Coliban Water Monitoring Program

Monitoring Program for Assessing the Benefits of Environmental Offsets on the Condition of the Campaspe River: Year 3 (2020)

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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 wastewater 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, monitoring river condition 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 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 3, 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 2020. While the benefits of SFM are not likely to be observed in three 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 (remain willow dominated with stock access).

At SFMW sites where works were conducted in Year 2 of Monitoring there are signs that works have led to initial increases in nutrient and sediment inputs, which has reduced water clarity. However, at sites where works were conducted prior to monitoring, vegetation has begun to establish and stabilise banks and abiotic conditions. For example, nutrient concentrations and water clarity appear to be stable or slightly improved. Similar abiotic conditions are observed at sites surrounded by established native vegetation. Across all SFMW and established native vegetation sites there has been an increase in macrophyte cover and a decrease in the occurrence of medium to long filamentous algae. At SFMW sites this is likely related to increased light and water temperatures, resulting from the lack of riparian shading. At native vegetation sites this is likely related to elevated nutrients delivered from upstream sources. 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.

In contrast, sites where no interventions have occurred are in poorest abiotic and biotic condition. Sites are characterised by elevated concentrations of dissolved nutrients, often exceeding guideline values, lower dissolved oxygen levels and water temperatures and poorer water clarity. The increase in macrophyte cover was due to floating macrophyte species which blanket the water surface. Filamentous algal cover and biofilm biomass declined, likely due to shading effects on algal growth and nutrient uptake. These factors result in reduced instream processing and greater export of nutrients from these sites. Poorest macroinvertebrate diversity and taxon richness is observed at these sites, likely a result of poor habitat, lack of quality food resources and elevated nutrients.

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, and wastewater inputs. Several 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 is expected at sites influenced by SFMW in subsequent monitoring years. 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. Sampling for Year 4 was completed during August to December 2021.

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 to enhance the health of the waterway and improve 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 and 2 of monitoring are provided in Myers *et al*. 2019 and 2020. This report presents the outcomes from Year 3 and makes comparisons with the first two 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 3 monitoring were to:

- Collect monitoring data which contribute to the study objectives.
- Compare the results of Year 3 with the results from Years 1 and 2 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 were 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).

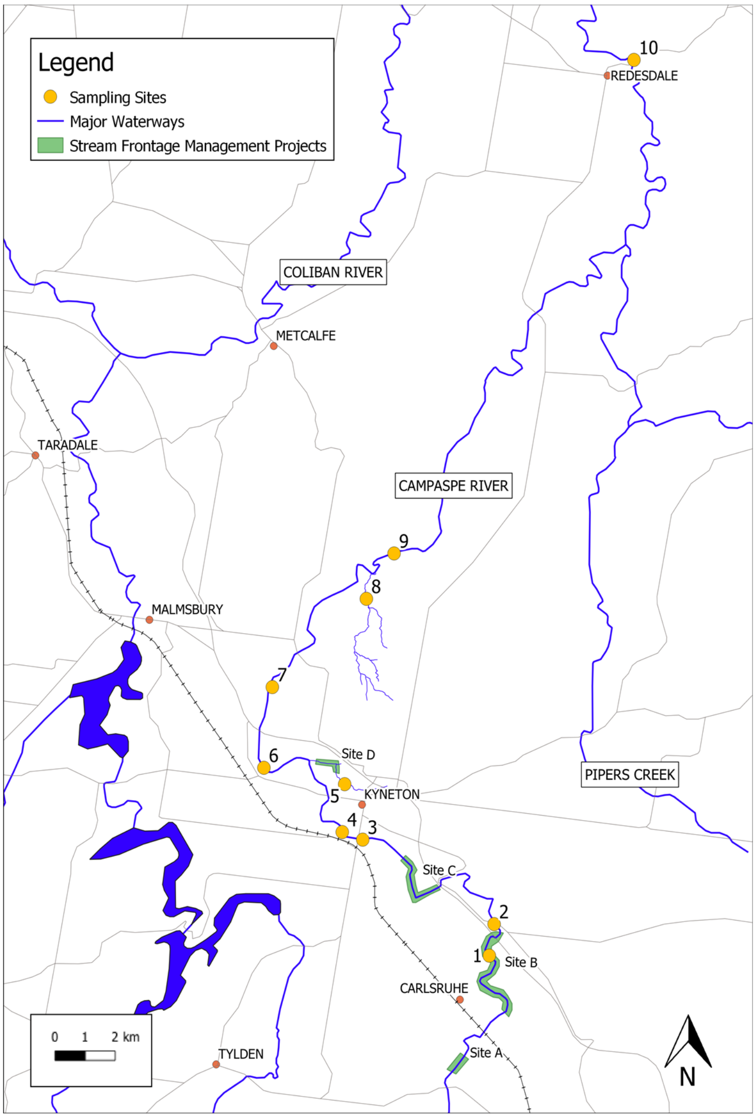


Figure 1: Locations of monitoring sites within the Campaspe River system and SFMW. Water flows from Site 1 downstream to site 10

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 (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 and 3 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										
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 3.

Monitoring	Aug	Nov	Dec	Jan	Feb	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 Bacteroides assay was used to determine the origin (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. At the end of Year 3 monitoring (February 2021), works across all SFM sites were completed and sites are now largely in the maintenance phase of the project.

Rainfall

In Year 3 monthly rainfall was highest in January (69.8 mm), and lowest in February (19 mm) (Figure 2). Below average monthly rainfall was reported for all monitoring months except for January. Mean monthly rainfall in Year 3 (44.3 mm) was similar to that of Year 1 (44.5 mm), and greater than that in Year 2 (34.8 mm).

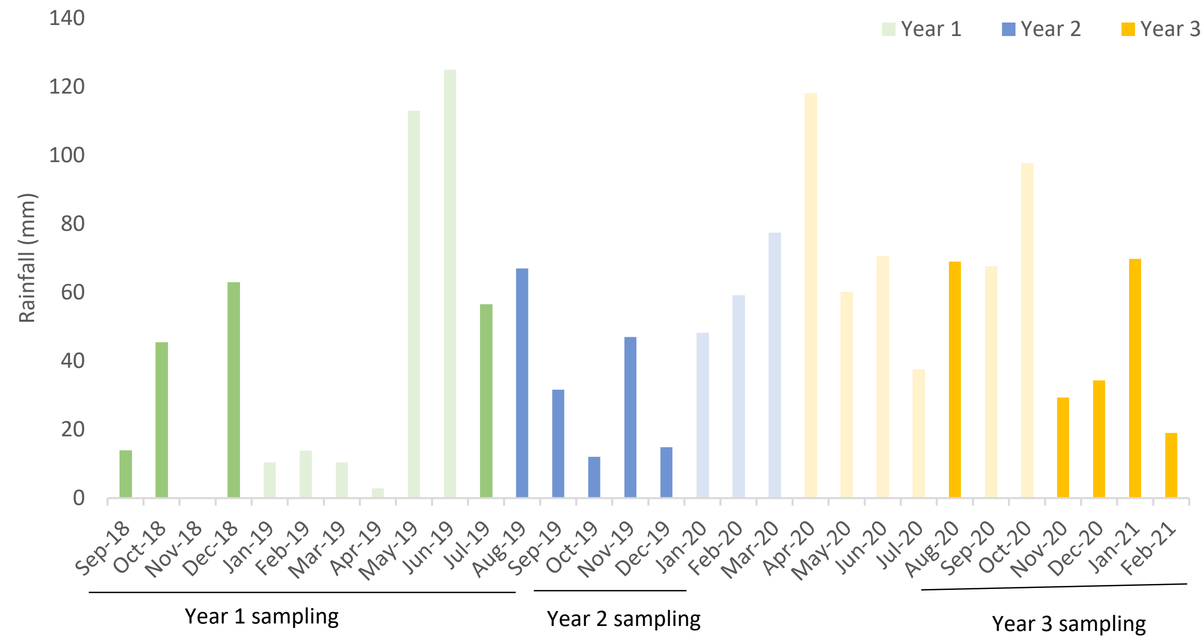


Figure 2: Monthly rainfall at Kyneton from year 1 (2018) to year 3 (2021) (weather observation station: 088036, Kyneton Post Office) (BOM, 2021). Solid coloured bars represent sampling months. No data was available for November 2018.

Surface Water Chemistry

Physico-chemistry

Surface water temperatures were temporally variable. Highest water temperatures (25.6°C) were recorded at Site 3, while lowest water temperature (7.2°C), was at Site 9 (Table 4); however, mean water temperatures were highest at Site 4 (19.5°C), and lowest at Site 8 (13.2°C). Overall, there has been a general trend of increased water temperatures over time, with the mean temperatures recorded being higher at most sites in Year 3 of monitoring, compared to previous years (Table 5).

Mean dissolved oxygen concentrations were highest at Site 4 (84.4% saturation) and lowest at Site 7 (30.3%) (Table 4). Comparing dissolved oxygen annually, shows Sites 7 and 8 tend to have lower dissolved oxygen concentrations (<66%), of all sites, however there are no distinct trends for the other 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 Site 4 which was 8.2pH units (Table 4). Exceedances of the ERS range were also observed at sites 3 and 9 where the maximum and minimum range were exceeded during January 2021, respectively (Table 4). An annual comparison of pH indicates that at Sites 1, 3, 6 it is stable (Table 5). At Sites 7, 9 and 10 on the Campaspe and tributary Sites 5 and 8 a decrease in pH is observed over the 3 years of monitoring and Site 4 where there has been an increase (Table 5).

Electrical conductivity was similar across sites in the Campaspe in year 3 monitoring ranging 379 to 579 µS/cm (Table 4). Electrical conductivity was noticeably greater in the tributary sites 5 and 8, being 823 and 1081 µS/cm respectively (Table 4). Site 8 has persistently exhibited high electrical conductivity throughout the three monitoring years (means ranging 1081 to 1333 µS/cm), while there has been an increase in electrical conductivity for site 5 over time. All other sites show relatively stable electrical conductivity annually (Table 5).

Mean turbidity measures exceeded the ERS trigger value of 15 NTU at Sites 1 and 7, while maximum measured turbidity exceeded at sites 2, 9, 10, 5 and 8 in Year 3 sampling (Table 4). Annually turbidity varies across sites, however Site 8 shows consistent exceedance of ERS trigger values in Years 1 and 2 of monitoring, with single exceedances in either Year 2 or 3 observed for sites 1, 7 and 5 (Table 5).

Table 4. Mean, minimum and maximum temperatures, dissolved oxygen, pH, electrical conductivity and turbidity measured across sites during Year 3 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	18.0	9.5	22.5	58.0	29.5	85.2	7.5	7.0	7.9	399.0	302.0	508.0	**23.4**	6.1	**52.2**
2	15.3	7.9	20.7	67.2	42.1	96.5	7.5	6.9	8.0	379.5	311.0	515.0	13.1	5.2	**19.2**
3	19.3	8.2	25.6	77.5	51.8	92.0	7.8	7.4	**8.4**	461.8	373.0	606.0	5.8	4.2	7.0
4	19.5	9.3	24.0	84.4	65.1	107.2	**8.2**	7.5	**8.7**	419.4	285.0	551.0	5.5	2.7	9.0
6	17.8	7.9	23.8	65.1	50.8	90.6	7.3	**6.7**	7.9	482.8	394.0	651.0	7.4	3.8	10.9
Kyneton WRP discharge between sites 6 and 7															
7	14.3	8.2	18.4	30.3	15.1	68.0	7.1	6.9	7.3	478.2	365.0	586.0	**22.2**	4.7	**35.5**
9	15.6	7.2	19.7	60.0	41.3	79.5	7.3	**6.7**	7.6	557.2	452.0	772.0	9.5	7.2	**113.2**
10	17.2	8.0	21.8	69.2	52.6	77.3	7.3	6.8	8.2	579.6	461.0	698.0	11.4	4.0	**20.6**
Tributaries															
5	18.5	8.0	23.6	61.7	34.0	88.0	7.3	7.1	7.8	823.2	441.0	1032.0	8.5	5.1	**15.7**
8	13.2	8.4	16.6	58.7	20.4	83.0	7.0	6.9	7.1	1081.0	948.0	1165.0	11.3	5.2	**20.5**
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 and 3 monitoring. Values in bold 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	1	2	3	1	2	3	1	2	3	1	2	3
Campaspe River															
1	13.9	15.9	18.0	60.1	79.0	58.0	7.65	7.8	7.5	400	391.4	399.0	7.79	8.8	**23.4**
2	13.5	16.1	15.3	55.5	85.5	67.2	7.59	**8.1**	7.5	383.8	395.4	379.5	6.25	14.3	13.1
3	14.2	18.4	19.3	85.3	86.1	77.5	7.73	7.7	7.8	459.6	445.8	461.8	4.98	9.0	5.8
4	13.2	18.0	19.5	87	86.6	84.4	7.76	7.8	**8.2**	433.6	424.6	419.4	4.68	9.7	5.5
6	15.5	18.4	17.8	71.8	78.9	65.1	7.56	7.2	7.3	412.4	431.2	482.8	9.93	9.1	7.4
Kyneton WRP discharge between sites 6 and 7															
7	13.9	13.0	14.3	66.1	56.3	30.3	**8.03**	7.3	7.1	430.8	452.2	478.2	10.13	9.0	**22.2**
9	14.1	14.6	15.6	79.8	78.0	60.0	**8.17**	7.4	7.3	491.6	494.9	557.2	6.35	7.9	9.5
10	15.8	15.6	17.2	82.3	81.2	69.2	7.73	7.3	7.3	591	580.6	579.6	7.65	8.7	11.4
Tributaries															
5	13.6	16.7	18.5	67	81.8	61.7	7.7	7.5	7.3	556.8	611.6	823.2	10.09	**15.4**	8.5
8	14.9	13.9	13.2	66.3	49.6	58.7	7.7	7.0	7.0	1173	1333.0	1081.0	**15.78**	**34.8**	11.3
Trigger Values	**-**			**max 130**			**6.8-8.0**			**≥2000**			**≥15**		

Nutrients

Total nitrogen concentrations ranged from 0.5 mg/L to 3.9 mg/L (Figure 3) in Year 3 of monitoring, and exceeded the ERS guideline value (≤1.05 mg/L) at Campaspe River sites on:

- One occasion for Site 3 and 4 (January 2021)
- Two occasions for Site 9 (January and February 2021)
- Three occasions for Sites 2 (December 2020; January and February 2021), 7 and 10 (November 2020, January and February 2021)

In the Tributary sites, exceedances were observed on:

- All months expect December 2020 for Site 5
- Two occasions at Site 8 (November 2020 and February 2021)

Mean total nitrogen concentrations was generally comparable across sampling years at Campaspe River sites 3 and 4 (Figure 4). However, at Campaspe River Sites 2 and 7, there has been a gradual increase in mean total nitrogen, while at Campaspe River Sites 1 and 6 and tributary Site 5 concentrations increased from Year 1 to Year 2 and have since stabilised. At Campaspe Sites 9 and 10 mean concentrations increased in Year 3, 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 3 of monitoring and ranged from <0.01 to 0.36 mg/L (Figure 3). Mean ammonia concentrations are generally stable at most Sites across monitoring years, with gradual declines observed for Sites 6, 7 and 8 (Figure 5).

Monthly mean total phosphorus ranged from 0.01 to 0.63 mg/L over the Year 3 monitoring period (Figure 6) and exceeded the ERS guideline value (≤0.055 mg/L) at Campaspe River sites on:

- Two occasions at Site 4 (November 2020 and January 2021)
- Three occasions at Site 10 (November 2020, January and February 2021)
- Four occasions at Sites 3 and 6 (November/December 2020 and January/February 2021)
- All occasions at Sites 1, 2, 7 and 9

In the Tributary sites, exceedances were observed on:

- Two occasions for Site 5 (January and February 2021)
- All sampling occasions at Site 8

Mean total phosphorus has fluctuated annually at Campaspe Sites 1, 2 and 3 (Figure 7), while at Sites 4, 6, 9 and 10 and the tributary Sites 5 and 8 has generally decreased (Figure 7). At Campaspe River Site 7 there has been a steady increase in mean total phosphorus (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 0.27 mg/L and were consistently lowest in the upper reaches Campaspe River (Sites 3-6) and at site 10 and Tributary Site 5, and highest at Campaspe River Sites 7 and 9 and Tributary Site 8 (Figure 6). Across all study sites, concentrations spiked in November 2020 (Figure 6).

On an annual basis, orthophosphate concentrations have remained stable across Campaspe River Sites 1, 2 and 4 (Figure 8). While at Campaspe Sites 3, 6 and 10 there have been steady increases and at Sites 7, 9 and Tributary Site 5 steady declines (Figure 8). The upper reaches of the Campaspe River (sites 1 to 6) and Site 10 consistently have lower concentrations of orthophosphate throughout the 3 years of monitoring, while Campaspe Sites 7 and 9 and Tributary Site 8 have seen declines since Year 3 monitoring, still consistently have the highest concentrations (Figure 8).

The ratio of orthophosphate to total phosphorus was highly variable across the Year 3 sampling period, highest ratios were observed in November 2020 at all sites while lowest ratios were observed at most sites in January 2021 (Figure 9). Orthophosphate ratios have generally increased annually at Campaspe Sites 1, 3, 4 and 6 as well as the two tributary sites (5 and 8), while they have tended to decrease at Campaspe Sites 2, 7, 9 and 10 (Figure 10).

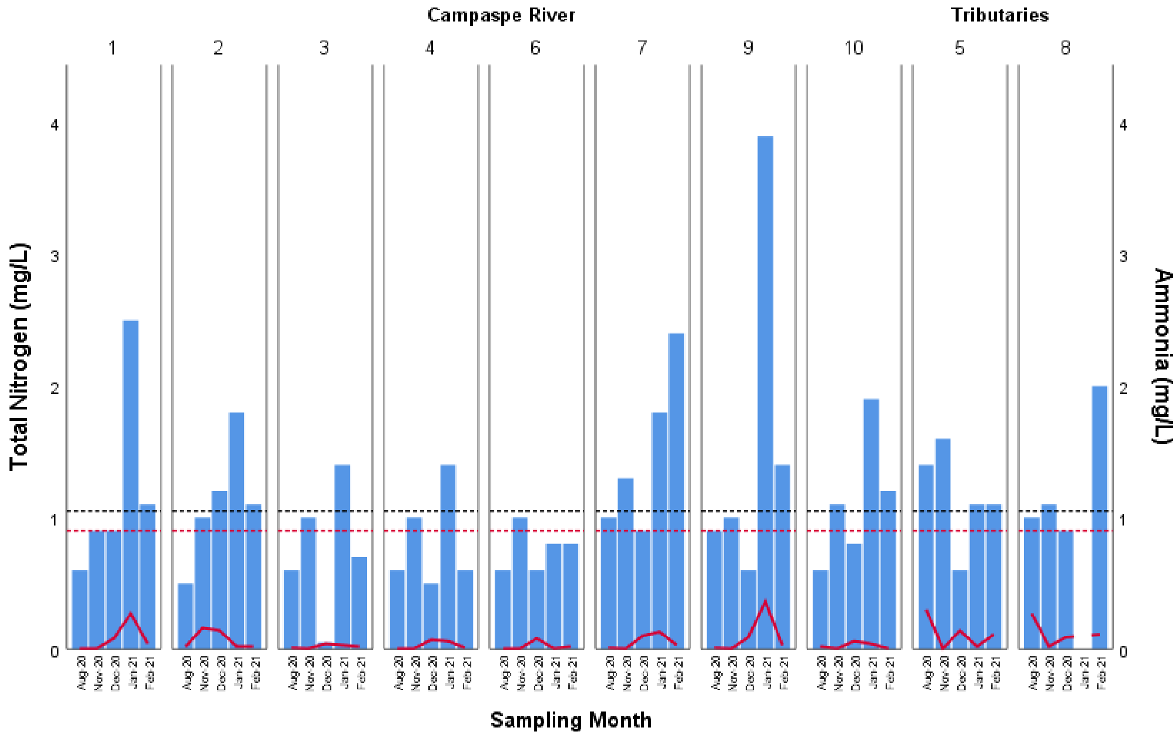


Figure 3. Monthly total nitrogen (blue bars) and ammonia (red line) concentrations in surface waters during Year 3 monitoring. The horizontal black and red dashed lines indicate ERS water quality guideline values for Total Nitrogen (1.05mg/L) and Ammonia (0.9mg/L) respectively. The Kyneton WRP discharge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

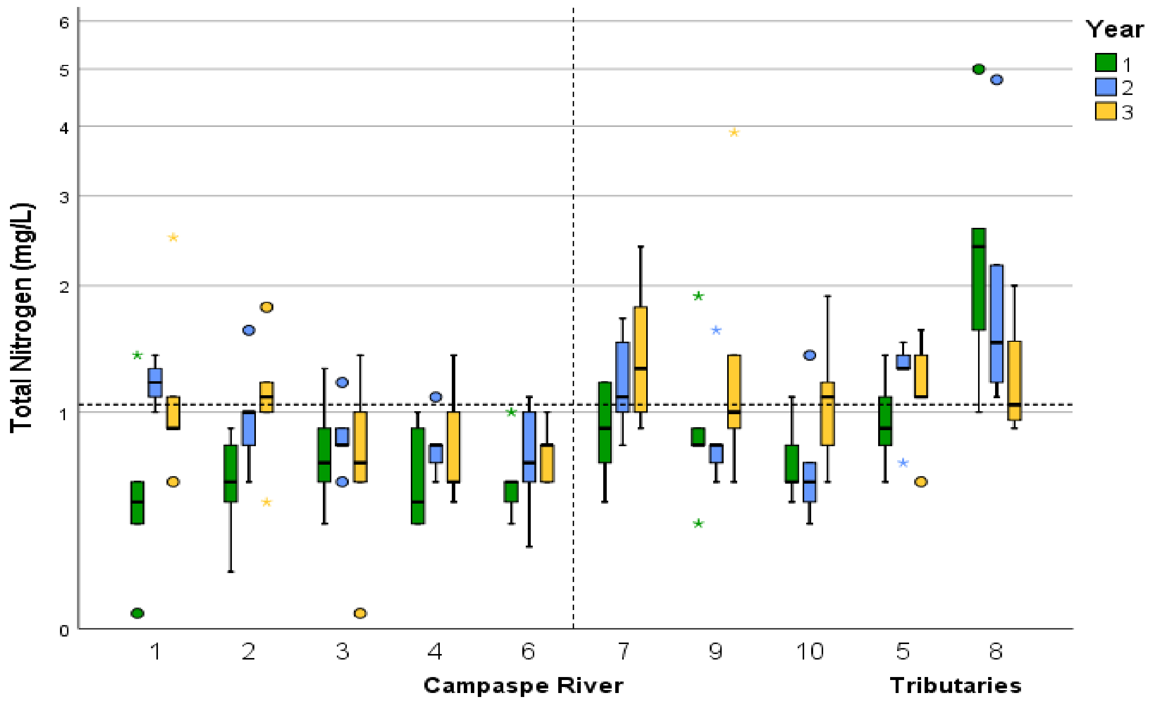


Figure 4. Total nitrogen concentrations in surface waters during Years 1 to 3 monitoring. The vertical dashed line indicates Kyneton WRP discharge between Sites 6 and 7. The horizontal dotted line indicates ERS water quality guideline value. N = 5.

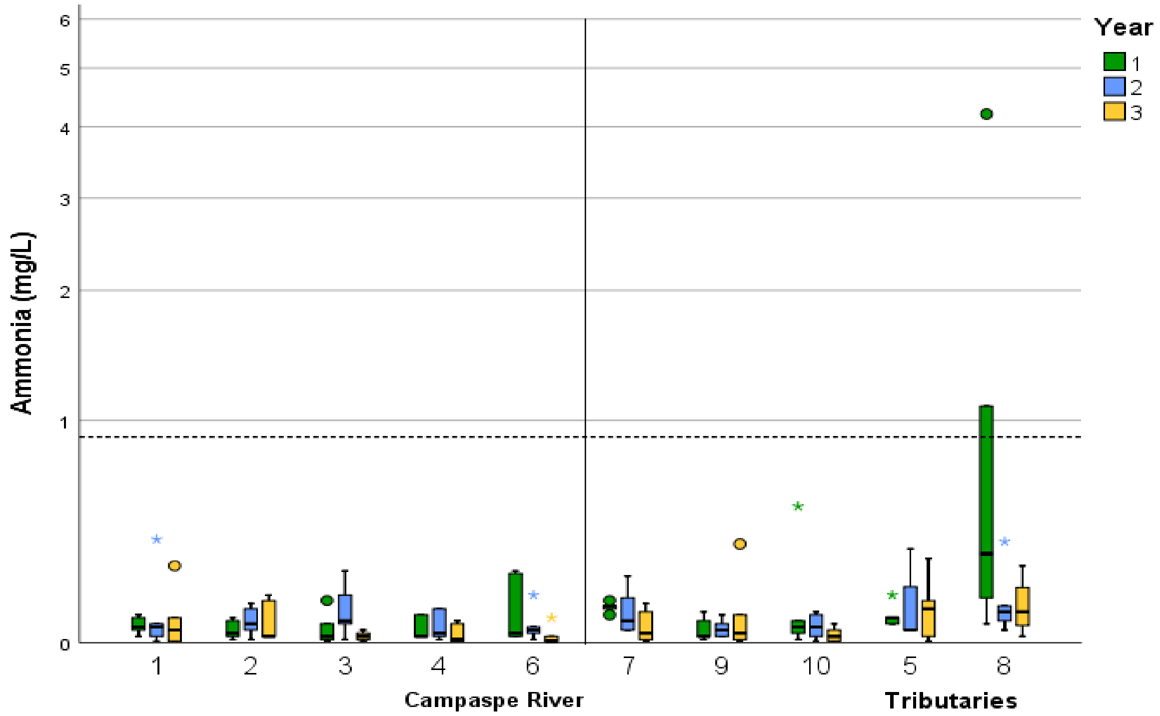


Figure 5. Ammonia concentrations in surface waters during Years 1 to 3 monitoring. The vertical dashed line indicates Kyneton WRP discharge between Sites 6 and 7. The horizontal dotted line indicates ERS water quality guideline value. N = 5.

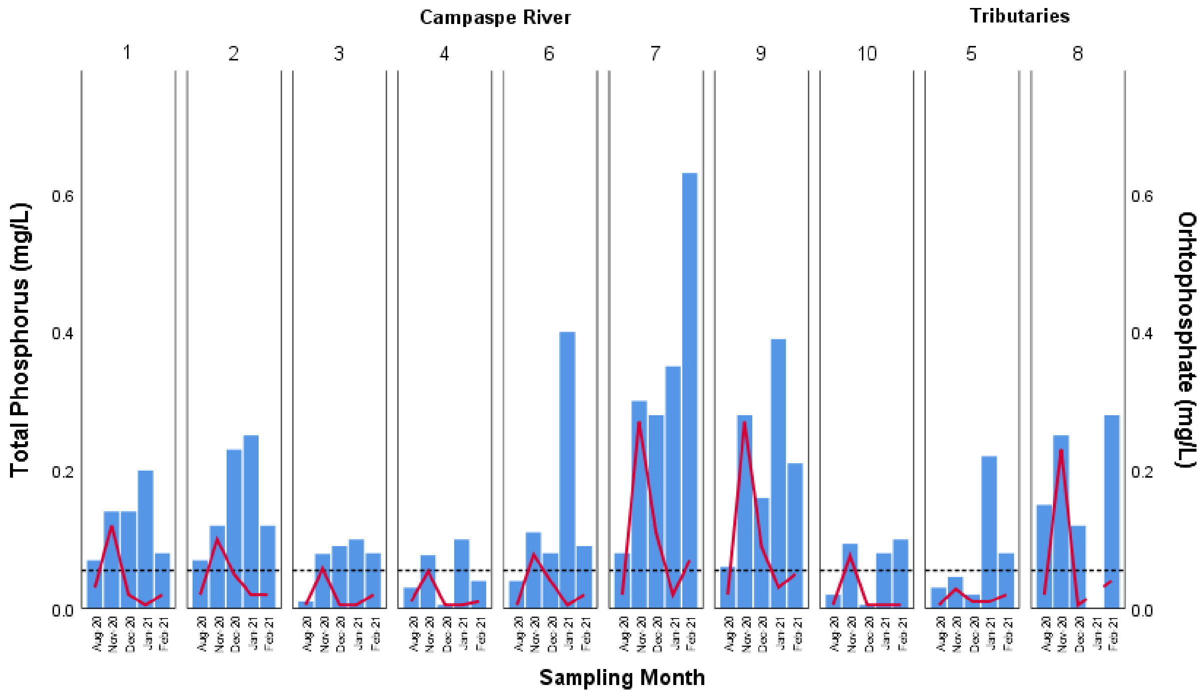


Figure 6. Monthly total phosphorous (blue bars) and orthophosphate (red line) concentrations in surface waters during Year 3 of monitoring. The horizontal black dashed line indicates ERS water quality guideline value for Total Phosphorus (0.055mg/L). The Kyneton WRP discharge is between Sites 6 and 7.

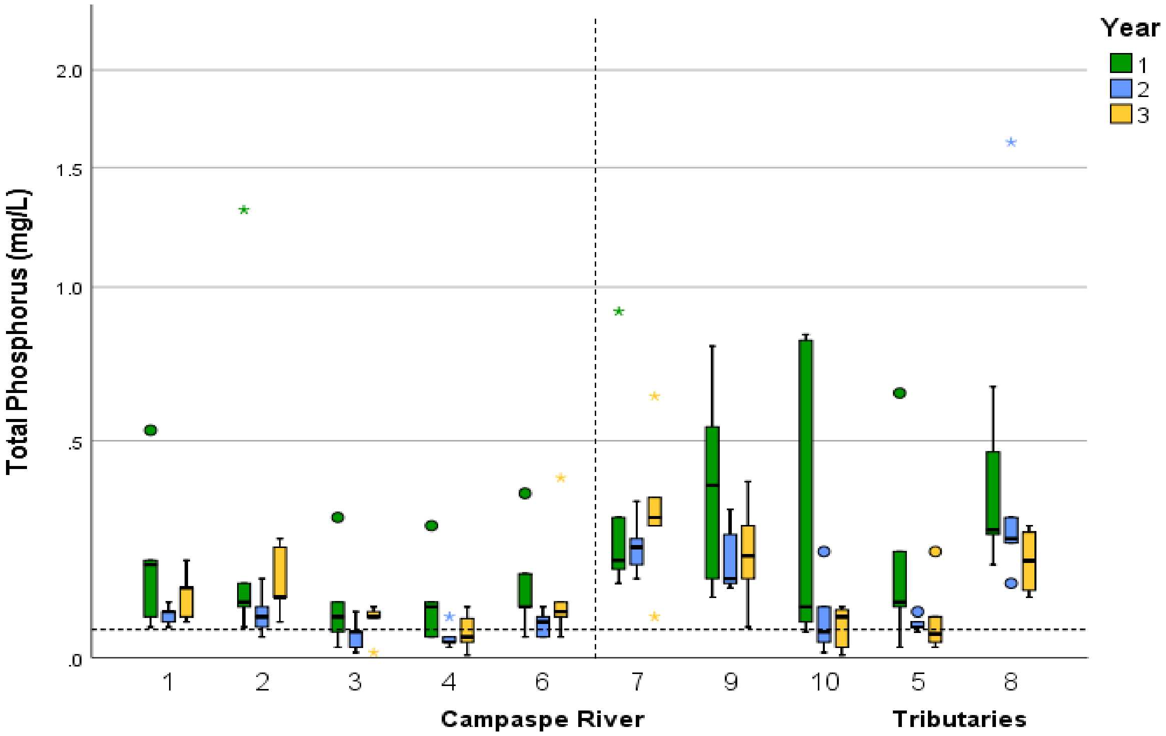


Figure 7. Total phosphorus concentrations in surface waters during Years 1 to 3 of monitoring. The vertical dotted line indicates Kyneton WRP discharge between Sites 6 and 7. The horizontal dotted line for total phosphorus indicates ERS water quality guideline value. N = 5.

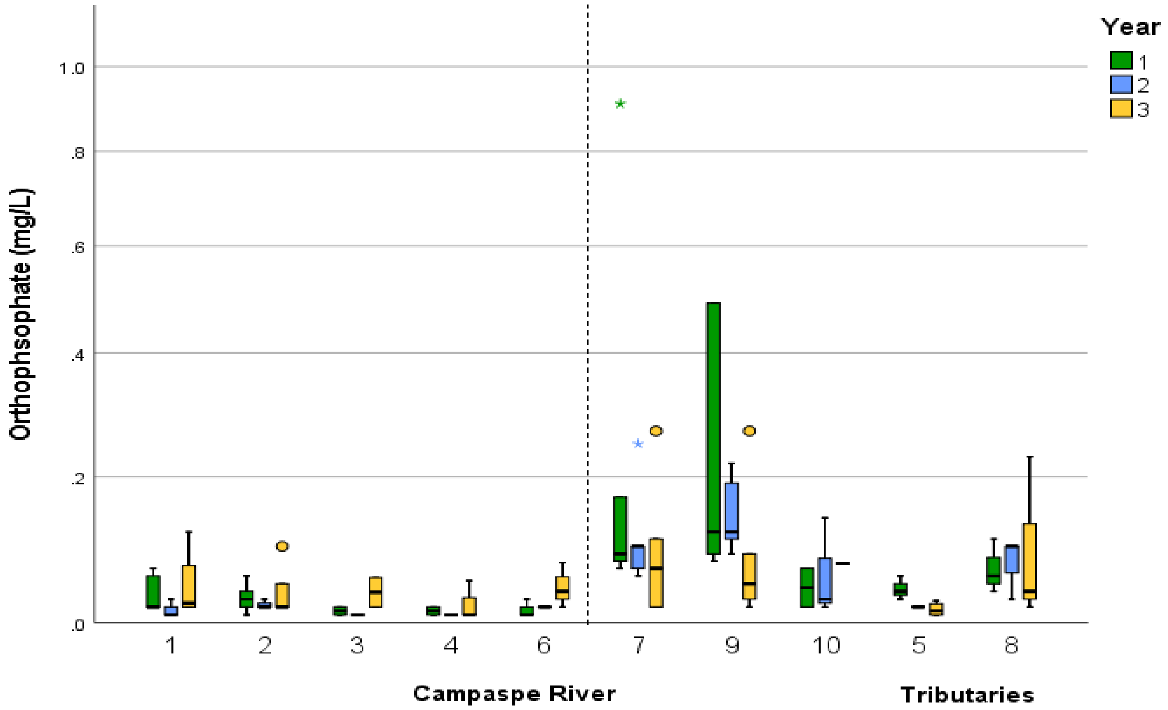


Figure 8. Orthophosphate (bottom) concentrations in surface waters during Years 1 to 3 of monitoring. The vertical dotted line indicates Kyneton WRP discharge between Sites 6 and 7. N = 5.

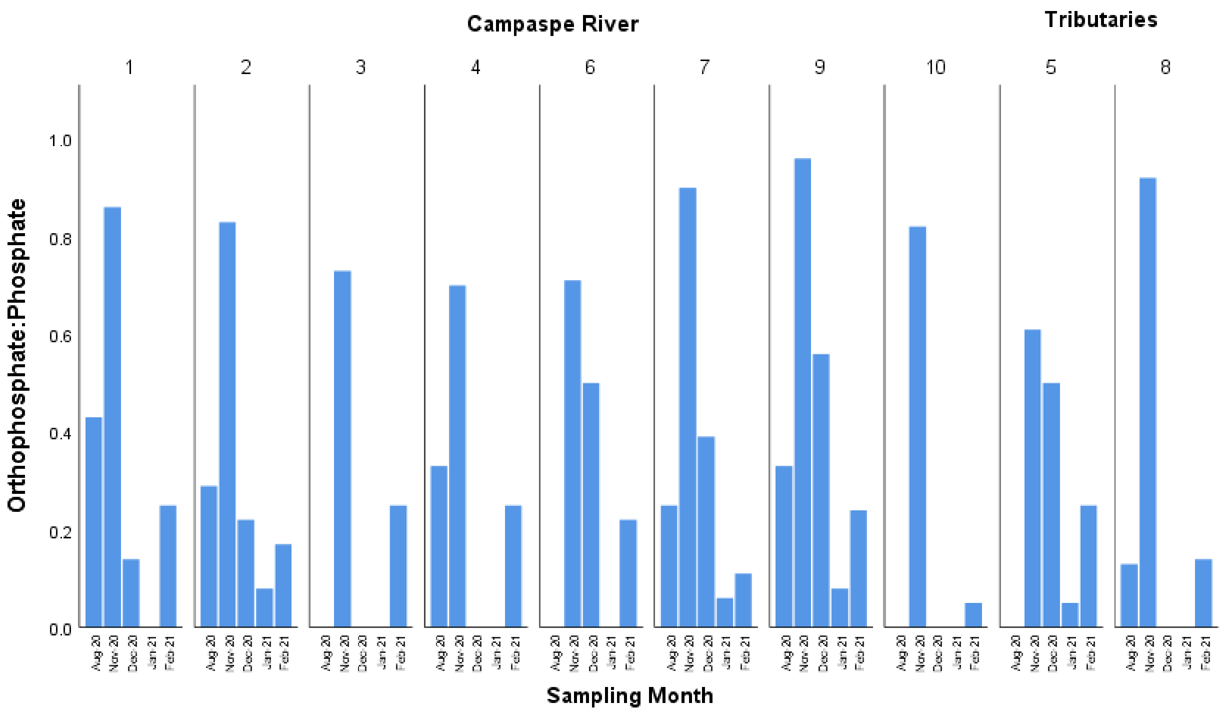


Figure 9. Ratio of orthophosphate to total phosphorus in surface waters at study sites from August 2020 to February 2021. No result is shown where orthophosphate was below the limit of reporting. The Kyneton WRP discharge is between Sites 6 and 7.

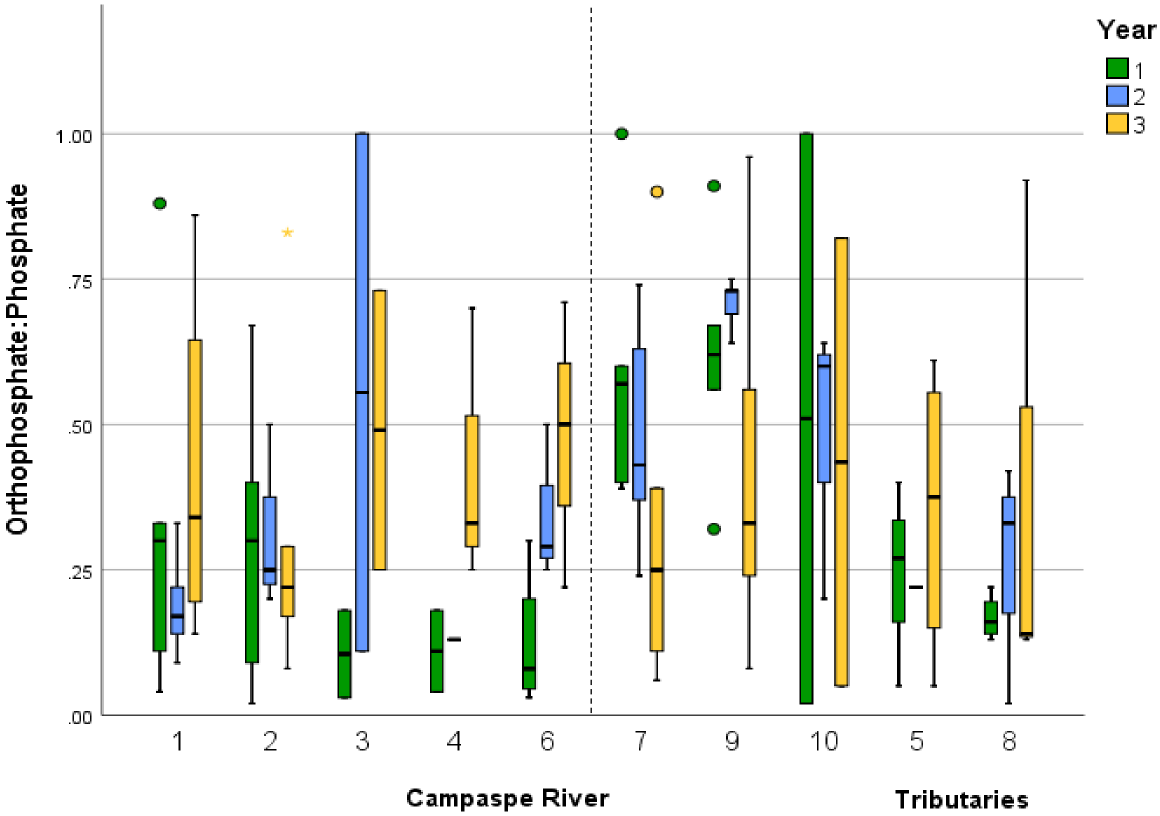


Figure 10. Ratio of orthophosphate to phosphorous in years 1 to 3 of monitoring. The vertical dotted line indicates Kyneton WRP discharge between Sites 6 and 7.

Faecal Monitoring

E. coli levels in Year 3 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 Site 4 in January 2021 and Tributary Site 8 in November 2020 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 were generally stable at Sites 1, 7, 9 and 8 across years 1 to 3 of monitoring (Figure 12). At Campaspe Site 4 a marked increase in mean *E. coli* was observed for Year 3, similar at Site 6 mean *E. coli* increased in Year 3, while at Tributary Site 5 there has been a stable decline across monitoring years (Figure 12). Tributary Site 8 has exceeded ERS guidelines for class A/B water in the last two monitoring years, while Campaspe Site 7 and Tributary Site 8 have also exceeded ERS guidelines for class C water (Figure 12).

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

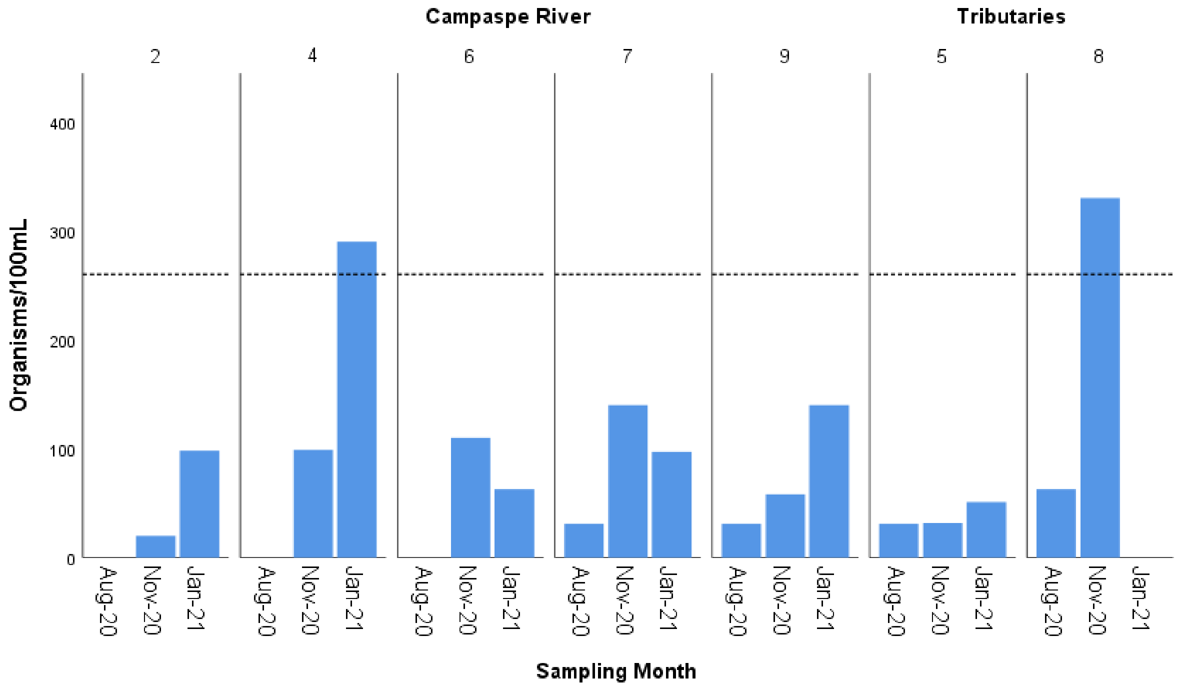


Figure 11: *E. coli* measured at select sites during year 3 monitoring. The horizontal dashed line indicates ERS guideline value for class A/B water. The Kyneton WRP discharge point is between Sites 6 and 7.

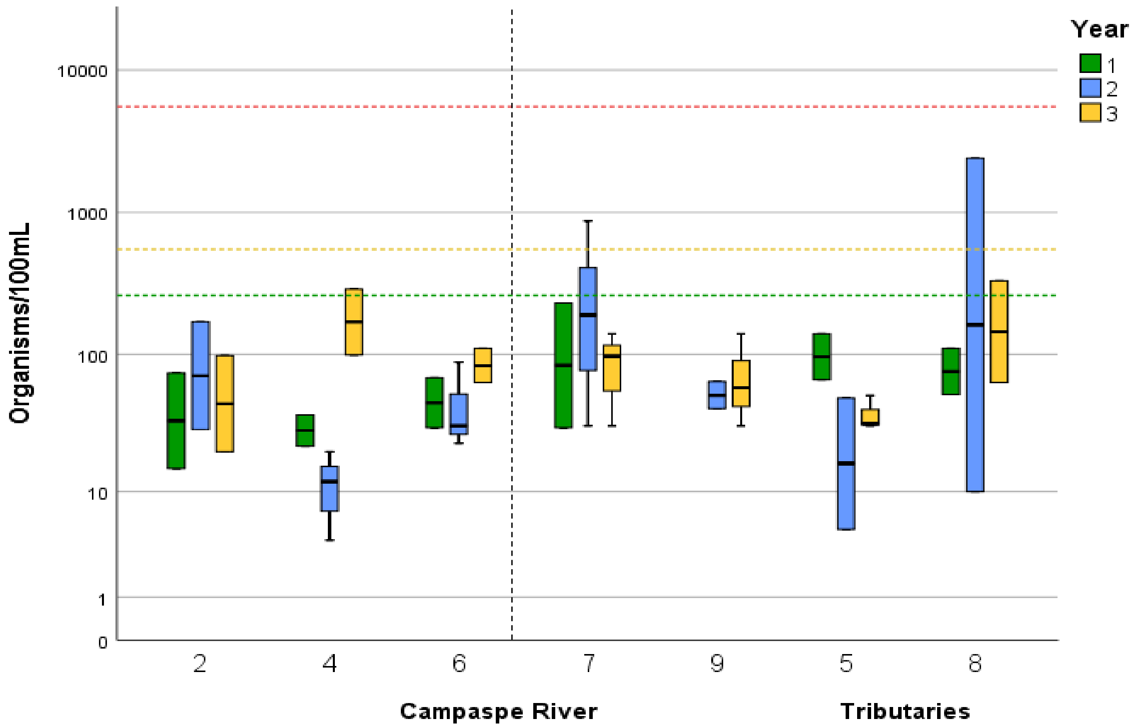


Figure 12. Mean *E. coli* concentrations during years 1, 2 and 3 of monitoring. The green, yellow and red horizontal dashed lines indicate ERS guideline values for class A/B, class C, and class D water respectively. The black vertical dashed line indicates Kyneton WRP discharge point between Sites 6 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 November 2019.

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	Sept-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

Bold indicates values that exceed the GI risk benchmark of 4.50 log10 GC/L (HF183) and 5.14 log10 GC/L (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 7 and 8. Seven different PPCPs and six pesticides were detected in Year 3 monitoring. This included the pharmaceuticals venlafaxine and oleandomycin at 3 sites (Campaspe Sites 7, 9, 10); sulphapyridine and sulfamethoxazole at 2 sites (Campaspe Sites 7, 9); trimethoprim at Campaspe Site 7, paracetamol at 3 sites (Campaspe Site 7 and Tributary sites 5 and 8) and carbamazepine detected at all sites except tributary Site 5 (Table 7). Two of these pharmaceuticals have been detected in previous monitoring years. Venlafaxine was detected during Year 1 monitoring at Campaspe Site 9 and Tributary Site 8, while carbamazepine was detected during Year 2 monitoring at Campaspe Site 1, 2, 3, 7, 9 and 10 (Table 7). Cholestrol was also detected during Year 2 monitoring at Campaspe Site 6 and 7 but has not been detected since.

Of the pesticides detected in Year 3, two were herbicides, three fungicides and one an insecticide (Table 8). The herbicide simazine was detected at all sites excluding Tributary Site 8, while diuron was detected at Campaspe Site 7 and Tributary Site 5. The fungicides carbendazim, and propiconazole and tebuconazole were detected at three sites (Campaspe Sites 8, 9 and Tributary Site 5) and one site

(Tributary Site 5) respectively (Table 8). The insecticide imidacloprid was detected at five sites (Campaspe Sites 6, 7, 9, and 10 and Tributary Site 5). Simazine, diuron and imidacloprid have been detected in previously monitoring years, while for the three fungicides Year 3 was the first time they have been detected (Table 8). In previous years several other herbicides (atrazine, MCPA, triclopyr and 2,4-D) and the insecticide carbaryl have been detected (Table 8).

Sediment Chemistry

Concentrations of heavy metals, petroleum hydrocarbons and pesticides found in the sediments of sites are presented in Table 9. ANZECC/ARMCANZ Sediment Quality Guidelines (2000) were exceeded for chromium, lead, mercury, nickel, zinc and total petroleum hydrocarbons (TPH).

Nickel exceeded the guideline value at all sites in Year 3 of monitoring, which is consistent with previous years monitoring (Table 9). While mercury exceeded at two sites (Campaspe Site 9 and Tributary site 5) and lead, zinc and chromium each exceed at a single site (Tributary site 5 for lead and zinc and Campaspe site 9 for chromium) during Year 3 (Table 9). Concentrations of lead and zinc in sediments collected from Site 5 have consistently exceeded the guideline value over the three years of monitoring. While mercury concentrations at Campaspe Site 9 have consistently exceeded the guideline value, they are lower in Year 3 than previous years (Table 9).

Petroleum hydrocarbons were above ANZECC/ARMCANZ Sediment Quality Guidelines (2000) at all sites in Year 3 (Table 9). Concentrations ranged from 420-1900 mg/kg, with the lowest concentrations recorded at Sites 7 and 10 (Table 9). While concentrations have consistently exceeded guideline values across all monitoring years, overall, concentrations were generally lower in Year 3 (Table 9).

There were no pesticides detected in sediments during Year 3, however two insecticides bifenthrin and permethrin have been detected in previous monitoring years (Table 9).

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 3.

Site number	Pharmaceuticals and Personal Care Products (µg/L)									
	Venlafaxine		Oleandomycin	Sulphapyridine	Sulfamethoxazole	Trimethoprim	Paracetamol	Carbamazepine		Cholestrol
	Yr1	Yr3	Yr3	Yr3	Yr3	Yr3	Yr3	Yr2	Yr3	Yr2
Campaspe River										
1	<1	<1	<1	<1	<1	<1	<1	1.67	2.2	<10
2	<1	<1	<1	<1	<1	<1	<1	2.38	4.9	<10
3	<1	<1	<1	<1	<1	<1	<1	1.71	3.3	<10
4	<1	<1	<1	<1	<1	<1	<1	<0.5	3	<10
6	<1	<1	2.8	<1	<1	<1	<1	<0.5	5.4	19.8
Kyneton WRP discharge between sites 6 and 7										
7	<1	40	21.5	34	4.8	9.45	4.15	38.33	43	84.6
9	4.6	16	9.1	13	1.4	<1	<1	14.25	46	<10
10	<1	1.1	1.1	<1	<1	<1	<1	4.42	9	<10
Tributaries										
5	<1	<1	<1	<1	<1	<1	7.7	<0.5	<1	<10
8	3.7	<1	<1	<1	<1	<1	1.4	<0.5	2.9	<10

Table 7. Pesticides detected in surface waters using POCIS passive samplers (µg/0.2 g sorbent) across sites for Years 1 to 3.

Site number	Herbicides									Insecticides			Fungicides		
	Simazine		Atrazine	Diuron		MCPA	Triclopyr		2,4-D	Imidacloprid		Carbaryl	Propiconazole	Tebuconazole	Carbendazim
	Yr1	Yr3	Yr1	Yr1	Yr3	Yr1	Yr1	Yr2	Yr2	Yr1	Yr3	Yr1	Yr3	Yr3	Yr3
Campaspe River															
1	<1	12	<1	<1	<1	<1	8.6	16.78	<5	1.3	<1	<1	<1	<1	<1
2	<1	18	<1	<1	<1	<1	9.5	6.84	<5	0.8	<1	<1	<1	<1	<1
3	<1	22	<1	<1	<1	<1	8	14.19	9.55	1.1	<1	<1	<1	<1	<1
4	1.8	58	<1	<1	<1	<1	7.3	9.33	10.97	<1	<1	1.8	<1	<1	<1
6	1.8	56	<1	1.8	<1	12.6	11.2	28.93	8.35	1.3	3.8	<1	<1	<1	<1
Kyneton WRP discharge between sites 6 and 7															
7	<1	42	<1	1.7	3.15	<1	7.5	14.5	<5	3.2	6.4	1.6	<1	<1	2.25
9	<1	22	<1	1.9	1	7.5	7.5	17.84	<5	6	4.5	<1	<1	<1	1.2
10	3.5	18	1.54	1.7	<1	<1	6.1	<2.5	<5	1.9	2.2	3.5	<1	<1	<1
Tributaries															
5	1.7	58	<1	4.8	4.6	<1	5.7	86.65	44.39	3.2	7.6	1.7	4.9	4.7	2.9
8	<1	<1	<1	1.7	<1	<1	15.1	10.28	6.7	<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			Cobalt			Copper			Lead		
	Yr1	Yr2	Yr3	Yr1	Yr2	Yr3	Yr1	Yr2	Yr3	Yr1	Yr2	Yr3	Yr1	Yr2	Yr3	Yr1	Yr2	Yr3	Yr1	Yr2	Yr3	Yr1	Yr2	Yr3
Campaspe River																								
1	<5	<5	<5		170	150	<1	1	1	<1	<1	<1		40	53		10	11		16	16		18	17
2	<5	<5	<5		180	200	<1	1	1	<1	<1	<1		45	49		18	12		30	20		37	23
3	5	<5	<5	210	180	210	1	1	1	<1	<1	<1	73	69	75	26	20	26	28	27	30	19	19	28
4	6	7	<5	200	250	260	1	1	1	<1	<1	<1	62	59	71	28	35	24	29	27	28	35	29	29
6	<5	<5	6	170	170	160	1	1	1	<1	<1	<1	64	66	73	25	27	23	34	37	39	20	22	31
Kyneton WRP discharge between Sites 6 and 7																								
7	<5	<5	<5	150	190	230	<1	1	1	<1	<1	<1	19	65	69	12	14	19	18	21	18	19	22	19
9	11	16	5	220	280	320	1	1	1	<1	<1	<1	17	66	**87**	31	42	18	25	27	29	17	28	20
10	11	8	<5	190	180	160	1	1	1	<1	<1	<1	31	41	37	19	16	28	23	21	18	31	25	18
Tributaries																								
5	10	11	11	260	280	310	1	<1	<1	<1	<1	<1	56	55	57	24	25	42	51	53	48	**87**	**83**	**86**
8	<5	19	<5	310	840	240	1	<1	1	<1	1	<1	54	37	66	43	206	13	24	17	19	12	8	12
Trigger Value*	20			-			-			1.5			80			-			65			50		

* Trigger value from ANZECC/ARMCANZ Sediment Quality Guidelines (2000)
Values **bold** and highlighted in orange exceed trigger value
Values showing < are at the limit of detection

Site Number	Heavy metals (mg/kg)															Petroleum Hydrocarbons (mg/kg)			Pesticides (mg/kg)	
	Manganese			Mercury			Nickel			Vanadium			Zinc			C10 - C36 Fraction (sum)			Bifenthrin	Permethrin
	Yr1	Yr2	Yr3	Yr1	Yr2	Yr3	Yr1	Yr2	Yr3	Yr1	Yr2	Yr3	Yr1	Yr2	Yr3	Yr1	Yr2	Yr3	Yr2	Yr2
Campaspe River																				
1		249	148	<0.1	<0.1	<0.1		**23**	**26**		30	42		100	73		**2190**	**540**	<0.01	<0.01
2		460	155	<0.1	<0.1	<0.1		**27**	**28**		38	39		135	86		**1300**	**970**	<0.01	<0.01
3	777	470	498	<0.1	<0.1	<0.1	**48**	**44**	**54**	62	54	72	171	146	124	**770**	**1020**	**830**	<0.01	<0.01
4	986	1980	767	<0.1	<0.1	<0.1	**46**	**43**	**45**	55	55	57	183	166	170	**550**	**1270**	**1120**	<0.01	<0.01
6	402	508	114	<0.1	<0.1	0.1	**38**	**38**	**40**	53	57	56	173	169	172	**690**	**1480**	**1360**	<0.01	<0.01
Kyneton WRP discharge between Sites 6 and 7																				
7	252	192	302	<0.1	<0.1	<0.1	**24**	**28**	**30**	39	46	50	120	132	125	210	**560**	**420**	<0.01	<0.01
9	1460	1990	1550	**0.7**	**1.2**	**0.2**	**48**	**48**	**64**	61	61	80	81	86	102	**330**	**1010**	**670**	<0.01	<0.01
10	674	479	253	<0.1	<0.1	<0.1	**36**	**33**	**28**	40	38	31	111	96	70	**490**	**3060**	**420**	<0.01	<0.01
Tributaries																				
5	451	427	548	0.1	0.1	**0.2**	**44**	**45**	**42**	50	49	53	**849**	**836**	**722**	**960**	**2050**	**1900**	0.016	0.011
8	1300	13400	554	<0.1	<0.1	<0.1	**35**	**45**	**36**	67	67	68	95	65	78	210	**3730**	**600**	<0.01	<0.01
Trigger Value*	-			0.15			21			-			200			280				

* Trigger value from ANZECC/ARMCANZ Sediment Quality Guidelines (2000)
Values **bold** and highlighted in orange exceed trigger value
Values showing < are at the limit of detection

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 met the biological standards outlined in the Environmental Reference Standards (ERS) objectives. SIGNAL2 scores were below the ERS objectives in Year 3 for all sites except Site 9 (with a score of 10). Sites 6 and 7 along the Campaspe River and tributary Site 5 have consistently had SIGNAL2 scores below 3.4 in the last three years of monitoring. SIGNAL2 scores at Campaspe Site 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 Site 7, and tributary Sites 5 and 8 were below the ERS objectives. Sites 7, 5 and 8 have been consistently below the ERS objectives in the last three years of monitoring (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 chironimidae) and Micronectiidae (true bugs) Chiltoniidae (sideswimmers) (Figure 14 a-f). In Year 3, 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, a number of the most common taxa have been recorded for the first time in Year 3 including Chiltoniiidae (sideswimmers), Leptoceridae (cased caddisflies), Leptophlebiidae (mayflys) and Physidae (bladder snails) (Figure 14 a,f,g,h). Atyidae (shrimp) and Dytiscidae larvae (diving beetles) were also found for the first time in Year 3 at tributary Site 5 and Leptoceridae (cased caddisflies) at Campaspe Site 9 (Figure 14 d, g, j). Chironominae (non-biting midge Chironomidae) were present at most sites in Year 1 and Year 3 while Gripopterygidae (stoneflies) were absent across all sites in Year 3 (Figure 14 i, k).

Table 910). Diversity was lowest at Campaspe Site 7 (12 families) and greatest at Campaspe Sites 4 (23 families), 6 (27 families) and 9 (24 families). The number of families present at tributary Site 8 has been consistently improving and was just below the ERS objective (19 families). Except for Campaspe Site 7, the number of families supported at each site has remained constant (sites 4, 10 and 5) or improved (sites 6, 9 and 8) since the first year of monitoring.

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 10 (5 families). In contrast, EPT taxa were absent from both Campaspe site 7 and tributary Site 5. Site 7 consistently has no or few EPT taxa over the 3 years. Improvements to the number of EPT taxa at Tributary Site 8, was also observed in Year 3.

All sites showed variation in the taxonomic composition of macroinvertebrate assemblages across the three years (Figure 133). 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 marginal change was seen at Campaspe Site 10 in Year 3 despite a large change in Year 2. Overall, there is a separation between Years, with Years 1 and 2 more like each other in comparison to Year 3 and Year 3 more like Year 1 compared to Year 2.

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 chironimidae) and Micronectiidae (true bugs) Chiltoniidae (sideswimmers) (Figure 14 a-f). In Year 3, 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, a number of the most common taxa have been recorded for the first time in Year 3 including Chiltoniiidae (sideswimmers), Leptoceridae (cased caddisflies), Leptophlebiidae (mayflys) and Physidae (bladder snails) (Figure 14 a,f,g,h). Atyidae (shrimp) and Dytiscidae larvae (diving beetles) were also found for the first time in Year 3 at tributary Site 5 and Leptoceridae (cased caddisflies) at Campaspe Site 9 (Figure 14 d, g, j). Chironominae (non-biting midge Chironomidae) were present at most sites in Year 1 and Year 3 while Gripopterygidae (stoneflies) were absent across all sites in Year 3 (Figure 14 i, k).

Table 9. Macroinvertebrate biological indices at each site sampled during Years 1 to 3.

Site	SIGNAL2			Number of families			Number of EPT families		
	2018	2019	2020	2018	2019	2020	2018	2019	2020
Campaspe River									
1	**3.00**	**3.17**	N/A	20	**19**	N/A	2	2	N/A
2	3.40	3.80	N/A	20	**15**	N/A	3	3	N/A
3	4.05	3.73	N/A	20	27	N/A	6	5	N/A
4	3.54	3.77	**3.18**	24	24	23	6	5	5
6	**3.13**	**3.38**	**3.18**	**15**	23	27	2	4	5
Kyneton WRP discharge between sites 6 and 7									
7	**2.94**	**3.31**	**2.67**	**18**	**16**	**12**	0	1	0
9	3.60	**3.23**	3.43	**16**	**14**	24	3	1	4
10	3.60	4.80	**3.20**	**19**	**10**	20	5	3	5
Tributaries									
5	**3.13**	**2.85**	**2.87**	**16**	**13**	**16**	3	1	0
8	**2.89**	3.67	**3.26**	**10**	**12**	**19**	1	1	3
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.

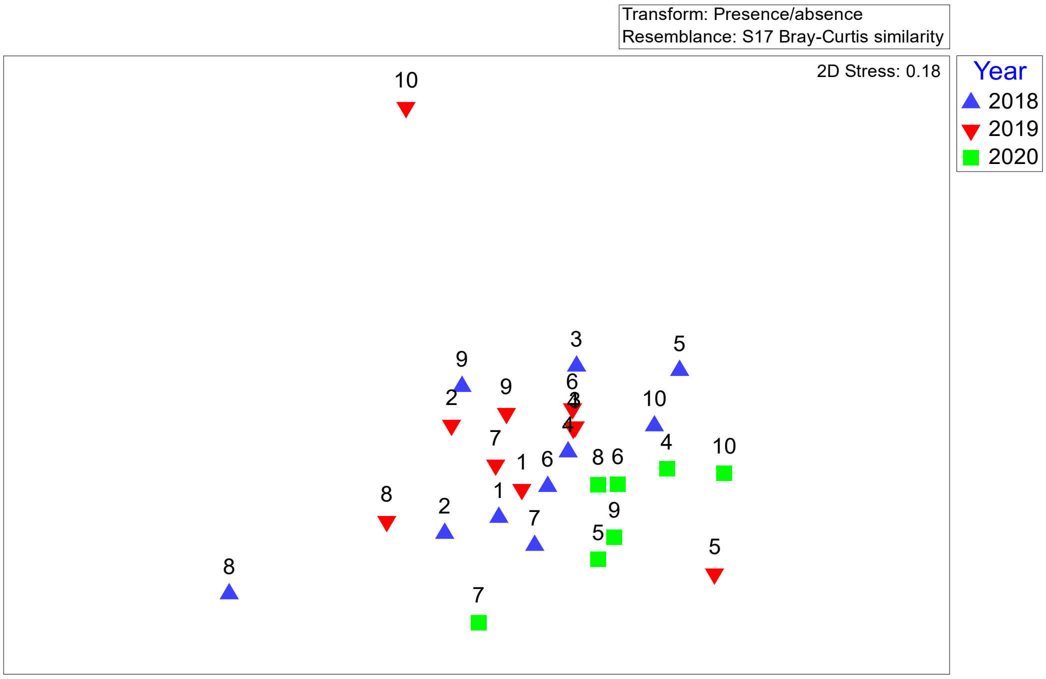
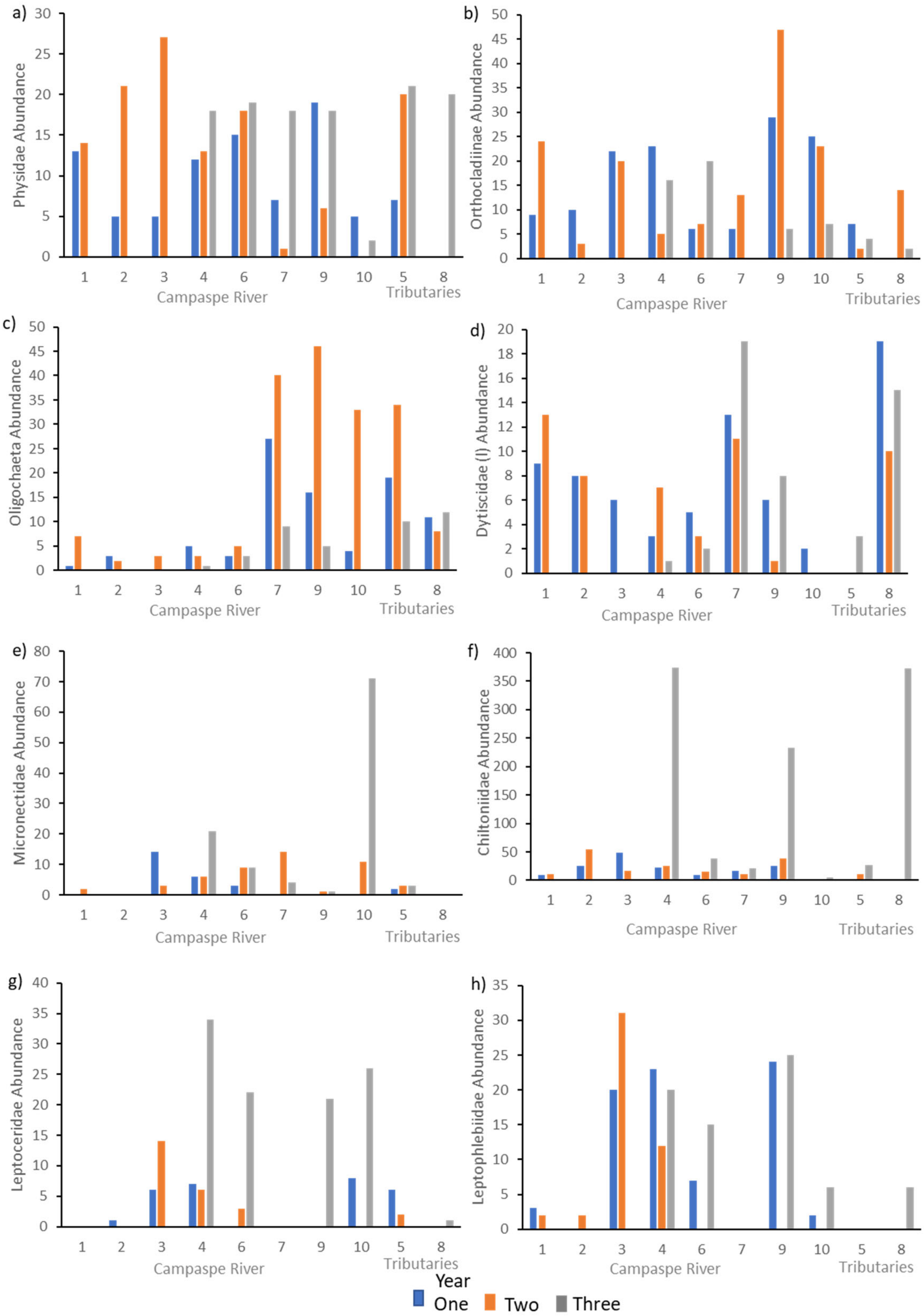


Figure 13. Non-metric multidimensional scaling ordination based on the presence or absence of macroinvertebrate taxa and the Bray-Curtis dissimilarity index. Points close together are similar in composition whereas points further apart have greater variation in their taxonomic composition.


a)
Physidae Abundance
b)
Orthocladiinae Abundance
c)
Oligochaeta Abundance
d)
Dytiscidae (l) Abundance
e)
Micronectidae Abundance
f)
Chiltoniidae Abundance
g)
Leptoceridae Abundance
h)
Leptophlebiidae Abundance
Campaspe River
Tributaries
Year
One
Two
Three

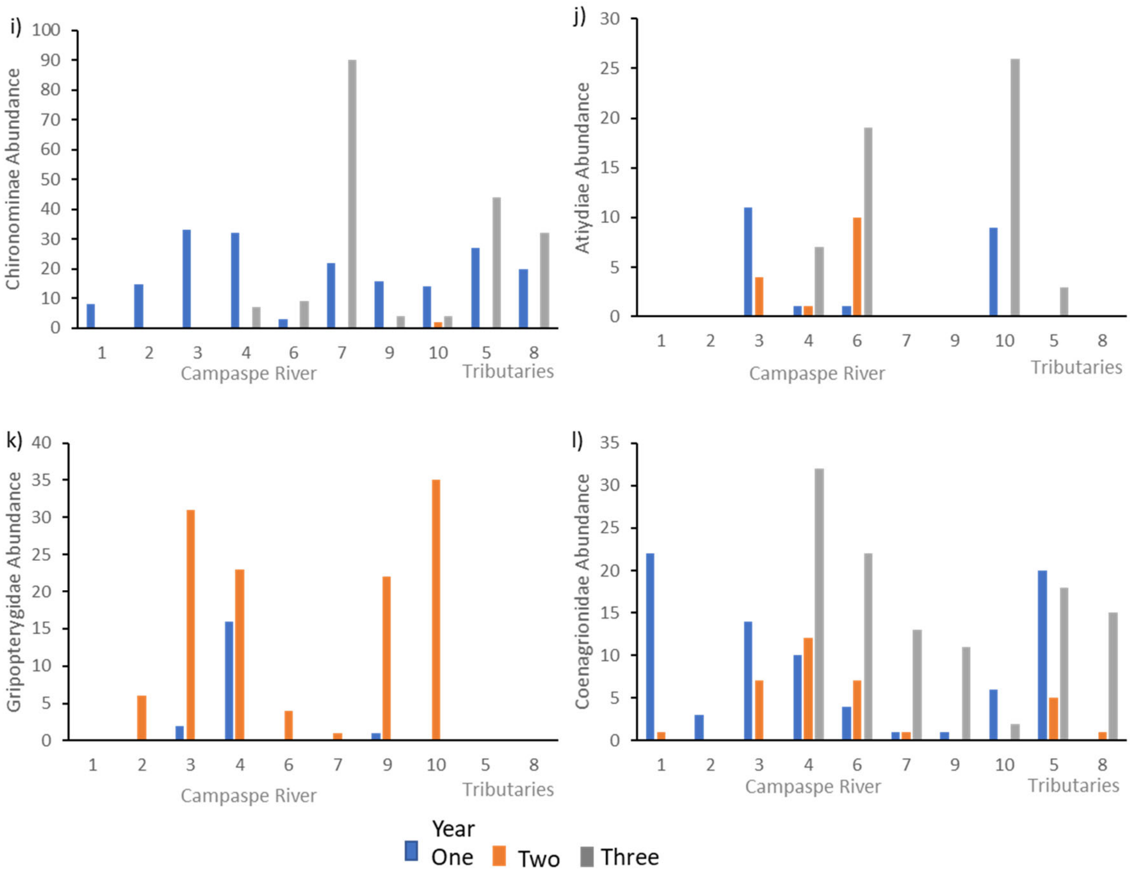


Figure 14: Abundance of common macroinvertebrate taxa at sites sampled during Years 1 to 3. a) Physidae b) Orthocladiinae c) Oligochaeta d) Dytiscdae larvae e) Micronectidae f) Chiltoniidae g) Leptoceridae, h) Leptophlebiidae, i) Chironominae, j) Atyidae, k) Gripopeterygidae, l) Coenagrionidae. Samples 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 November 2020 and January 2021 (Figures 14 and 16). Chlorophyll-a, an indicator of the autotrophic component of algal biofilms, significantly differed between sites during the two sampling months (November 2020 Kruskal-Wallis, H=24.68, df=9, p=0.003 and January 2021 Kruskal-Wallis, H=16.32, df=8, p=0.038) (Figure 14). In general chlorophyll-a concentrations at Campaspe Sites 1, 4, 6, 9, 10 and tributary Site 5 were greater than those at Sites 2, 3, 7 and tributary Site 8 in November 2020, whereas in January 2021 chlorophyll-a concentrations at Campaspe River Site 6 and tributary Site 5 were greater than those at all other sites, expect Site 10. Concentrations of chlorophyll-a at Campaspe Sites 7 and 9 were lower than all other sites (Figure 14).

Seasonal variation within a site was observed, with higher chlorophyll-a measured in November 2020 at Campaspe sites 1, 4, 7, 9 and 10 and tributary Sites 5 and 8, while at Campaspe sites 2, 3 and 6 higher concentrations occurred in January 2021 (Figure 14).

Mean chlorophyll-a concentrations at Campaspe Sites 3, 4, and 7 and tributary Sites 5 and 8 declined in Year 3 compared to previous years (Figure 15). At all other sites concentrations have fluctuated across years or are comparable to previous year's results (Figure 15).

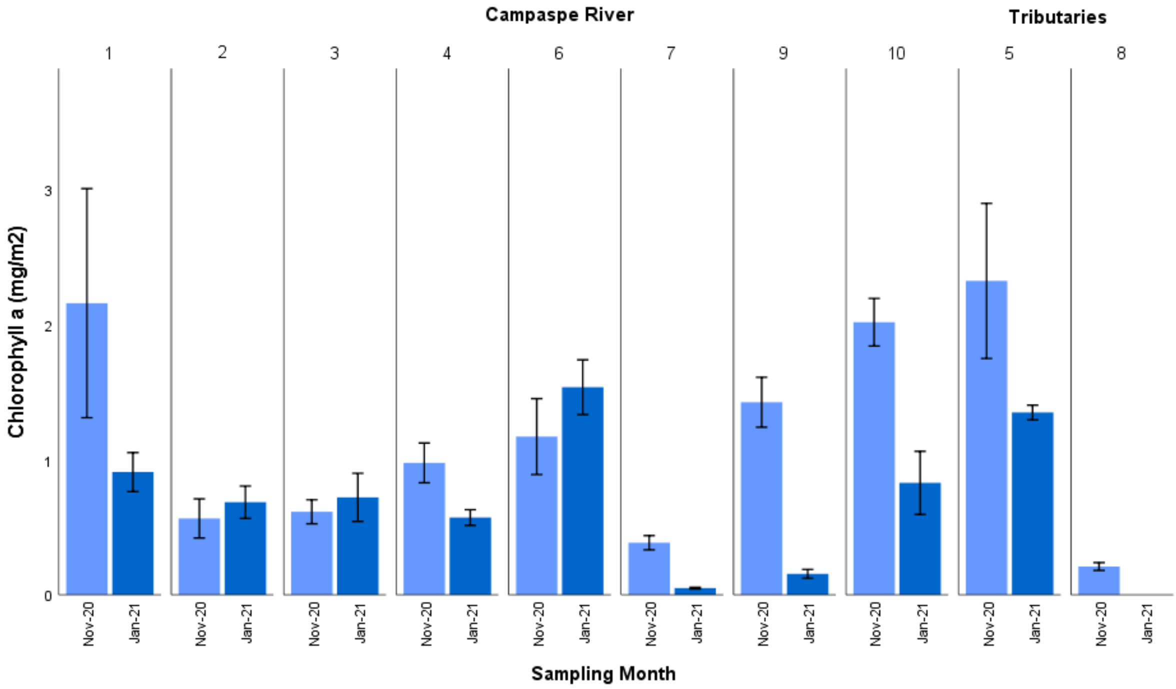


Figure 14: Chlorophyll-a (±SE) of biofilms on artificial substrates deployed across sites during November 2020 and January 2021. The Kyneton WRP discharge point is between Sites 6 and 7.

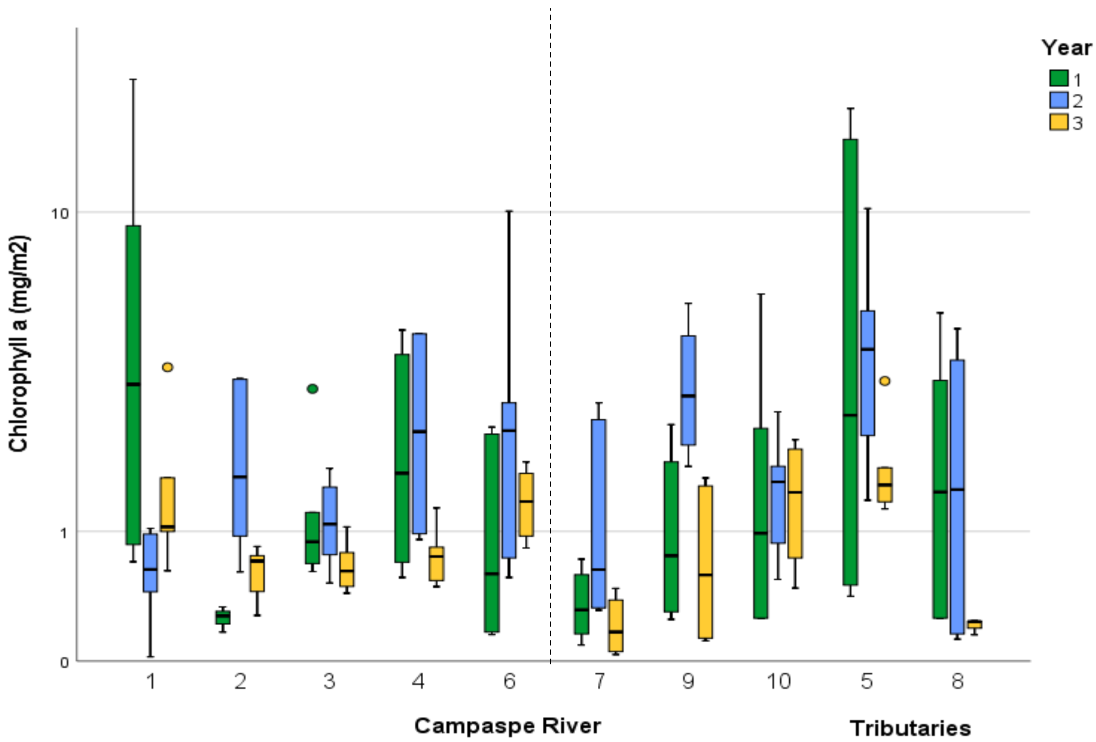


Figure 15. Mean chlorophyll-a of biofilms on artificial substrates deployed across sites during Years 1, 2 and 3 of monitoring. The vertical dashed line indicates Kyneton WRP discharge point between Sites 6 and 7.

AFDM, a measure of total amount of organic material, including autotrophic, heterotrophic and detrital carbon, significantly differed between sites in November 2020 (Kruskal-Wallis, H=20.99, df=9, p=0.013), but not in January 2021 (Kruskal-Wallis, H=10.98, df=8, p=0.203). In November 2020, highest AFDM concentrations were measured at Campaspe Site 1, which were significantly greater than all other sites. Similarly, higher concentrations were observed at Campaspe Site 3 and tributary Site 6 compared to most other sites. Lowest AFDM levels were observed at Campaspe Site 7 and tributary Site 8, although only site 8 significantly differed to all other sites (Figure 16). Highest concentrations were observed at Campaspe Site 3, while lowest concentrations were observed at Site 7 in January 2021, but these were not statistically significant (Figure 16).

Seasonal variation was observed at Campaspe Sites 1 and 10, with higher AFDM occurring in November 2020 at Site 1 and January 2021 at Site 10 (Figure 16). AFDM showed little seasonal variation at all other sites (Figure 16).

Overall mean AFDM was generally lower in Year 3 of monitoring relative to previous years (Figure 17). At Campaspe Site 2, 10 and tributary Site 8 there has been a general decline in AFDM with time, while at Sites 4, 7, 9 and tributary Site 5 there was a large decline in AFDM in Year 3 (Figure 17). AFDM at Campaspe Sites 1, 3 and 6 are comparable to previous year's results (Figure 17).

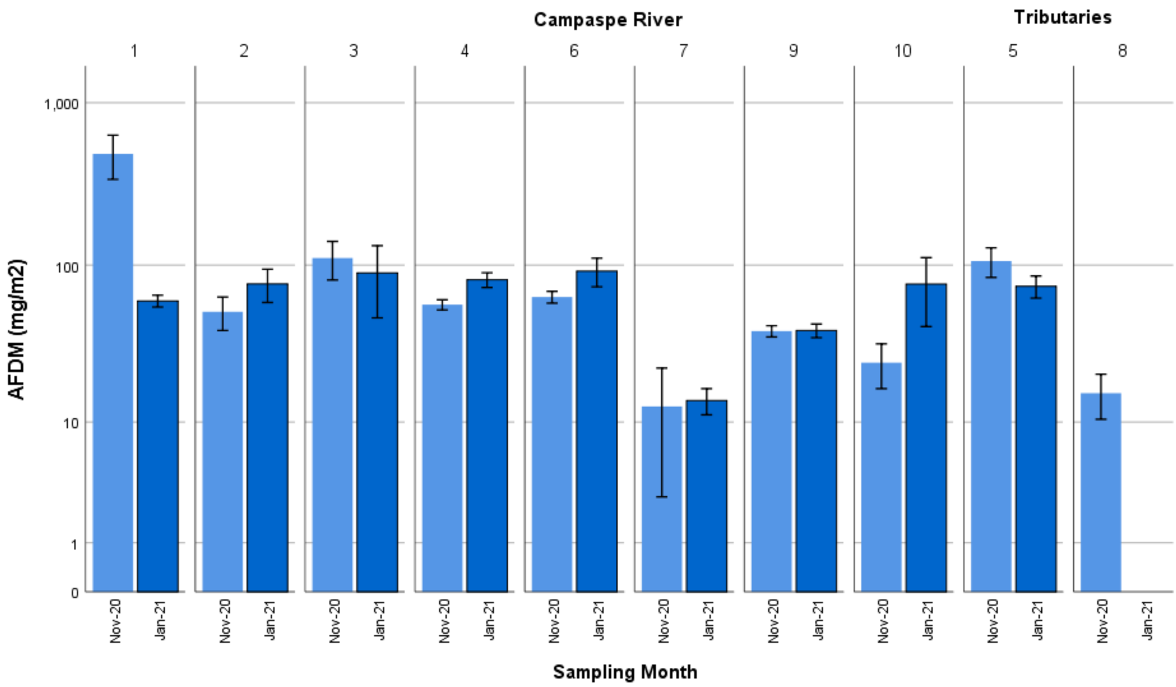


Figure 16: Mean ash-free dry mass (AFDM) (±SE) of biofilms on artificial substrates deployed across sites during November 2020 and January 2021. The Kyneton WRP discharge point is between Sites 6 and 7.

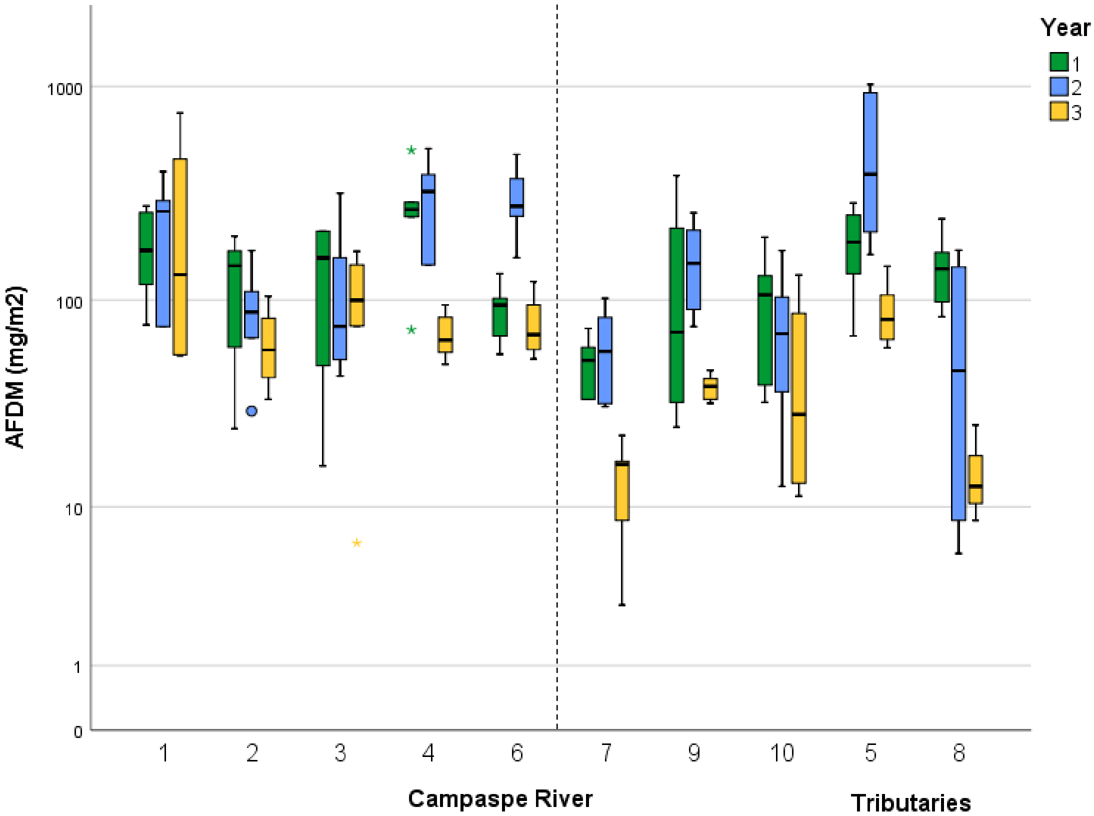


Figure 17. Annual change in ash-free dry mass (AFDM) (±SE) of biofilms on artificial substrates deployed across sites. Vertical dashed line indicates Kyneton WRP discharge point between Sites 6 and 7.

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 January 2021 (Kruskal-Wallis, H=16.32, df=8, p=0.038), but not in November 2020 (Kruskal-Wallis, H=14.00, df=9, p=0.122). At Campaspe Sites 3, 4, 7, 9 and 10 the AI values were generally higher than other sites, exceeding 100, in January 2021 indicating a dominance of heterotrophic organisms (Figure 18). Mean AI values at all sites were below 400 for both sampling months, although they did exceed the threshold on some deployed substrates at Site 1 in November 2020 (Figure 18).

Seasonal variation was observed across several sites. At Campaspe Sites 1 and 3 higher AI values occurred in November 2020, while at Sites 4, 7, 9 and 10 higher values occurred in January 2021 (Figure 18). AI values have generally remained stable across monitoring years at most sites, except for Campaspe Site 3 where there has been a gradual increase across sampling years, and Sites 6 and 10 where a gradual decrease is observed (Figure 19).

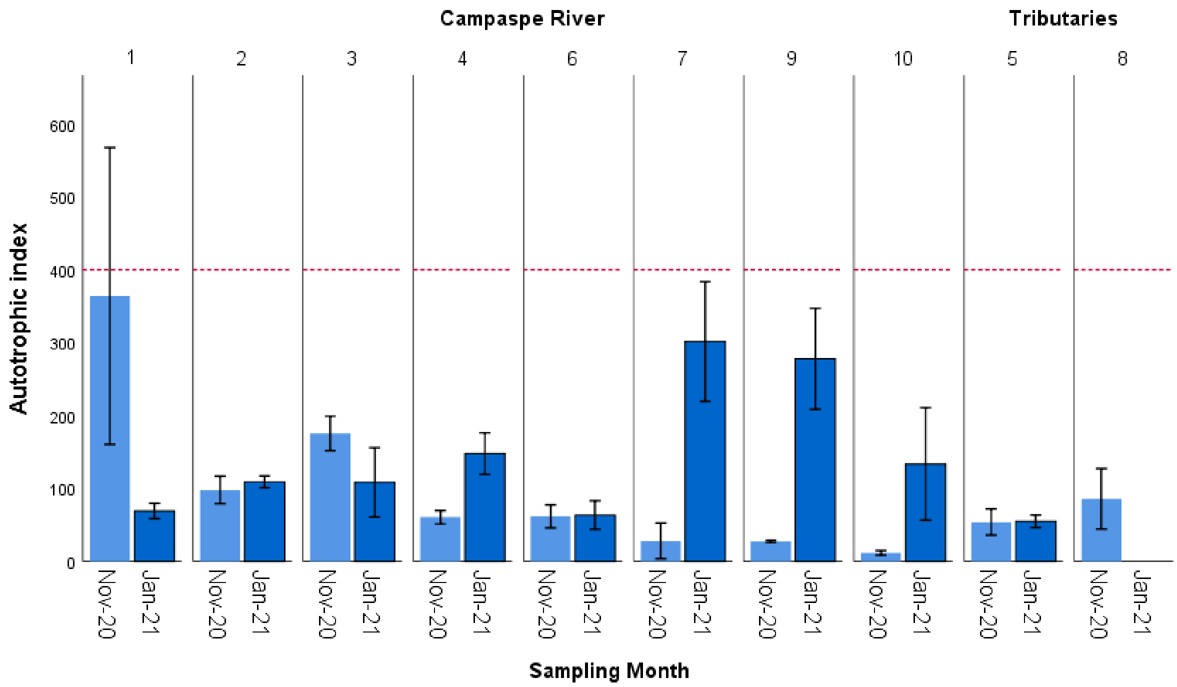


Figure 18: Autotropic index (±SE) of biofilms on artificial substrates deployed across sites during November 2020 and January 2021. The horizontal red dashed line indicates level above which communities are impacted by organic pollution. The Kyneton WRP discharge point is between Sites 6 and 7.

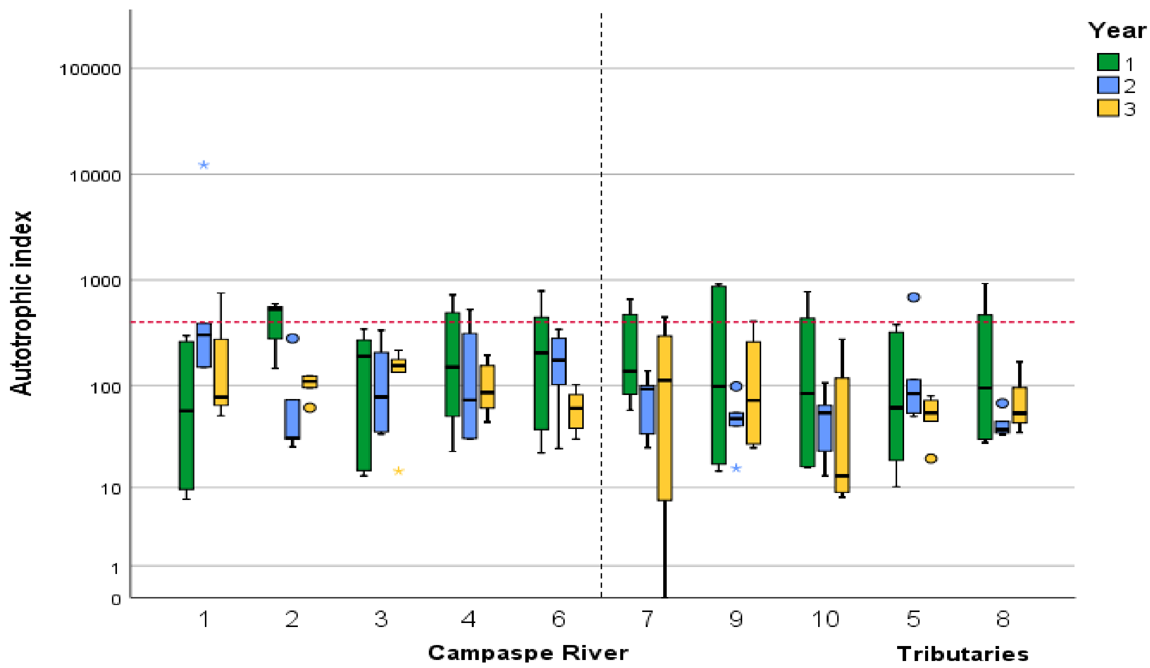


Figure 19: Annual autotropic index (±SE) of biofilms on artificial substrates at monitoring sites. The horizontal dashed line at 400 indicates level above which communities are impacted by organic pollution. Vertical dashed line indicates Kyneton WRP discharge point between Sites 6 and 7.

The community structure of cyanobacteria, chlorophytes (green algae) and diatoms provides an indicator of ecosystem health, with communities dominated by cyanobacteria 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 November 2020 and January 2021 were comprised of cyanobacteria and diatoms, with chlorophytes (green algae) only present at Campaspe Site 1 in January 2021 (Figure 20). In both sampling months sites in the upper reaches of the Campaspe had slightly higher dominance of cyanobacteria (60-80 % total composition), compared to sites in lower reaches, Sites 7-10, where they made up 45-60 % total composition (Figure 20).

The proportion of cyanobacteria to diatoms was relatively consistent between seasons at Campaspe Site 10 and tributary Site 5, while there was a higher contribution of cyanobacteria in January 2021 compared to November 2020 at all other sites (except tributary Site 8, which was not sampled in January 2021) (Figure 20).

Compared with previous years, there is a clear absence of chlorophytes in Year 3 monitoring across all sites (Figure 21). At most sites this has been replaced with an increased contribution of cyanobacteria (Figure 21).

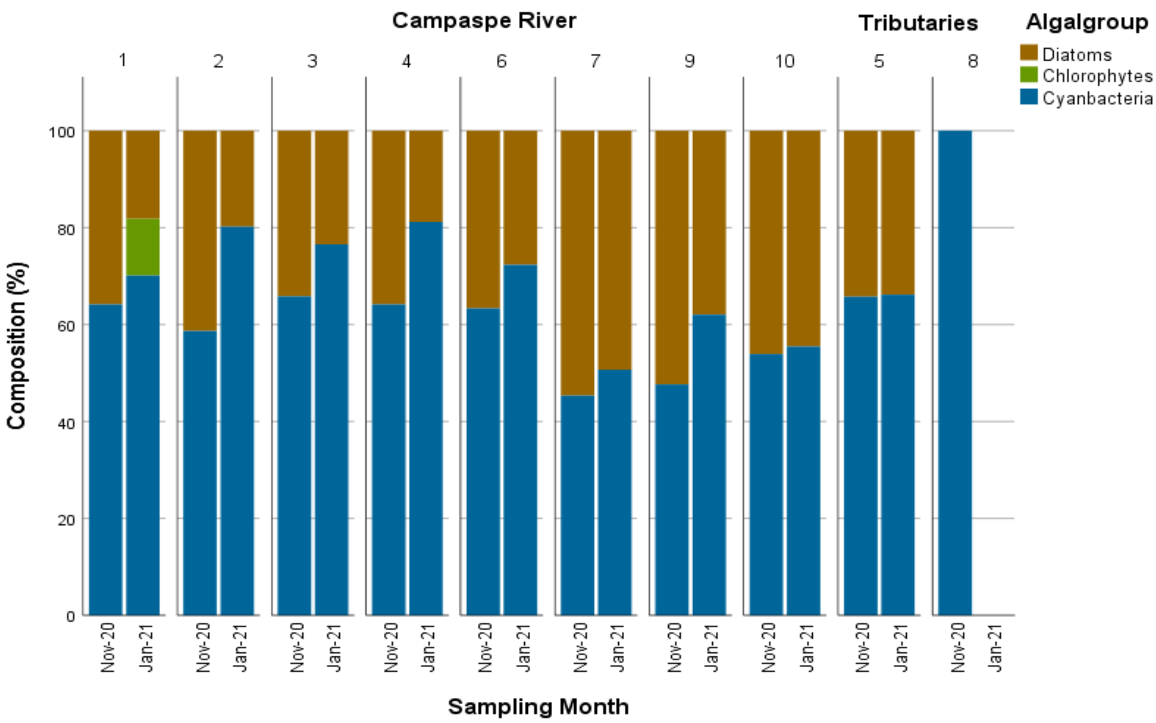


Figure 20: Relative composition of algal groups (Cyanobacteria, Chlorophytes and Diatoms) on artificial substrates deployed in November 2020 and January 2021. The Kyneton WRP discharge point is between Sites 6 and 7.

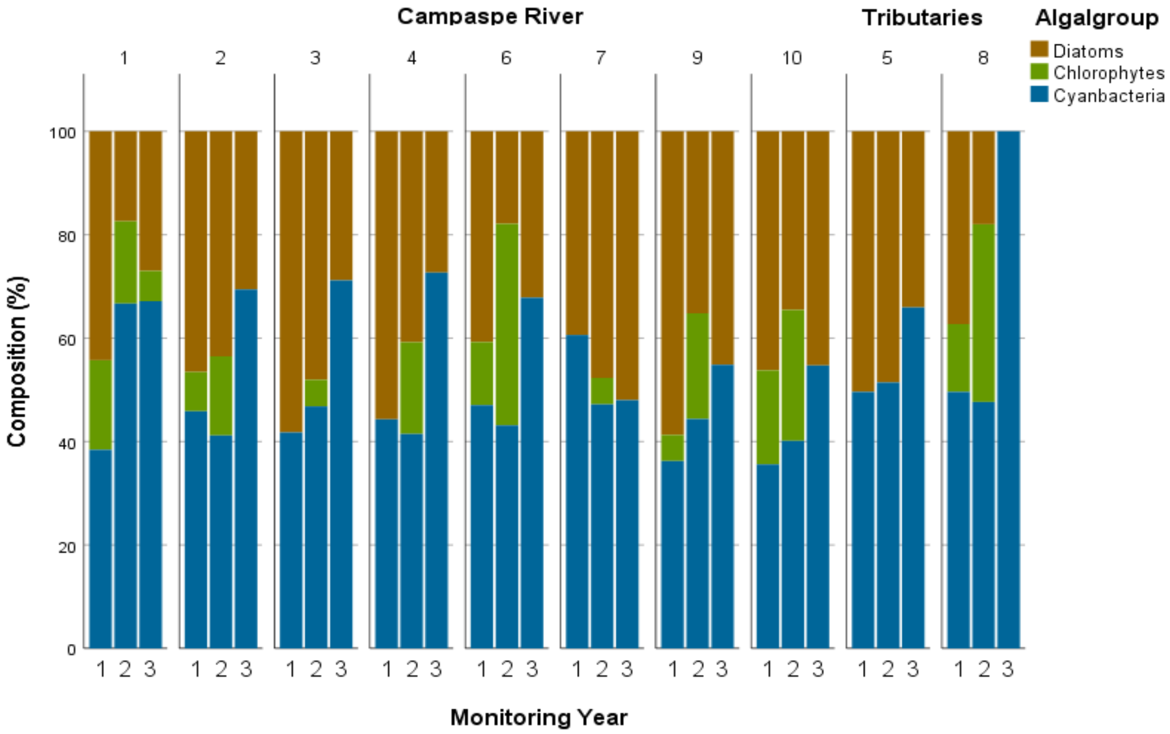


Figure 21: Annual relative composition of algal groups (Cyanobacteria, Chlorophytes and Diatoms), on artificial substrates. The Kyneton WRP discharge point is between Sites 6 and 7.

Physical Habitat

Instream habitat Assessment

Monthly mean macrophyte cover, an indicator of instream habitat availability, ranged 0-100 % at sites during Year 3 monitoring and is shown in Figure 22. Campaspe Sites 1, 2, 3 and 4 consistently had greatest monthly mean macrophyte cover (range 73-100 %) (Figure 22). While at Campaspe Sites 6, 7, 9 and 10 monthly mean cover ranged 0 to 100 % but was mostly below 74 %. Campaspe Site 10 has consistently low monthly mean cover (<5 %) (Figure 22). At tributary Sites 5 and 8, mean cover ranged from 2.3 to 54 %, with a drop in cover at tributary Site 8 in February 2021 (Figure 22).

Mean macrophyte cover has generally increased over time at the monitoring sites, except for Campaspe site 10 where it has declined (Figure 23). Mean macrophyte cover is consistently highest in the upper reaches of the study area, Campaspe Sites 1-6, and lowest at Campaspe Site 10 and tributary Site 8 (Figure 23).

Monthly filamentous algae (>2cm length) cover, an indicator of nutrient enrichment, ranged from 0 % to 24 % at sites during Year 3 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 or 2 months (Figure 23). The exception was at Campaspe Site 3 where filaments >2cm were observed in 4 of 5 sampling months. Greatest cover of filamentous algae was generally observed in August 2021 across all sites, except Campaspe Site 6 and tributary Site 5 where greatest cover was observed in January 2021 and December 2020 respectively. Highest cover across all sites was observed at tributary Site 8 in August 2020 (24 %) (Figure 23).

The occurrence of filamentous algae >2cm has decreased over time at all sites, except tributary Site 8 where it remained stable (Figure 24). Since sampling in Year 1 where mean cover reached nuisance levels at three sites (6, 9 and 5), levels have remained generally below 10% (Figure 24).

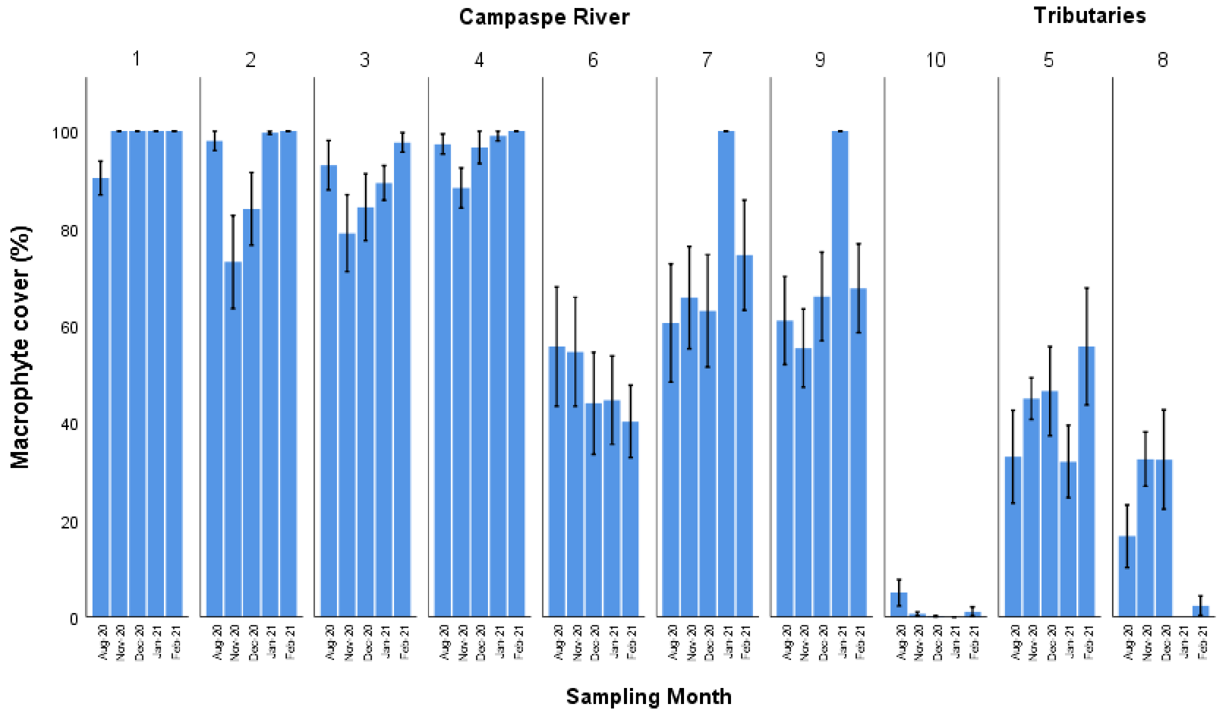


Figure 22. Monthly mean macrophyte cover at monitoring sites during Year 3. The Kyneton WRP discharge point is between Sites 6 and 7.

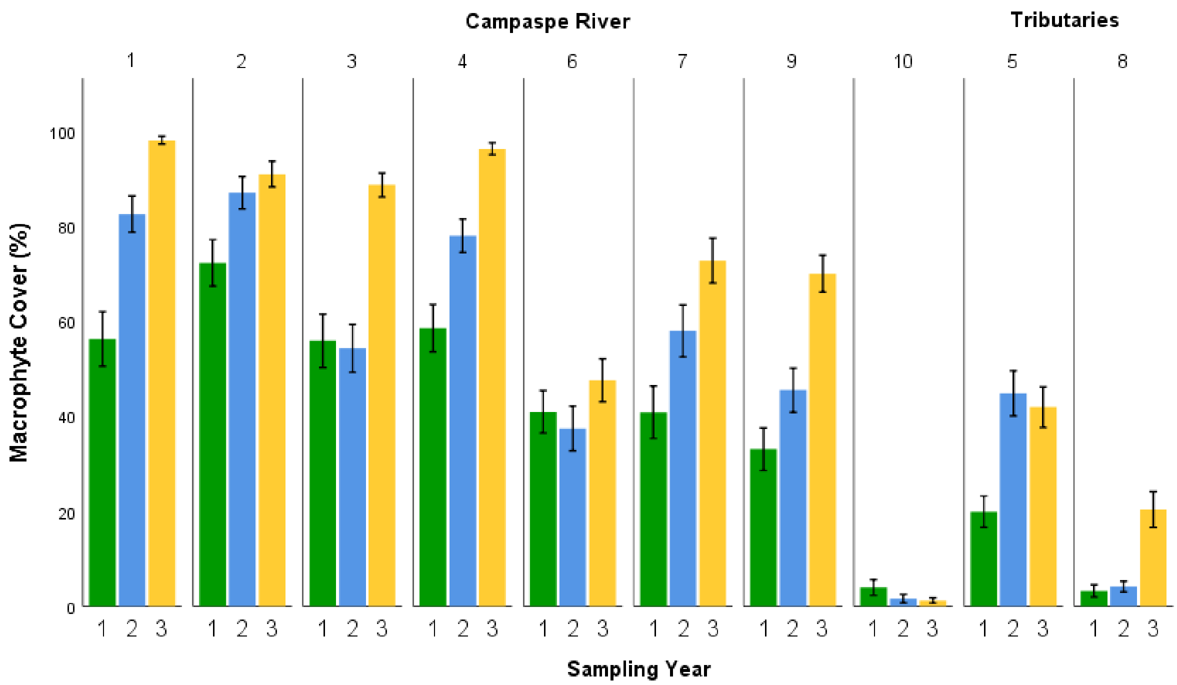


Figure 23. Mean macrophyte cover at sites in Years 1 to Years 3 of monitoring. The Kyneton WRP discharge point is between Sites 6 and 7.

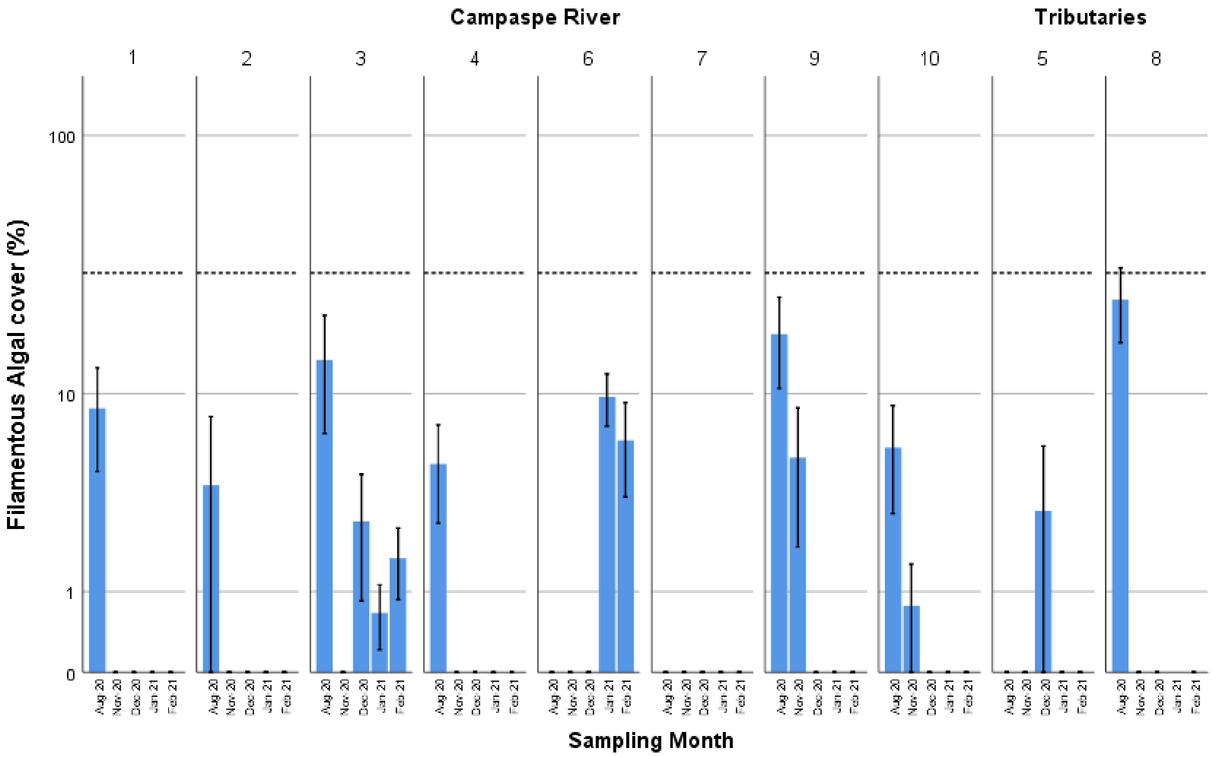


Figure 24: Monthly mean filamentous algal (>2cm length) cover at sites during Year 3. The Kyneton WRP discharge point is between Site 6 and 7. Horizontal dashed line represents nuisance algal cover threshold (30%).

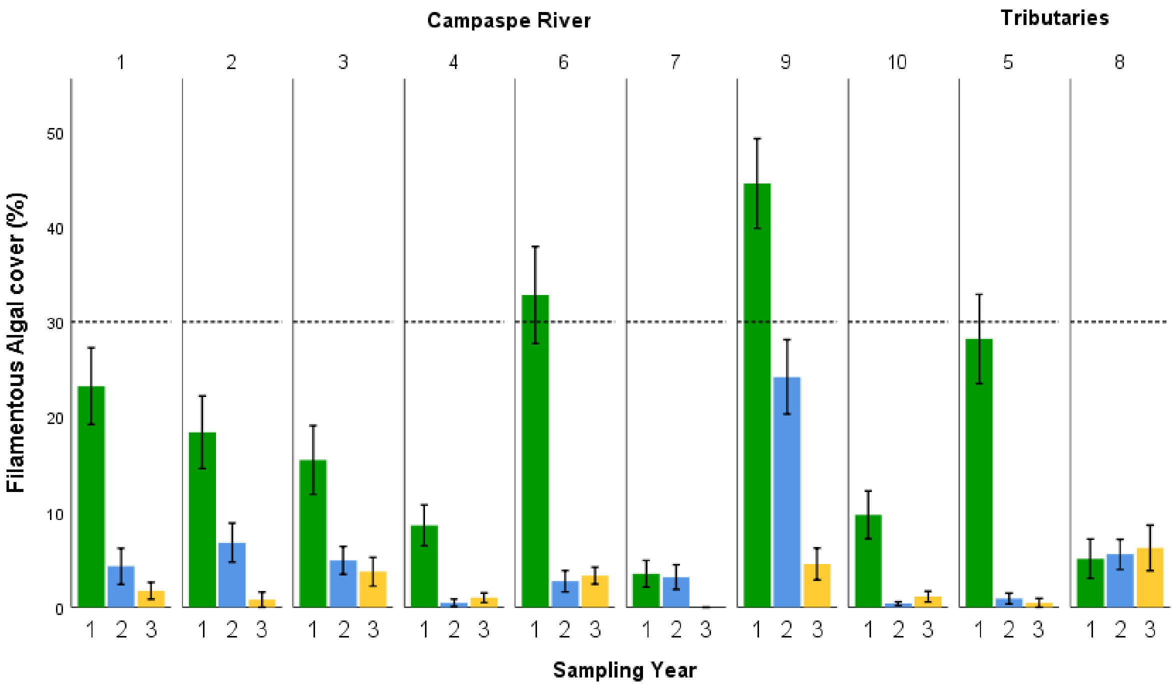


Figure 25. Mean filamentous algal (>2 cm length) cover in Years 1 to 3 of monitoring. The Kyneton WRP discharge point is between Sites 6 and 7. Horizontal dashed line represents nuisance algal threshold (30%).

Surface Water Toxicity

Faunal toxicity - Snails

Moderate impacts to Mud snail survival were observed at Campaspe River sites 3, 4 and 10 and tributary Site 8 (Figure 26). No impact on mud snail survival was observed at any of the other study sites, where the average survival range was 83 to 100 %. (Figure 26). Average snail survival has varied annually (Table 12). For Campaspe Sites 1,2 6 and 7 and tributary Site 5 there has been a general improvement in survival with time, while at Campaspe Sites 3, 4 and 10 there was a significant decline in snail survival in Year 3 (Table 12).

Embryonic production, an indicator of reproductive success and potential exposure to endocrine disrupting chemicals, ranged from 29 % to 55 % in Year 3 (Figure 27, Table 12). Lowest embryonic production was observed at Sites 4 and 7 in the Campaspe and tributary Site 5 where production was <36 %, while highest rates were observed at Campaspe Sites 9 and 10 (52 % and 55 %, respectively) (Figure 27). Yearly assessments of embryonic production show varied results across sites (Table 12). General declines in embryonic production have been observed at Campaspe Sites 3, 4, 6, and 7 (Table 12). At all other sites production rates have fluctuated between sampling years (Table 12).

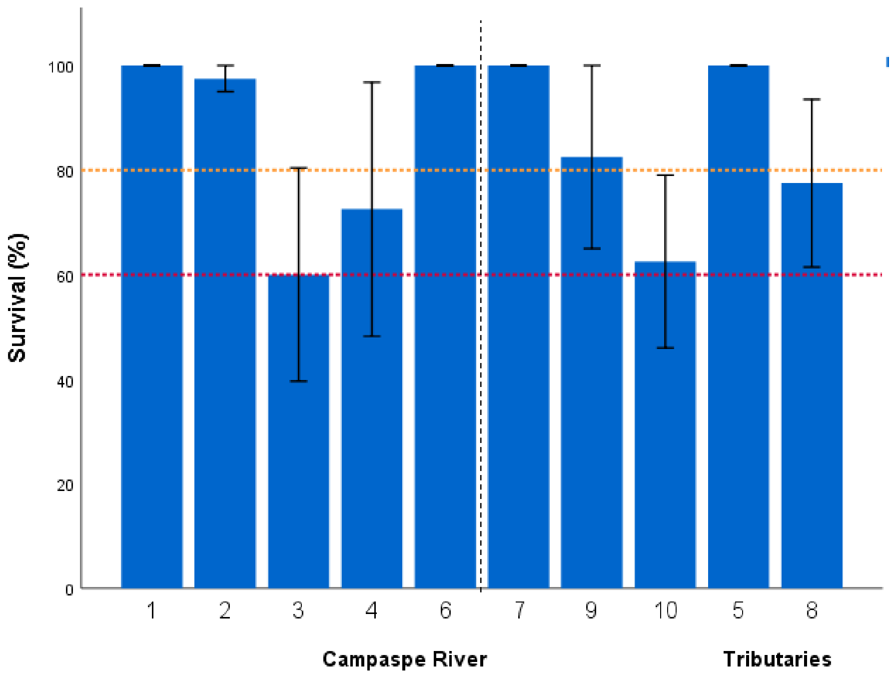


Figure 26: Mean survival (±SE) of mud snails, *P. antipodarum*, across sites during December 2020. Vertical dashed line indicates Kyneton WRP discharge point between Sites 6 and 7. Orange and red horizontal dashed lines represent the <80% and <60% thresholds below which moderate and high impacts are occurring, respectively.

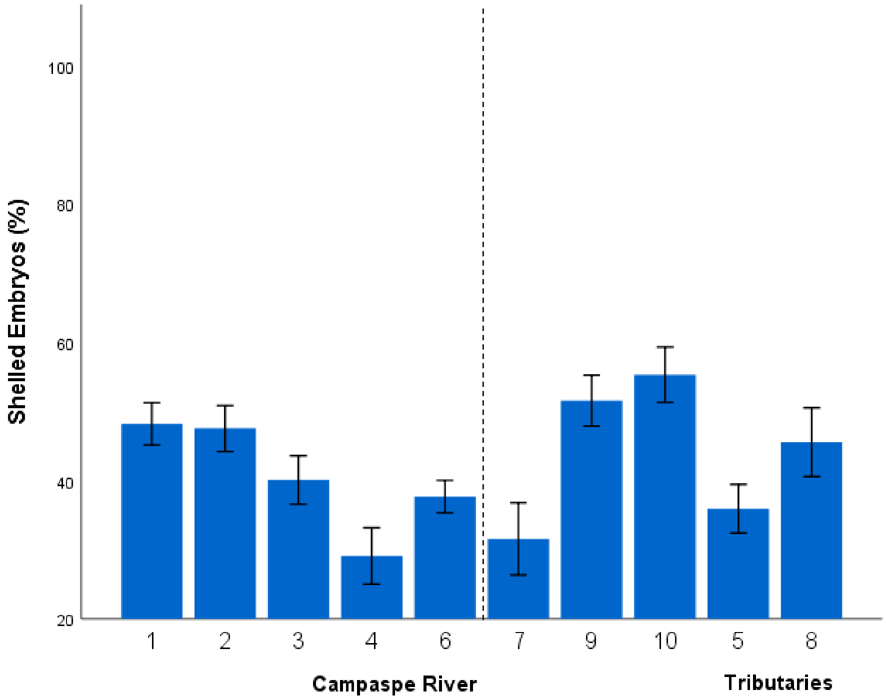


Figure 27: Mean percentage (±SE) of mud snails, *P. antipodarum*, with shelled embryos present across sites in 2020. The vertical dashed line indicates Kyneton WRP discharge point between Sites 6 and7.

Table 10: Mean survival of mud snails, *P. antipodarum*, and percentage of snails with shelled embryos present during Years 1 to 3 of monitoring.

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

Floral Toxicity - Algae

During Year 3, algal toxicity was assessed in November 2020 and January 2021. High inhibition of algal growth was observed in January 2021 at Site 6 (52%) in the Campaspe and tributary Site 5 (66%), while moderate growth inhibition occurred at Campaspe River Sites 4 (26%) and 10 (21%) in November 2021 and Site 7 (35%) in January 2021 (Table13). Substantial stimulation of algal growth, ranging 43-336% of site controls, was observed during November 2020 at all sites, except for Campaspe Sites 4 and 10 (Table 13).

Yearly monitoring indicates consistent moderate to high growth inhibition at Campaspe Sites 4 and 6 with 4 of 6 months exceeding 20% or 50% inhibition (Table 13). While at Campaspe Sites 1 and 10, moderate to high growth inhibition was observed in 3 of 6 months sampled, and at Campaspe Sites 3 and 9 and tributary Site 5, moderate to high inhibition was observed in two of 6 months sampled (Table 13). In contrast, Campaspe Site 7 and tributary Site 8 consistently show stimulation of algal growth (Table 13).

Table 11: Mean inhibition of algal growth (relative to site control) across sites during Years 1 to 3 of monitoring.

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

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.

Discussion

The principle focus of the SFM program is to reduce the delivery of sediment, nutrients, and pathogens (related to stock) to the Campaspe River, thus improving ecological health. While the benefits of SFM are not likely to be observed in three 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 (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, that remain dominated by willows with free stock access, are generally 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 native species which have a more continuous leaf fall (Lester et al., 1994). They 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 SFMW activities. Established native vegetation sites at Boundary Rd Langley and at Redesdale (Sites 9 and 10), are characterised by stable water clarity, dissolved oxygen, and water temperatures. There have been improvements in TP, and ammonia at Site 10 in Redesdale, however TN concentrations have increased over time. At Site 9 at Langley, consistently elevated nutrients (TN, TP, orthophosphate) occur. The elevated nutrients are likely the result of downstream nutrient transport. While Sites 9 and 10 have a good established native riparian vegetation, upstream areas of the catchment are characterised by poor riparian vegetation to armour 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 stabilise. 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 a result of sediment mobilisation and bank disturbance from installation of stock exclusion fencing, and willow removal and revegetation work 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. Monitoring in Years 4 and 5 will provide a better indication of whether the works will lead to improvements in water clarity and nutrient levels at these sites. In comparison, at SFMW sites 3-6, where willow removal and revegetation works occurred prior to Year 1 monitoring, there is 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. Water temperatures at all SFMW sites have 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.

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). Over time, 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. 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 at Sites 1 and 2 in Carlsruhe, where macrophytes cover >90% of the wetted area of the river and dissolved oxygen levels have 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). Overtime as the riparian becomes more established, it's possible we will see an improved balance of macrophyte and algal communities and possibly improved instream nutrient processing.

There has been significant increases in macrophyte cover at sites where no SFM interventions have taken place, Campaspe River Site 7 at Old School Rd and tributary Site 8 on Snipes Creek, however the species composition differs significantly to that at all other sites. Floating macrophytes such as *Azolla* sp and *Lemna* sp. dominate at Sites 7 and 8, while all other sites comprise a mix of rooted submerged and emergent macrophytes such as *Myriophylliom* sp., *Triglochin* sp, *Allisma* Sp. The high abundances of these more opportunistic macrophyte species are likely related to the high nutrient availability, notably phosphates at these sites. While nutrient concentrations are the highest across the study area at these sites, and algal toxicity testing suggests availability for significant growth stimulation, there has been a general decline in filamentous algal cover and biofilm biomass. 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 growth of macrophytes and algae. This subsequently impacts on instream nutrient processing, resulting in poor instream processing of nutrients and high export from these reaches. This is reflected in the conditions at Site 9 at Boundary Rd Langley, where we also see elevated nutrients and increased 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 7 and 8, present great opportunities for SFM works to improve overall river health.

Are we seeing changes in faecal contamination?

In agricultural catchments the major source of faecal contamination is animal excreta. Reductions in faecal contamination have been shown 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 adjacent land 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, however at sites where stock access remains we see more frequent and higher exceedance of *E. coli* guidelines. For instance, *E. coli* were observed in all monitoring years at the subset of sites monitored (Campaspe Sites 2, 4, 6, 7, 9, tributary Sites 5 and 8), however exceeded guideline values (100 organisms per 100mL) at Site 4 in Year 3, and at Sites 7 and 8 during multiple years. That said, *E. 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, with results available for Years 1 and 2. The markers indicated faecal contamination, attributable in part to stock, occurred at Sites where stock graze adjacent to the river with restricted access (2, 4, and 9) and at Site 7 where stock have unrestricted access.

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 1-6), 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, have shown variable macroinvertebrate diversity and taxon richness over the three sampling years. It is possible that water availability is a strong influence 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, and willows remain the dominant riparian vegetation and stock access is allowed and at the urban site 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 during Year 3 which could be a result of increased macrophyte cover, 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 including poor habitat availability, lack of different habitat types such as riffles or runs, most of the site is characterised by deep water, and 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 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 and 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 have been detected at concentrations of concern for aquatic life. Additionally, hydrocarbon concentrations have been consistently elevated across the study area, particularly at sites directly surrounded by heavy traffic roads and rail tracks.

Several pesticides have been detected in waters and sediments. 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. Six herbicides, three fungicides and two insecticides have been detected in surface waters, while two synthetic pyrethroid insecticides have been detected in sediments. The detection of pesticides in both urban and agriculturally dominated sites suggest applications in both land use contexts are contributing to pesticide contamination. Toxicology assessments indicate some of these pesticides, particularly the herbicides and insecticides, are at levels that may be adversely impacting stream biodiversity.

Eight pharmaceuticals, including an antidepressant, anticonvulsant, and antibacterial medication, several antibiotics and a veterinary medication have been detected in surface waters. 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. In addition to the detection of the human

Bacteroides markers, several pharmaceuticals were detected in monitoring from Years 1 and 2, which further indicates wastewater contributions to the Campaspe River and tributaries.

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 initial three years of monitoring has provided information from which to assess the short-term benefits of the SFMW to the Campaspe River and provided insight into other factors influencing river health. Benefits of the SFMP will continue to be seen over the next few years as initial impacts from willow removal and revegetation and fencing activities dissipate and riparian becomes more established. Immediate benefits have started 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, poor ecological health is evident and results in impacts downstream.

The presence of toxicants and impacts from treated and untreated wastewater, including elevated nutrients and faecal contamination are observed across the study region and may be contributing to the ecological health of these sites. Continued monitoring will provide a better understanding of the longer-term benefits of the SFMP as the riparian vegetation becomes more established, providing a more optimal microclimate and improving habitat quality, reducing nutrient inputs, and improving food availability and 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 4 was completed between August to December 2021, with results available in mid-2022. This schedule, however, may be affected by restrictions introduced to address the COVID-19 pandemic.

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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