMW activities. Established native vegetation sites at Boundary Rd, Langley, and at Redesdale (Sites 9 and 10), are characterised by generally stable water clarity, dissolved oxygen, and water temperatures. Nutrient concentrations vary temporally, with elevated TN occurring at Site 10, and consistently elevated TP and orthophosphate at Site 9. These elevated nutrients are likely the result of downstream nutrient transport from upstream sites. While Sites 9 and 10 have a good established native riparian vegetation, upstream areas of the catchment are characterised by poor riparian vegetation that protect stream banks against erosion, and stock have unrestricted access to large areas of the river. These factors lead to increased sediment and nutrient transport to the waterway, and contribute to poor instream nutrient retention.

At SFMW sites, there is evidence that the works have led to increases in nutrient and sediment inputs, but once vegetation has become more established, and banks more stabilised, concentrations appear to have stabilised. The removal of woody weeds, revegetation and fencing works can lead to initial increases in sediment and nutrient delivery to sites, due to destabilisation of banks and removal of vegetation, which is usually followed by trending improvements as streamside vegetation grows and stabilises banks (Hughes and Quinn et al 2014). This was evident at Site 1 at Cheveley Rd, in Carlsruhe, during Year 3 monitoring where there was a significant decline in water clarity and elevated TN, possibly as a result of sediment mobilisation and bank disturbance from the installation of stock exclusion fencing, and willow removal and revegetation work that were completed during Year 2. Water clarity was also significantly reduced, and TN remained elevated at the next downstream site at Cobb and Co Rd, Carlsruhe (Site 2). This site is dominated by established native vegetation and thus it's possible the works upstream resulted in downstream sediment and nutrient transport leading to reduced water clarity and elevated TN. Year 4 monitoring has shown improvement at these two sites, despite a year of high rainfall compared to Year 3. Water clarity has improved and there have been declines in both TN and TP and orthophosphates at these sites. At SFMW Sites 3-6, where willow removal and revegetation work occurred prior to Year 1 monitoring, we continue to see evidence of improved or stable abiotic conditions. The riparian vegetation at these sites is more established and provides greater bank stabilisation. Nutrient levels (TN, TP, orthophosphate) are elevated, but have remained stable or slightly improved. Similarly, water clarity and dissolved oxygen levels remain steady. Up to Year 3, water temperatures at all SFMW sites tended to increase, which is likely a result of the lack of riparian canopy to provide shade. As the plantings become more established, it is predicted water temperatures will stabilise. In Year 4, water temperatures did decline, however, this is likely due to increased water in the river due to higher rainfall, or decreased overall air temperatures in the area, rather than increased shading as the riparian vegetation has not grown enough to significantly change shade levels at this point in time.

While aquatic macrophytes and algae are important structural and biological components of rivers, supporting ecosystem health by processing instream nutrients and providing habitat and food resources for aquatic biota (Paice et al 2017), excessive growth can lead to choking of the channel, reduced light, low oxygen, and poor habitat and food resources (Rutherford and Cuddy 2005; McKergow et al., 2016). Since monitoring started in 2018, macrophyte cover has become less temporally variable and substantially increased across much of the study area. In contrast, filamentous algal cover, particularly medium and long filamentous algae, has simultaneously declined, with nuisance levels not observed since Year 1 monitoring. Macrophyte and algal abundance is generally strongly correlated with nutrients and light (Biggs, 2000; Rutherford and Cuddy, 2005). Fluxes of nutrients and sediments from land can stimulate the growth of nuisance algae and macrophytes in rivers (McKergow et al 2016), while shade from riparian canopy plays an important role in reducing water temperatures, which controls nuisance plant and algal growth and instream nutrient processing (Quinn et al., 1997; Cox and Rutherford, 2012; Matheson et al., 2012; Hughes and Quinn, 2014; McKergow et al., 2016).

At SFMW sites, river canopy cover, and thus shade, has significantly reduced or is non-existent following willow removal, which, when coupled with sufficient availability of nutrients, has resulted in stimulated macrophyte growth. This is evidenced particularly over the first three years of monitoring at Campaspe Sites 1 and 2 in Carlsruhe, where macrophytes cover of >90% of the wetted area of the river and dissolved oxygen levels declined. Assessments of algal growth indicate that nutrient levels at the SFMW sites are sufficient to support algal growth, however, the occurrence of filamentous algae and abundance of biofilms has declined. It is possible the increase in macrophyte cover is shading the water column and controlling algal growth and nutrient uptake. Several studies have attributed reductions in nutrient uptake and algal growth due to stream shading (Quinn et al., 1997; Cox and Rutherford, 2012; Matheson et al., 2012). Year 4 monitoring showed a stabilisation in macrophyte cover, an increase in biofilm communities and an improvement in dissolved oxygen levels across SFMW sites from Year 3. These observations could be indicating an improved balance of macrophyte and algal communities, resulting in improved instream nutrient processing across these sites, or it could be due to a reduction in nutrient inputs, with stabilisation of the banks and improved establishment of the revegetated riparian zone.

Up until Year 3, there had been significant increases in macrophyte cover at sites where no SFM interventions have taken place, that is, Campaspe River Site 7, at Old School Rd, and tributary Site 8, on Snipes Creek, however, in Year 4, declines in overall macrophyte cover were observed at these sites. The macrophyte species composition at these sites differs significantly to that at all other sites. Floating macrophytes, including *Azolla* sp and *Lemna* sp., dominate at Sites 7 and 8, while all other sites comprise of a mix of rooted submerged and emergent macrophytes, such as *Myriophylliom* sp., *Triglochin* sp, and *Allisma* Sp. The high abundances of *Azolla* and *Lemna*, which are more opportunistic macrophyte species, during monitoring Years 1-3 is likely related to the high nutrient availability, notably orthophosphates, observed at these sites. In Year 4 monitoring, while these species still dominated the macrophyte assemblage, the decline in cover was likely related to a combination of the decline in nutrients that was also observed at the sites, and higher rainfall. Algal toxicity results further support this, with reduced nutrient availability observed for algal growth in Year 4 compared to other monitoring years.

While there was a slight increase in the algal biomass at both Sites 7 and 8, and filamentous algal cover at Site 8, in Year 4, likely in part a result of lower macrophyte cover, both sites still show limited algal biomass availability compared to all other sites. Studies in New Zealand streams have shown that stream channel shading by weeds and indigenous vegetation results in reductions in biofilm biomass and/or the growth of healthy macrophyte stands (Hughes and Quinn, 2014; McKergow et al., 2016). It is likely that the dominance of willows, covering between 60-100% of these two sites, together with the blanketing of the water surface by floating macrophytes, results in poor light conditions for the growth of healthy macrophyte and algae communities. This subsequently adversely impacts on instream nutrient processing, resulting in poor instream processing of nutrients and high nutrient export from these reaches. This is reflected in the conditions at Site 9, at Boundary Rd, Langley, where monitoring continues to show elevated nutrients and high macrophyte growth, including an increasing number of floating species, such as *Azolla* and *Lemna.* It is likely that shading from riparian vegetation also plays a role in elevated nutrients at Site 9 (>70% shade cover), by reducing macrophyte and algal nutrient uptake. Improvements in upstream reaches is likely the only way to improve nutrient levels at Site 9. Thus, sites such as Sites 7 and 8, present great opportunities for SFM works to improve overall river health.

Are we seeing changes in faecal contamination?

The major source of faecal contamination in agricultural catchments is animal excreta. Reductions in faecal contamination have been shown to occur when stock are excluded from within the riparian zone, however, when cattle still graze adjacent to rivers, results can be variable (Hughes and Quinn 2014). Stock has unrestricted access to the river at Sites 7 and 8, while stock graze on land adjacent to the Campaspe River at Sites 1, 2, 4, 6, 9 and 10. At this point there are no clear indicators that the removal of cattle has resulted in reductions in faecal contamination to the Campaspe River, with monitoring to date showing that *E. coli* occurs at all sites where it is monitored (Campaspe Sites 2, 4, 6, 7, 9, tributary Sites 5 and 8). However, *E. Coli* levels consistently exceed guideline values (100 organisms per 100mL) at sites where stock have unrestricted waterway access (Campaspe Site 7 and Snipes Creek Site 8). *Escherichia coli* is a general marker of faecal contamination in waterways and cannot be linked to a particular source. To provide a better understanding of the sources of faecal contamination, markers for the presence of Bacteroides, a bacterium that inhabit the digestive tracts of animals, were applied. The markers indicated faecal contamination, attributable to stock, occurred at sites where stock graze adjacent to the river with restricted access (Campaspe Sites 2, 4, 6 and 9) and at Campaspe Site 7 and Snipes Creek Site 8, where stock have unrestricted access. However, marker levels were more elevated at sites where stock had unrestricted access to the waterway, which suggests that reducing access to the waterway can reduce levels of stock faecal contamination in the waterway.

How is ecological health responding?

Macroinvertebrate communities provide a picture of ecological health in the Campaspe River, with increased richness and diversity, indicating better water quality and ecological condition. However, it's important to note that the Campaspe River is in the central foothills and coastal plains zone, and requires a SIGNAL2 score of ≥3.4 and 20 families to meet Environmental Reference Standard objectives. But these guidelines are intended for permanently flowing streams. The upper Campaspe River, and the tributaries of Snipes Creek and Post Office Creek, are ephemeral, and so long-lived macroinvertebrates are less likely to occur where water is not permanent. Monitoring to date shows a general improvement in macroinvertebrate taxon richness and diversity across most sites, which is likely related to several factors, including improved habitat complexity, improved food sources and availability, but also rainfall.

Highest diversity and taxon richness, as well as the presence of sensitive taxa, consistently occurs at sites in the upper to mid reaches of the study area (Sites 2-6), where SFMW have been conducted. This is likely related to the diversity of instream plants providing good habitat structure for invertebrate communities, a developing native riparian zone, which will provide a constant and improved food source, and due to the more permanent and stable water levels through this section of the river during dry periods. The two sites dominated by native riparian vegetation (Sites 9 and 10) generally have good habitat available for macroinvertebrate communities; however, they have shown variable macroinvertebrate diversity and taxon richness over the four sampling years. With higher rainfall over the last few years, however, diversity and taxon richness has increased at these sites, particularly Site 9. It is likely that water availability strongly influences communities at these sites, which dry up during summer months. There are also signs of higher nutrient enrichment at these sites compared to SFMW sites.

Poorest ecological health is consistently observed at sites were no SFM interventions have occurred, willows remain the dominant riparian vegetation, and stock access is allowed, as well as at the urban Site 5 on Post Office Creek. Willows are known to restrict macroinvertebrate diversity (Lester et al., 1994; McInerney et al., 2016), because of increased nutrient concentrations, and a lack of a continual supply of organic matter of appropriate quality as a food source. There was a slight increase in diversity and richness at tributary Site 8, on Snipes Creek, over the last few years (Year 3 and 4), which could be a result of increases in food and water availability, but condition is still rated as "low", compared to most other sites. At tributary Site 5 on Post Office Creek, it is likely that several factors play a role in the reduced macroinvertebrate diversity and taxon richness seen at this site, including poor habitat availability, lack of different habitat types, such as riffles or runs, most of the site is characterised by deep water, as well as the presence of other pollutants.

What else is influencing waterway health?

To achieve overall improvements in river water quality and biodiversity, an understanding of all factors impacting waterway health across the catchment is required. Monitoring of several pollutants in surface waters and sediments, paired with toxicological assessments, provides us with an understanding of the different pressures influencing waterway health. Several additional pressures have consistently been detected across the study area, including the presence of toxicity and a range of pollutants associated with urban, industrial and agricultural runoff, as well as wastewater inputs.

Urban, industrial and agricultural runoff can result in the contribution of a range of pollutants to waterways, including heavy metals, hydrocarbons and pesticides. Heavy metals and hydrocarbons are usually related to anthropogenic activities, such as rail and road transport, industrial activities (e.g.: metal recyclers, old mining) and housing (e.g.: zinc roofing). These contaminants have the potential to impact on aquatic ecosystem health, reducing biodiversity and causing toxicity to both flora and fauna. Several heavy metals, including zinc, mercury, lead, and chromium continue to be detected at concentrations of concern for aquatic life. Sites of greatest concern for these metals include Campaspe Sites 3 (chromium) and 9 (chromium and mercury) and urban Site 5, on Post Office Creek (lead, zinc and mercury). Additionally, hydrocarbon concentrations have been consistently elevated across the study area, particularly at sites directly surrounded by heavy traffic roads and rail tracks.

Year 4 monitoring showed the continued detection of complex mixtures of pesticides in surface waters and sediments across the Campaspe River and tributary sites. Pesticides enter waterways via various pathways, including surface runoff during irrigation and/or rainfall, aerial deposition during application (spray drift), and via infiltration from groundwater. Eight herbicides, three fungicides and two insecticides have been detected in surface waters, while two synthetic pyrethroid insecticides and two fungicides have been detected in sediments during the four years of monitoring. The detection of pesticides in both urban and agriculturally dominated sites suggest applications in both land use contexts are contributing to pesticide contamination. The most frequently occurring pesticides are the herbicides simazine, atrazine, triclopyr and hexazinone, the insecticide imidacloprid, and the fungicide carbendazim. Toxicology assessments have indicated some of these pesticides, particularly the herbicides and insecticides, are at levels that may be adversely impacting stream biodiversity.

Nine pharmaceuticals, including an antidepressant, an anticonvulsant, and antibacterial medication, several antibiotics, and a veterinary medication, have been detected in surface waters during the four years of monitoring. The most frequently occurring pharmaceutical is the anticonvulsant, carbamazepine, which has been detected in multiple sampling years across nearly all study sites. The source of these chemicals is usually wastewater, which could include licensed discharges or through infiltration from septic systems. Bacteroides markers, HF183 and Lachno3, also indicated impacts from wastewater, both treated and untreated, across the study area. Water samples collected during the baseflow periods had higher concentrations of the marker genes, compared with samples collected during wet periods, indicating dry weather or septic leakages may be occurring. Further sanitary inspections would be required to confirm these findings.

Continued monitoring of the occurrence of these ‘other’ pollutants provides a greater understanding of potential risks posed to river health, and a better understanding of their sources, so that management actions can be identified.

Conclusions

The four years of monitoring has provided information from which to assess the short-term benefits of the SFMW to the Campaspe River, and provides insight into other factors influencing river health. Immediate benefits are continuing to present at SFMW sites, with stabilizing nutrient levels, improving water clarity and improved aquatic environment for macroinvertebrates. However, at sites where no SFM interventions have occurred, where willows remain and dominate the entire riverbed, and stock have free river access, we see sustained evidence of poor ecological health, which continues to result in impacts to downstream river health.

The presence of toxicants, including pesticides, heavy metals, hydrocarbons and pharmaceuticals and impacts from treated and untreated wastewater, including elevated nutrients and faecal contamination, are observed across the study region, and are likely to be contributing to the ecological health of these sites.

Monitoring provides a better understanding of the longer-term benefits of the SFM Program, as the riparian vegetation becomes more established, and provides a more optimal microclimate for macroinvertebrates, and improves habitat quality, thereby reducing nutrient inputs, and improving food availability. Monitoring also provides a better understanding of how other factors influence overall instream improvements.

Future Sampling

The five-year monitoring and assessment program is due for completion in 2022. Sampling for Year 5 will commence in August 2022 and be completed December 2022, with the final report available June 2023.

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Appendices

Appendix 1: List of pesticides screened in sediment samples and their detection limits. H=herbicide, I=Insecticide, F=Fungicide, MISC=miscellaneous

Pesticide	Type	Detection Limit mg/kg	Pesticide	Type	Detection Limit mg/kg
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_Endosulfan	I	0.01
Metalaxyl	F	0.01	beta_Endosulfan	I	0.01
Atrazine	H	0.01	Endosulfan_sulfate	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_methyl	I	0.01
Tebuconazole	F	0.01	Dichlorvos	I	0.01
Diazinon	I	0.01	Chlorpyrifos_methyl	I	0.01
Dimethoate	I	0.01	Fenthion	I	0.01
Propiconazole_II	F	0.01	Ethion	I	0.01
Boscalid	F	0.01	Chlorfenvinphos_E	I	0.01
Fenamiphos	F	0.01	Chlorfenvinphos_Z	I	0.01
Difenoconazole	F	0.01	Parathion_ethyl	I	0.01
Propiconazole_I	F	0.01	Parathion_methyl	I	0.01
Cyprodinil	F	0.01	Pirimiphos_methyl	I	0.01
Carbaryl	I	0.01	Pirimiphos_ethyl	I	0.01
Pirimicarb	I	0.01	Bromophos_ethyl	I	0.01
Buprofezin	I	0.01	Carbophenothion	I	0.01
Metribuzine	H	0.01	Coumaphos	I	0.01
Propiconazole_I_II	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_methyl	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_epoxide	I	0.01	Vinclozolin	F	0.01
Aldrin	I	0.01	o_Phenylphenol	F	0.01
gamma_BHCLindane	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_Chlordane	I	0.01	Piperonyl_Butoxide	SYN	0.01
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: List of personal care products (PPCP), pharmaceuticals and pesticides screened in surface waters and their detection limits

Type	Limit of detection (ug/L)
PPCP and Pharmaceuticals	
Caffeine	<5
Venlafaxine	<1
Carbamazepine	<0.5
DEET	<1
ketoprofen	<5
TCS	<1
Diclofenac	<2
Ibuprofen	<5
BPA	<2
Paracetemol	<5
cholesterol	<10
Pesticides	
Pirimicarb	<1
Simazine	<1
Metalaxyl	<1
Atrazine	<1
Carbaryl	<1
Diuron	<1
Pyrimethanil	<2
indoxacarb	<5
Metolachlor	<1
Pyraclostrobin	<1
Trifloxystrobin	<1
Prochloraz	<1
MCPA	<1
2,4-D	<5
Dicamba	<5
Myclobutanil	<5
Difenconazole	<2
Benzotriazole	<2
Imidacloprid	<1
Triclopyr	<2.5
Artificial sweeteners	
Acesulfame	<1
Saccharin	<5
Cyclamate	<1