Coliban Water Monitoring Program

Monitoring Program for Assessing the Benefits of Environmental Offsets on the Condition of the Campaspe River: Year 2 (2019)

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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

A stream frontage management program (SFMP) has been established along the Campaspe River and two tributaries by the North Central Catchment Management Authority (NCCMA). This program aims to improve the ecological condition of the Campaspe River by improving riparian vegetation (through native vegetation plantings and the removal of exotic flora) and providing stock exclusion fencing. Coliban Water has provided additional funding to expand the program to improve a further 14 km of land along the Campaspe River and Post Office Creek. The Aquatic Environmental Stress Research Group (AQUEST), from RMIT University, was commissioned by Coliban Water to assess the outcomes of the SFMP on the ecological condition of the Campaspe River. This report assesses results from the second year of monitoring and identifies short-term benefits of the program on water quality and aquatic ecosystem health in the Campaspe River.

As at December 2019, woody weed control, revegetation planting, fencing and installation of off stream watering had been mostly completed across the four stream frontage management (SFM) sites included in the additional funding provided by Coliban Water for the Caring for the Campaspe project. These sites are now largely in a management phase for the next few years.

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 2019. Sites located along the Campaspe River are generally of good quality and show signs of reducing nutrient inputs, particularly in the mid reaches between Sites 3 and 6. However, sites in the upper reaches around Carlsruhe, and downstream of Site 7, indicate multiple impacts from wastewater(treated discharges and untreated such as septic leakage), agricultural and urban runoff, poor habitat condition and unrestricted stock access, including elevated nutrient concentrations, faecal contamination, and the presence of pharmaceuticals, herbicides and insecticides, heavy metals and/or elevated hydrocarbons.

Post Office Creek (Site 5), Snipes Creek (Site 8), and Site 7 along the Campaspe River at Old Station Road, were in poorest condition during initial surveys completed in Year 1. This was observed again in Year 2 monitoring.

The full benefits to the ecological health of sites from the SFMP are expected to be seen in subsequent monitoring years now that SFMP works have been completed and are mostly in a management phase. Improvements in the condition of many of these sites, however, is complicated by the surrounding residential, industrial and agricultural land-uses which create additional challenges for stream management.

Additional longer-term benefits are expected as the riparian vegetation becomes more established, creating advantageous microclimatic conditions, and improving habitat quality and food availability for aquatic taxa. Sampling for Year 3 is expected to be completed during August to December 2020, subject to state COVID-19 restrictions.

Introduction

In 2012, the North Central Catchment Management Authority (NCCMA) established a stream frontage management program (SFMP) along the Campaspe River. The “Caring for the Campaspe” project aimed to enhance the health of the waterway and improve the biodiversity of the river. Coliban Water has provided additional funding to continue the program and expanded improvement works to include a further 14 km of land along the Campaspe River and Post Office Creek. Twenty-one hectares of river frontage will be revegetated with native trees and shrubs, and 13 km of fencing will be installed to keep livestock out of the waterway. This is expected to reduce faecal contamination and nutrient pollution instream via the removal of livestock, especially cattle, from the river, and to create better riparian habitat via the removal of exotic willows and revegetation with indigenous species. Longer term benefits will occur as the riparian vegetation becomes more established and provides shade, habitat, and food for aquatic animals. Further details on the program and its location is 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 uses assessments of water quality, together with aquatic ecology surveys and toxicology techniques to investigate improvements to water quality and biodiversity in the Campaspe River, from Carlsruhe to Redesdale, along with two associated tributaries. Results from Year 1 monitoring are provided in Myers *et al*. 2019. This report presents outcomes from Year 2 and makes comparisons with the Year 1 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 fencing) will:

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

The objectives of Year 2 monitoring were to:

- Collect monitoring data which contribute to the above objectives.
- Compare the results of Year 2 with the results from Year 1 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 assess the potential to achieve the desired long-term outcomes.

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. Ten sites (Table 1 and Figure 1), including eight along the Campaspe River (Plate 1) and one in each of Post Office Creek (Plate 2) and Snipes Creek (Plate 2) were selected to assess the benefits of the SFMW. Detailed descriptions of each sampling site are provided in the Year 1 Monitoring Report (Myers *et al.* 2019).

Stream Frontage Management Works undertaken as part of the funding provided by Coliban Water to the NCCMA, as part of the Caring for the Campaspe Project, were targeted at four locations along the Campaspe River and Post Office Creek (Figure 1). These works include initial woody weed control, revegetation, fencing and off stream watering installations undertaken from February 2019 to June 2020. These works will be followed by weeding, supplementary watering and revegetation maintenance programs from Spring/Summer 2020 to Spring/Summer 2023.

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.

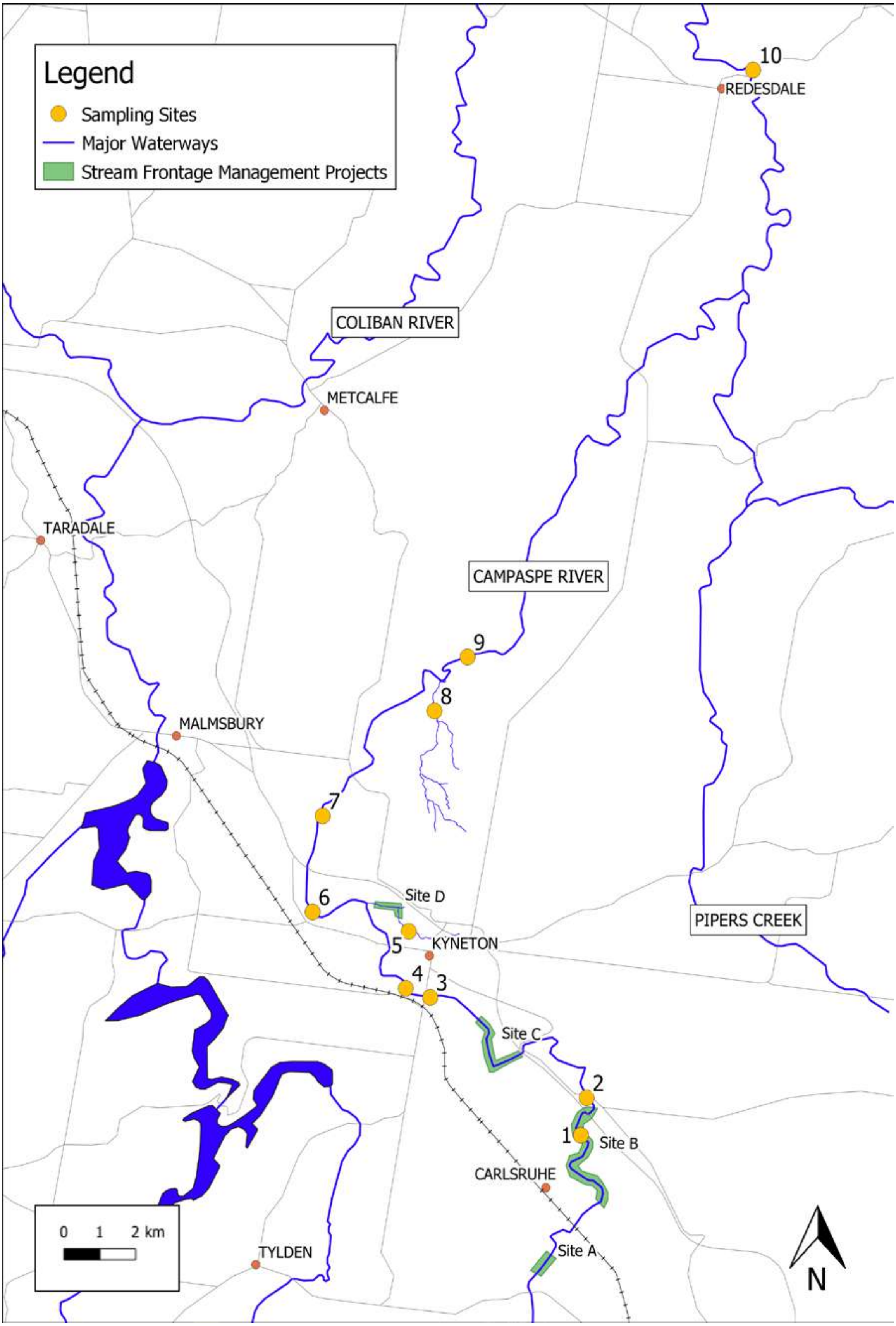

Figure 1: Locations of monitoring sites within the Campaspe River system and SFMW.

Plate 1: Study sites situated on the Campaspe River. a) and b) Site 1, Cheveley Rd, Carlsruhe; c) and d) Site 2, Cobb & Co Rd., Carlsruhe; e) and f) Site 3, Mollison St., Kyneton; g) and h) Site 4 Kyneton Botanical Gardens; i) and j) Site 6 Burton Ave., Kyneton; k) and l) Site 7 Old Station Rd.; m) and n) Site 9 Boundary Rd.; o) and p) Site 10 Redesdale.

Plate 2: Study sites situated on Tributaries a) and b) Site 5, Post Office Creek, Wedge St., Kyneton; c) and d) Site 8, Snipes Creek, Barbower Rd.

Monitoring

Samples are collected annually from all ten sites at five time points to assess seasonal trends. As the Campaspe River and associated tributaries are ephemeral, and much of the study area is dry during summer and autumn. Sampling is, therefore, concentrated around periods when the river is connected and flowing. Year 2 monitoring surveys were conducted in August, September, October, November and December 2019. Myers *et al* (2019) provides further detail on previous sampling times.

Monitoring at each site included:

- Surface water physico-chemistry, nutrient and faecal analysis
- Aquatic macroinvertebrate survey
- Determination of nutrient bioavailability through visual assessments, artificial substrates and *in situ* algal growth measurements
- Water toxicity assessment
- Sediment chemistry including hydrocarbons, heavy metals and pesticides
- Surface water microcontaminants including personal care products (PCP), pharmaceuticals, herbicides, insecticides and fungicides

The monitoring methods applied are summarised 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 and 2 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*								▨	▨	▨	▨	▨
2021	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
2022	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨

* Sampling may be 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.

Monitoring	Aug	Sep	Oct	Nov	Dec	Description
Physico-chemistry	▨	▨	▨	▨	▨	Parameters included water temperature, dissolved oxygen (% saturation), pH, electrical conductivity and turbidity.
Nutrients	▨	▨	▨	▨	▨	Water samples were sent to ALS Global for nutrient analysis; including ammonia as N (NH_4-N), total nitrogen (TN), total Kjeldahl Nitrogen (TKN), nitrate and nitrite (NOx), orthophosphate (OP) and total phosphorus (TP).
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). Results were compared to guideline values (State Environment Protection Policy SEPP (Waters) and EPA Use of Reclaimed Water).
Macroinvertebrate survey		▨				Sampled using the Rapid Bioassessment (RBA) method. Collection and identification took place according to EPA Victoria guidelines (EPA Victoria, 2003). Biological indices (number of families, and SIGNAL and EPT indices) were compared to guideline values (State Environment Protection Policy SEPP (Waters)).
Visual assessment	▨	▨	▨	▨	▨	Percentage cover of aquatic macrophytes and filamentous algae. The length of filamentous algae was also noted (short <2cm, medium 2-10cm, long >10cm).
Artificial substrates		▨		▨		Thin discs were suspended, in triplicate, in the water column (Plate 3) for a 4-week period. Artificial substrates were analysed for biofilm biomass (biomass of algae, cyanobacteria, heterotrophic microbes and detritus), photosynthetic health (photosynthetic efficiency) and community composition.
In situ algal growth		▨		▨		Algal balls, containing algae immobilised in alginate beads, were deployed in exposure or control cages in triplicate at each site (Plate 4 and 5) to examine nutrient bioavailability and the toxicity of surface waters to floral species. Cages were deployed for a 10-day period and the resulting biomass was used to assess algal health.
Water toxicity				▨		The survival and reproductive ability of the mud snail, *Potamopyrgus antipodarum* (Plate 5) was used to assess surface water toxicity. Snails were deployed in cages for 4 weeks. Survival and number of embryos present was recorded.
Sediment chemistry				▨		Heavy metals, petroleum hydrocarbons and multi-residue pesticides (see Appendix 1 for full list) were analysed from sediment samples. Results were then compared to guideline values for the protection of aquatic ecosystems, where available (Australian and New Zealand Water Quality Guidelines 2000).
Other pollutants	▨			▨		Polar Organic Chemical Integrated Samplers (POCIS) (Plate 5 and 6) were deployed at each site for a 4-week period to detect other pollutants. These included personal care products, pharmaceuticals, herbicides, insecticides and fungicides, in surface waters (see Appendix 2 for full list).

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

Plate 3: (a) Artificial substrate samplers, and (b) deployment of artificial substrates.

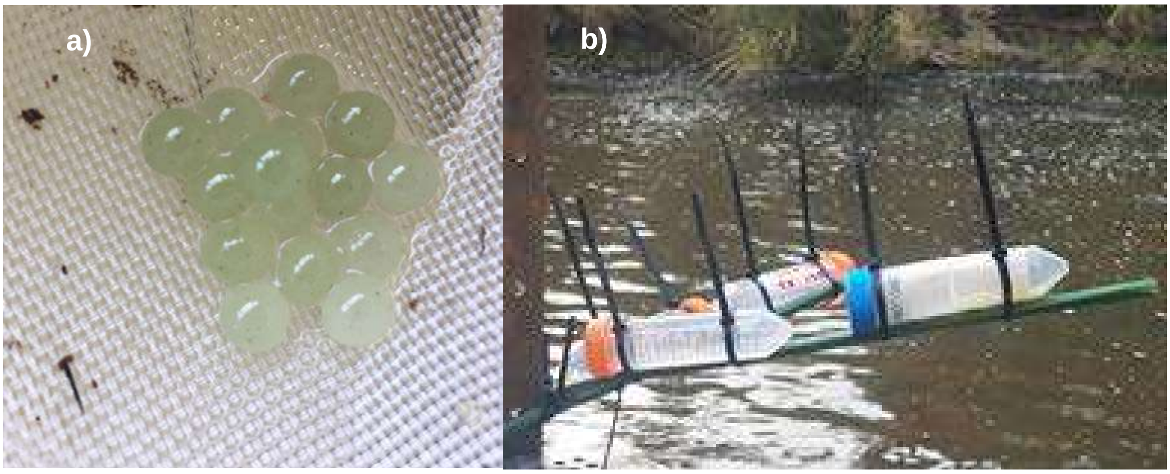

Plate 4: (a) In situ algal balls to assess algal growth, and (b) algal balls in cages prior to deployment.

Plate 5: (a) Embryos present in mud snail (*P. antipodarum*) and (b) mud snails, algal balls and POCIS passive samplers during deployment.

Plate 6: POCIS passive samplers following retrieval.

Results

Stream Frontage Management Works

Stream frontage management works across the four sites began in February 2019. Initial woody weed control (56 ha) and revegetation (15 ha) was undertaken from April to October 2019, and September to November 2019, respectively. Livestock exclusion fencing (12 km), and off stream watering works began in August 2019. The Campaspe River monitoring study Site 1 provides an example of the SFMP, with willows at the site removed during the Year 2 monitoring program (Plate 7). At the end of sampling for Year 2, the majority of works across all SFMW sites were completed, except for Site 3 where revegetation will occur in Spring 2020. Sites are now largely in the maintenance phase of the project.

Plate 7: Campaspe River Site 1 before (left) and after (right) willow removal as part of stream frontage management works undertaken in 2019.

Rainfall

Weather conditions permitted sampling to occur in five consecutive months, from August to December 2019. The highest monthly rainfall recorded during the sampling period was in August 2019 (67 mm, Figure 2), with the lowest recorded in October 2019 (12 mm). This range in rainfall is comparable to the previous year of monitoring, which received 13.9 mm (September 2018) to 63 mm (December 2018). Below average monthly rainfall was reported for all monitoring months.

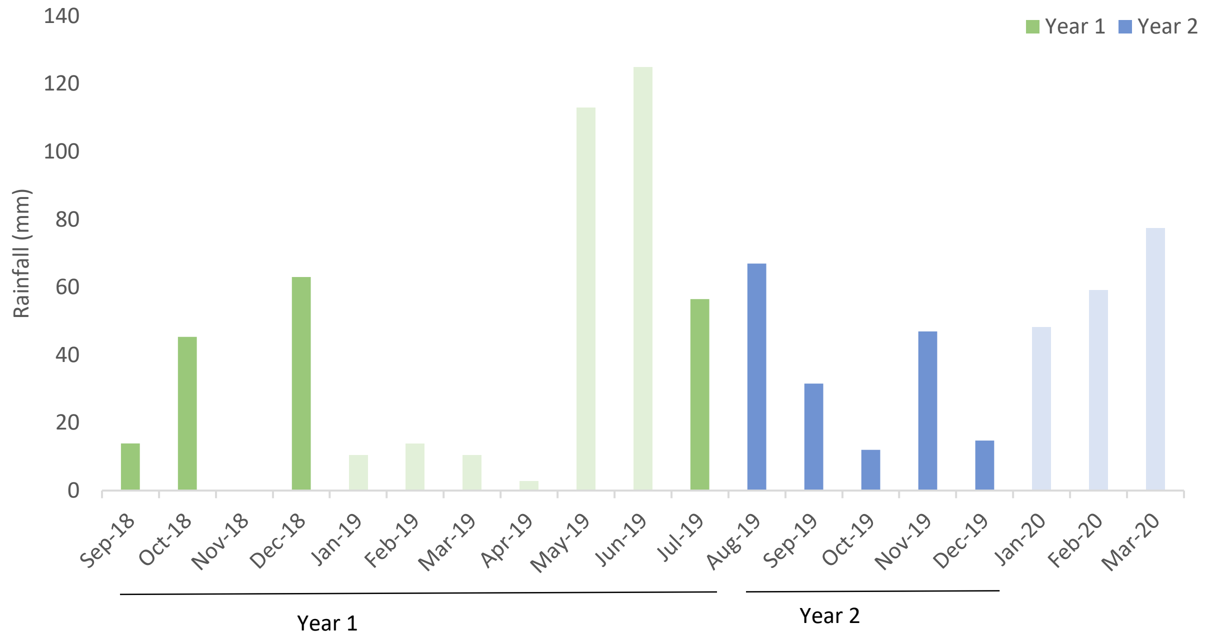

Figure 2: Monthly rainfall at Kyneton during 2018 and 2019 monitoring. Monitoring years are indicated by the green (Year 1) and blue (Year 2) bars. The darker green and blue bars represent months where sampling occurred (source: BOM, 2020).

Water Quality

Physico-chemistry

Surface water temperatures varied monthly. Site 2 recorded the highest temperature (26.7°C), while Site 10 recorded the lowest temperature (9.6°C) (Table 4); however, mean temperatures were highest at Sites 4 and 6 (18.4°C), and lowest at Site 7 (13.0°C). Overall, the maximum and mean temperatures recorded were considerably higher at Sites 1 to 6 in Year 2 of monitoring, compared with Year 1. In contrast, monitoring Sites 7 to 10 had marginally lower or equal mean temperatures in Year 2, as compared to Year 1 (Figure 3). In general, temperatures progressively increased downstream.

Mean dissolved oxygen concentrations were highest at Site 4 (86.6% saturation) and lowest at Site 8-Snipes Creek (49.6%) and exhibited less variation than the Year 1 monitoring period (Figure 4). Dissolved oxygen concentrations exceeded the SEPP (Waters) trigger value of 130% at Site 5 (140.0%) during September 2019, whereas all other sites were below the trigger value (Table 4).

Mean pH levels were generally comparable across all sites, and ranged between 7.0 and 8.1 units, a similar range to that observed in Year 1 monitoring (Table 4; Figure 5). The pH of all sites was within the recommended SEPP (Waters) range of 6.8 to 8.0 units, except for three sites (Sites 1, 2 and 4), which exceeded the maximum trigger value of 8.0 units in December 2019, but this was still within acceptable limits for most aquatic organisms. In comparison to the results of Year 1 monitoring, this is a reduction in the overall number of exceedances of the maximum trigger value.

Electrical conductivity was similar across sites and tended to increase downstream (Table 4). Site 8, however, continually exhibited high electrical conductivity (722 to 2160 µs/cm) and exceeded the SEPP (Waters) trigger value of 2000 µs/cm in December 2019 (2160 µs/cm). Similar results were observed during Year 1 monitoring, although no sites exceeded the SEPP (Waters) trigger value (Figure 6).

Considerable variation in turbidity was observed in the tributary streams, that is Sites 8 (17.0 to 89.5 NTU) and 5 (7.6 to 31.1 NTU). Mean turbidity at these two sites exceeded the SEPP (Waters) trigger value, however, mean turbidity did not exceed the trigger value at any site along the main river. Maximum recorded turbidity exceeded the SEPP (Waters) trigger value at all sites, except Site 1, which is located nearest the headwaters. Minimum recorded turbidity at Site 8 also exceeded the SEPP (Waters) trigger value. Site 8 was also identified as a turbid site during Year 1 of monitoring (Figure 7).

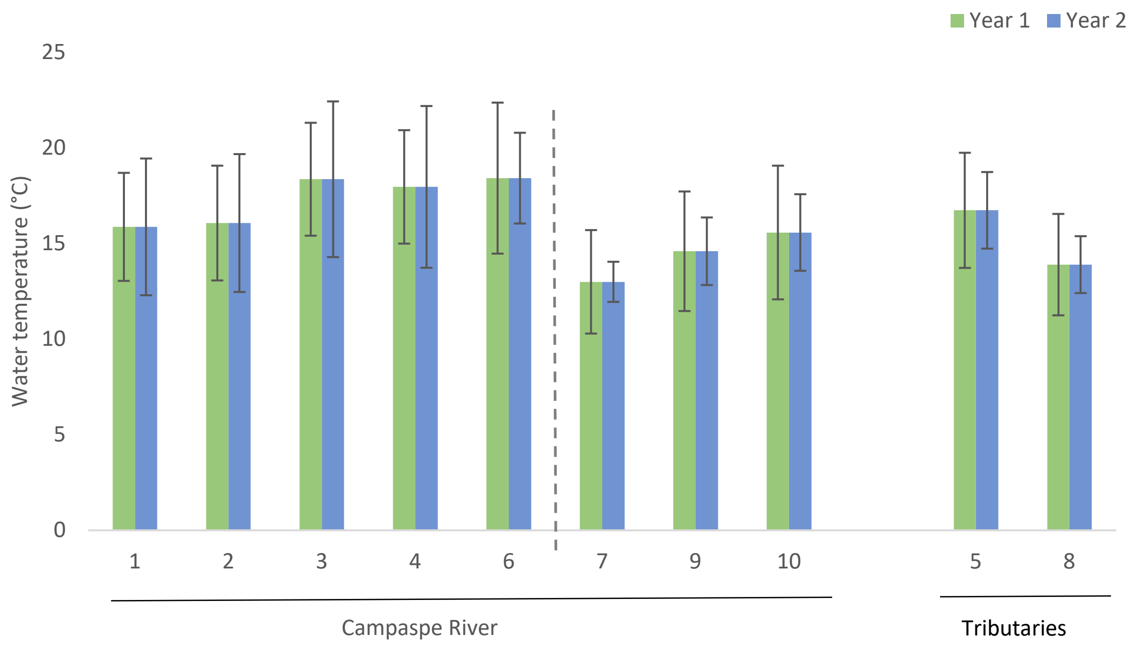

Figure 3. Mean surface water temperatures at Campaspe River and tributary sites during Year 1 and 2 monitoring. The vertical dashed line indicates location of Kyneton WRP discharge between sites 6 and 7.

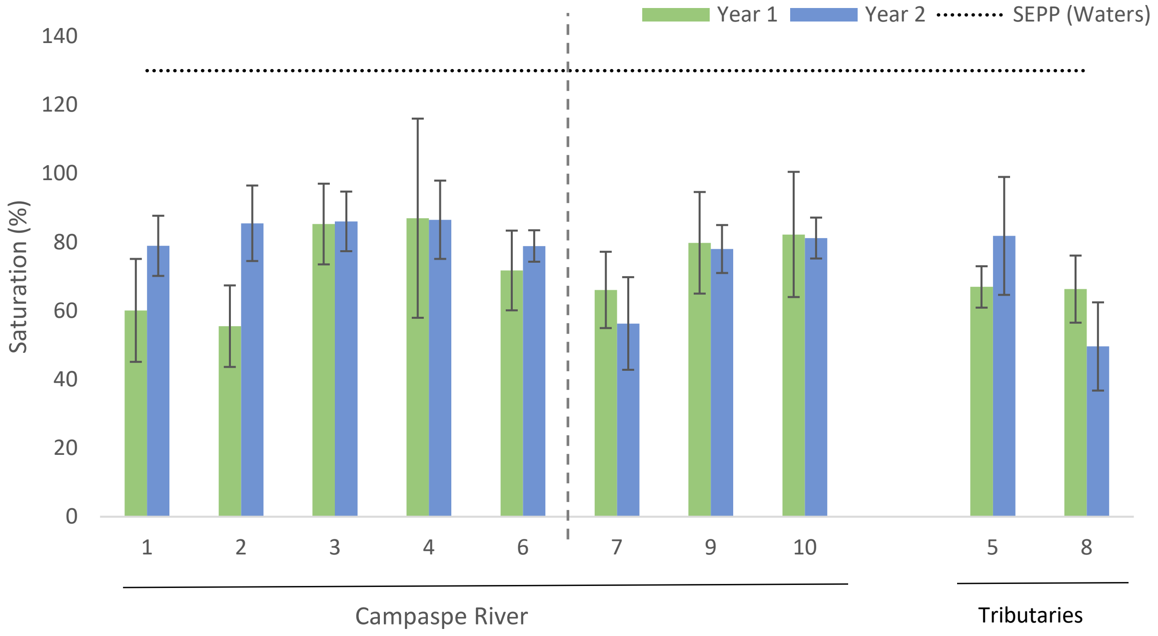

Figure 4. Mean dissolved oxygen saturation at Campaspe River and tributary sites during Year 1 and 2 monitoring. The vertical dashed line indicates location of Kyneton WRP discharge between sites 6 and 7. The horizontal dotted line indicates SEPP (Waters) trigger value.

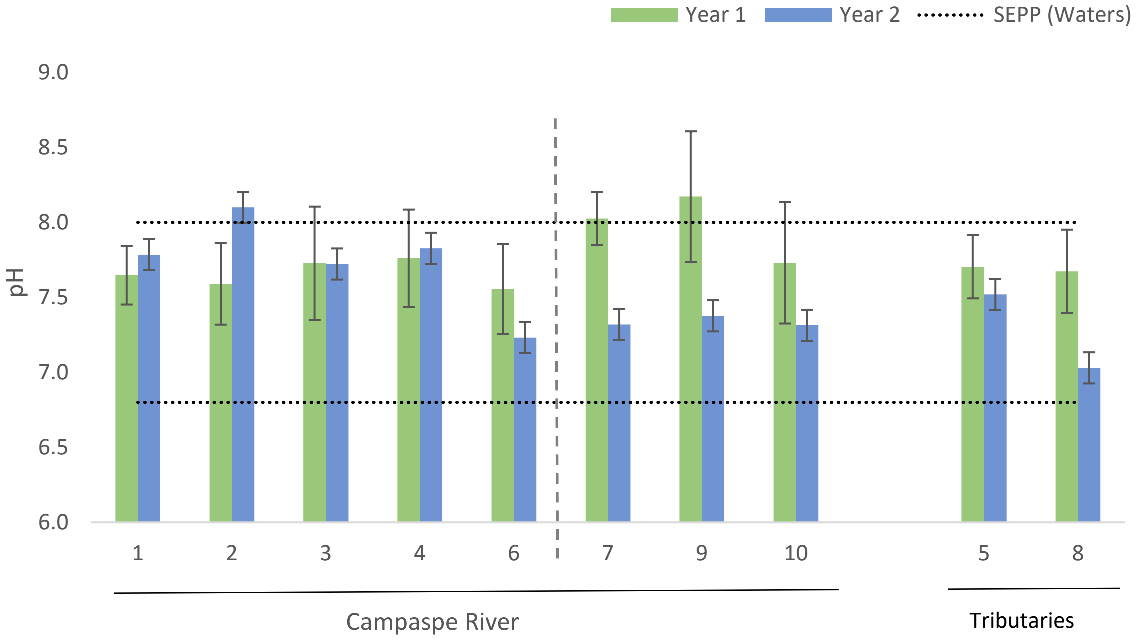

Figure 5. Mean water pH at Campaspe River and tributary sites during Year 1 and 2 monitoring. The vertical dashed line indicates location of Kyneton WRP discharge between sites 6 and 7. The horizontal dotted line indicates SEPP (Waters) trigger value.

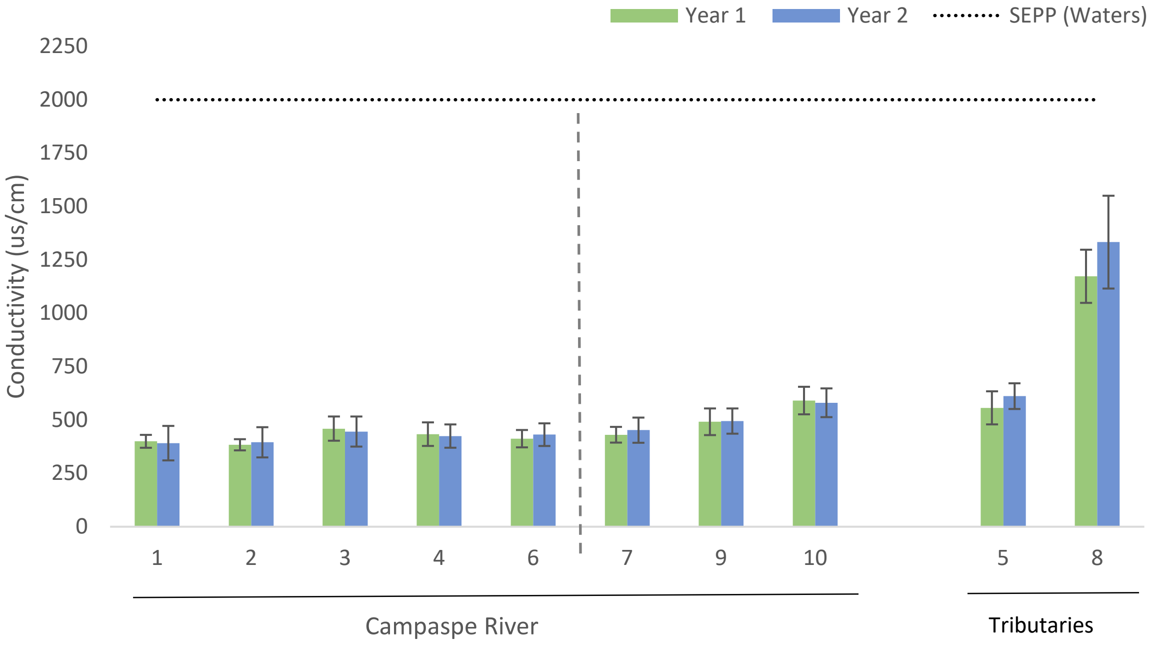

Figure 6. Mean water conductivity at Campaspe River and tributary sites during Year 1 and 2 monitoring. The vertical dashed line indicates location of Kyneton WRP discharge between sites 6 and 7. The horizontal dotted line indicates SEPP (Waters) trigger value.

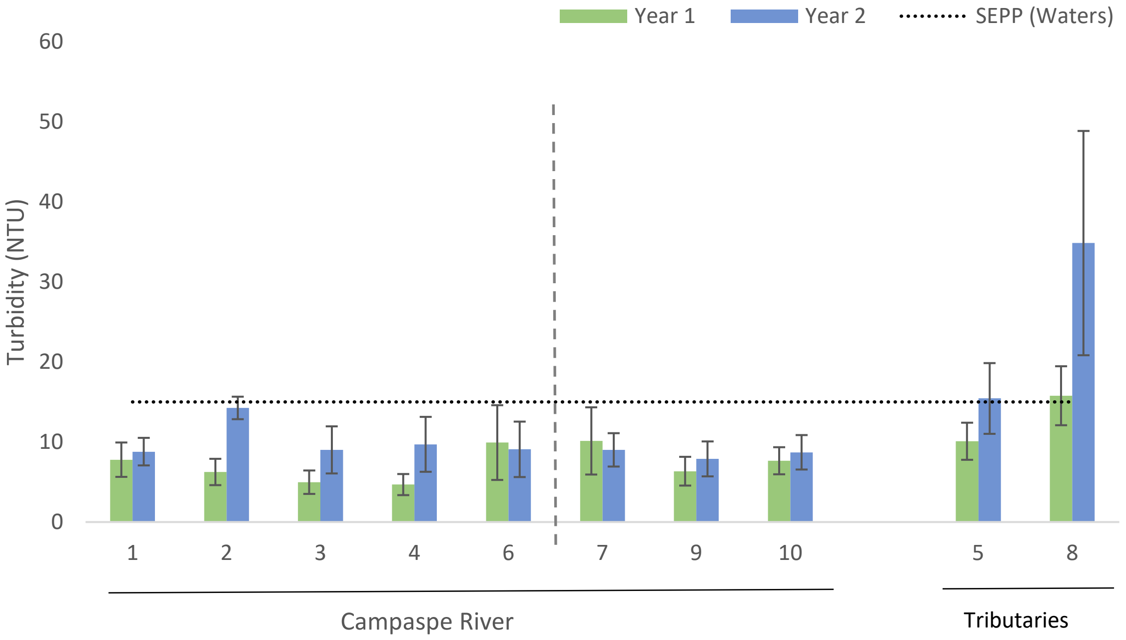

Figure 7. Mean turbidity at Campaspe River and tributary sites during Year 1 and 2 monitoring. The vertical dashed line indicates location of Kyneton WRP discharge between sites 6 and 7. The horizontal dotted line indicates SEPP (Waters) trigger value.

Table 4. Mean, minimum and maximum temperatures, dissolved oxygen, pH, electrical conductivity and turbidity measured across sites during year 2 monitoring. Values in bold exceed State Environment Protection Policy (Waters) 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	15.9	9.7	25.6	79.0	57.8	100.0	7.8	7.6	**8.1**	391.4	240.0	559.0	8.8	4.9	12.8
2	16.1	11.2	26.7	85.5	55.4	108.0	**8.1**	7.7	**9.1**	395.4	253.0	562.0	14.3	10.2	**16.5**
3	18.4	11.2	25.3	86.1	60.9	100.0	7.7	7.3	7.9	445.8	276.0	670.0	9.0	5.0	**17.7**
4	18.0	10.1	24.6	86.6	54.0	107.0	7.8	7.4	**8.2**	424.6	283.0	634.0	9.7	3.7	**18.1**
6	18.4	11.0	23.6	78.9	68.0	91.6	7.2	6.8	7.7	431.2	291.0	602.0	9.1	4.7	**22.8**
Kyneton WRP discharge between sites 6 and 7															
7	13.0	9.9	15.4	56.3	30.0	96.0	7.3	6.9	7.5	452.2	284.0	606.0	9.0	5.5	**17.2**
9	14.6	10.3	19.6	78.0	62.0	97.0	7.4	6.8	7.8	494.9	311.0	645.0	7.9	4.6	**16.3**
10	15.6	9.6	20.4	81.2	64.5	96.6	7.3	6.9	7.9	580.6	363.0	802.0	8.7	4.0	**15.8**
Tributaries															
5	16.7	10.6	20.8	81.8	36.9	**140.0**	7.5	7.2	7.8	611.6	386.0	834.0	**15.4**	7.6	**31.1**
8	13.9	10.0	18.1	49.6	19.0	91.1	7.0	7.0	7.2	1333.0	722.0	**2160.0**	**34.8**	**17.0**	**89.5**
Trigger Values	**-**			**max 130**			**6.8-8.0**			**≤2000**			**≤15**		

Nutrients

Total nitrogen ranged from 0.6 mg/L to 4.92 mg/L, and exceeded the SEPP (Waters) environmental quality indicator (≤1.05 mg/L) at:

- All sites in August 2019
- Sites 1, 5, 7 and 8 in September 2019
- Sites 1, 2, 3, 5, 6 and 8 in October 2019
- Sites 1, 2, 5, 7 and 8 in November 2019
- Sites 1, 7 and 8 in December 2019

Total nitrogen concentrations at Sites 1 and 8 consistently exceeded the environmental quality guideline (Figure 8). All ammonia concentrations met the SEPP (Waters) guideline value (≤0.9 mg/L) in Year 2 of monitoring and ranged from <0.01 to 0.38 mg/L (Figure 9).

A comparison of mean total nitrogen and ammonia concentrations between Years 1 and 2 are presented in Figures 10 and 11, respectively. Mean total nitrogen concentrations were slightly higher at Campaspe River Sites 1, 2 and 7, and Post Office Creek in Year 2 monitoring. No change was observed at other sites (Figure 10). A substantial decline in mean ammonia was observed at Site 8 in Year 2 of monitoring. No change was observed in mean ammonia at any other sites between Years 1 and 2 (Figure 11).

Monthly total phosphorus and orthophosphate concentrations are shown in Figures 12 and 13, respectively. Total phosphorus ranged from 0.01 to 1.62 mg/L over the monitoring period and exceeded the SEPP Waters environmental quality indicator[1] (≤0.055 mg/L) at all sites in August 2019, and on all occasions at Sites 1, 7, 8 and 9. Total phosphorus, and to a lesser extent orthophosphate, was generally more elevated at the downstream sites (Sites 7-10) and in Snipes Creek (Site 8).

Mean total phosphorus and mean orthophosphate between Years 1 and 2 of monitoring are shown in Figures 14 and 15. Mean total phosphorus was lower in Year 2 of monitoring at all sites, except Site 8. Mean orthophosphate was relatively consistent between Years 1 and 2 of monitoring at all sites, except at Sites 7 and 9, where concentrations were lower in Year 2.

The ratio of orthophosphate to total phosphate (Table 5) provides an indication of the bioavailability of phosphorus. Relatively low proportions of orthophosphate were observed at Sites 1, 2, 3, 4 and 6 (except Site 3 in October 2019); Post Office (Site 5) and Snipes Creeks (Site 8) also had low relative orthophosphate levels. Sites 7, 9 and 10 had higher proportions of orthophosphate, which indicates a rich source of phosphorous at these sites. This pattern corresponds with results from Year 1 of monitoring, albeit results from Sites 7, 9 and 10 are elevated, compared to Year 1 of monitoring.

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

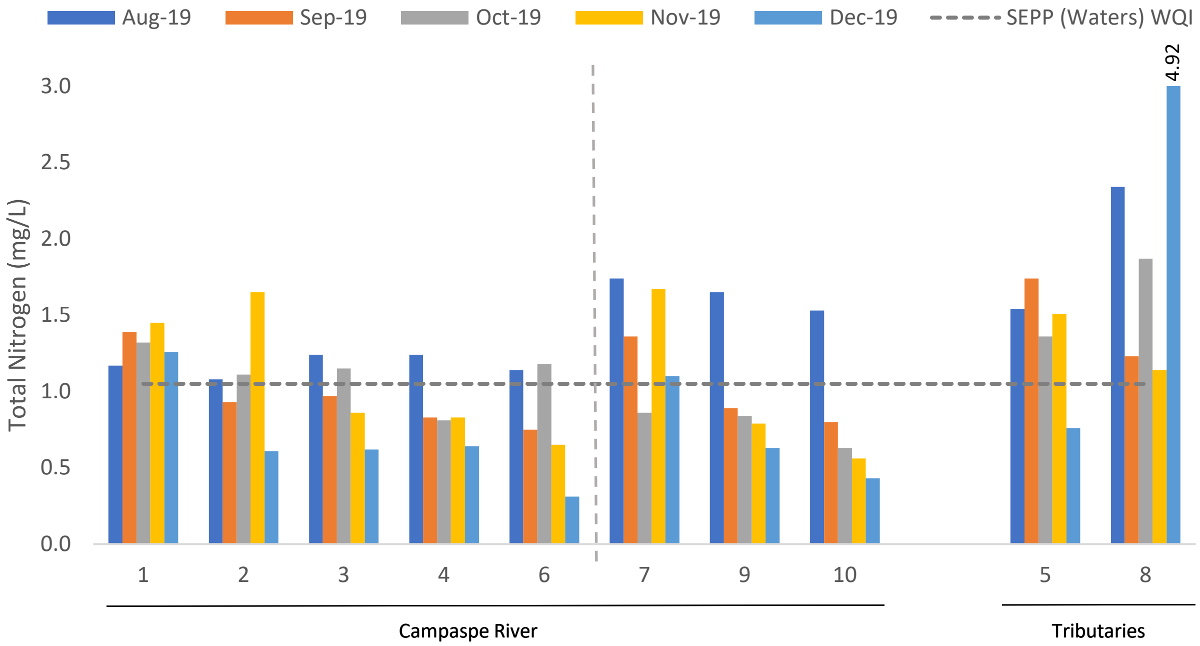

Figure 8. Monthly total nitrogen concentrations in surface waters during Year 2 monitoring from August to December 2019. The vertical dashed line indicates Kyneton WRP discharge point between Sites 6 and 7. Where concentrations were below the limit of reporting these have been presented as zero.

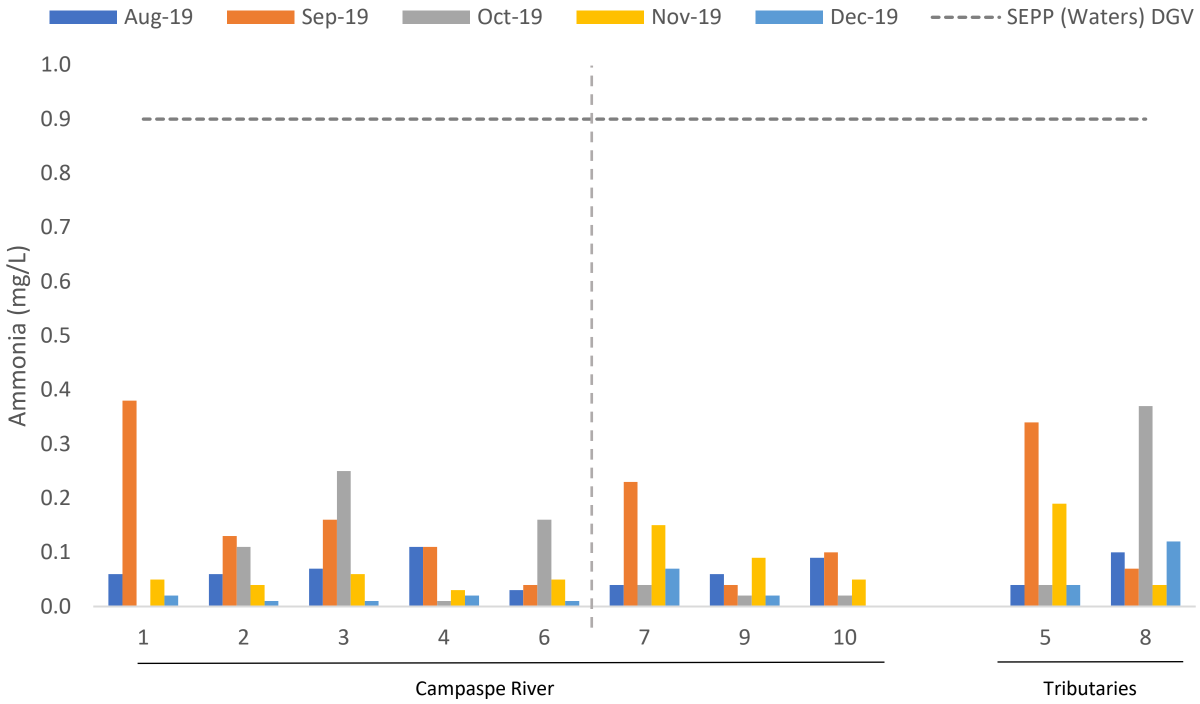

Figure 9. Monthly ammonia concentrations in surface waters during Year 2 monitoring August to December 2019. The vertical dashed line indicates Kyneton WRP discharge point between Sites 6 and 7. Where concentrations were below the limit of reporting these have been presented as zero.

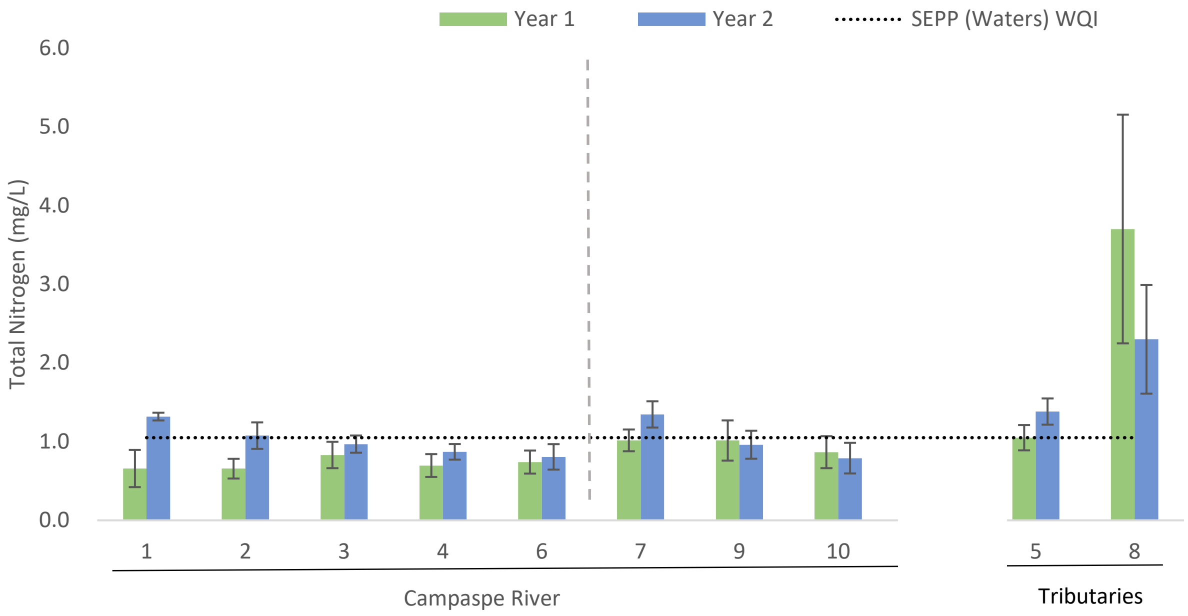

Figure 10. Mean total nitrogen concentrations in surface waters during Years 1 and 2 of monitoring. The vertical dotted line indicates Kyneton WRP discharge between Sites 6 and 7. The horizontal dotted line indicates SEPP (Waters) water quality indicator value.

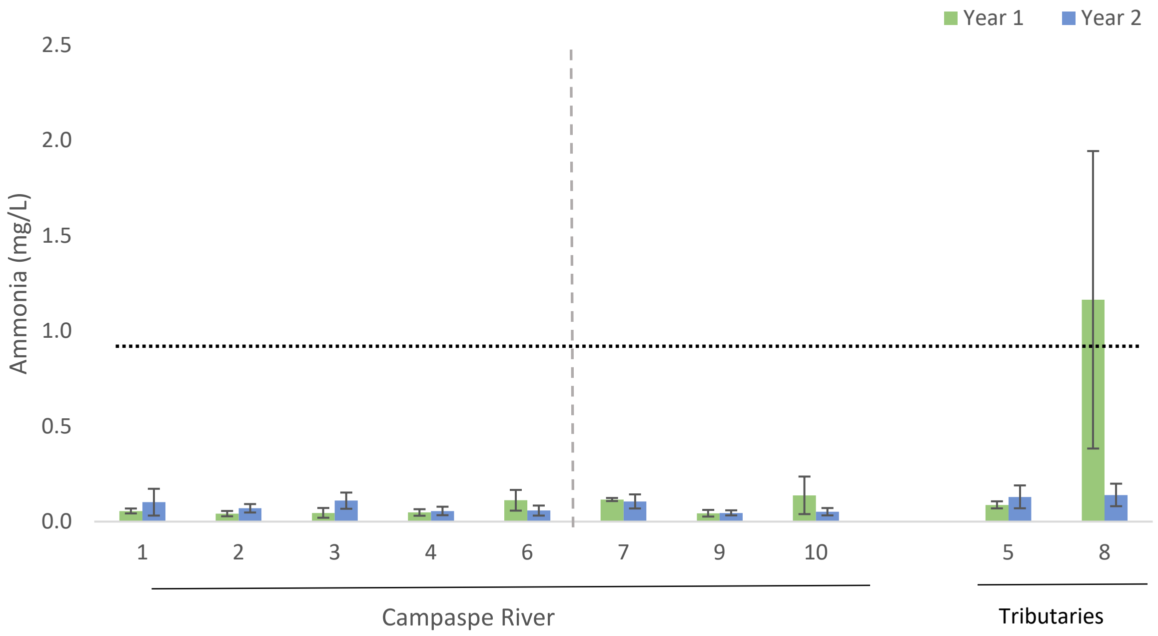

Figure 11. Mean ammonia concentrations in surface waters during Years 1 and 2 of monitoring. The vertical dotted line indicates Kyneton WRP discharge between Sites 6 and 7. The horizontal dotted line indicates SEPP (Waters) water quality indicator value.

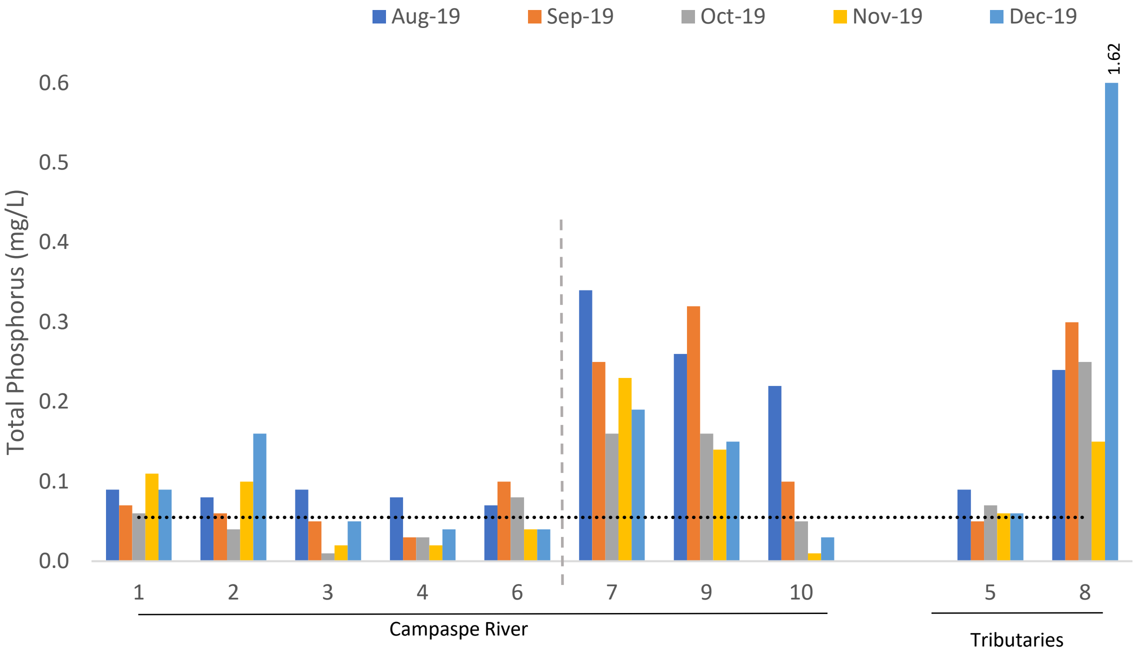

Figure 12. Monthly total phosphorous concentrations in surface waters during Year 2 monitoring from August to December 2019. The vertical dashed line indicates Kyneton WRP discharge point between Sites 6 and 7. Where concentrations were below the limit of reporting these have been presented as zero. The horizontal dotted line indicates SEPP (Waters) water quality indicator value.

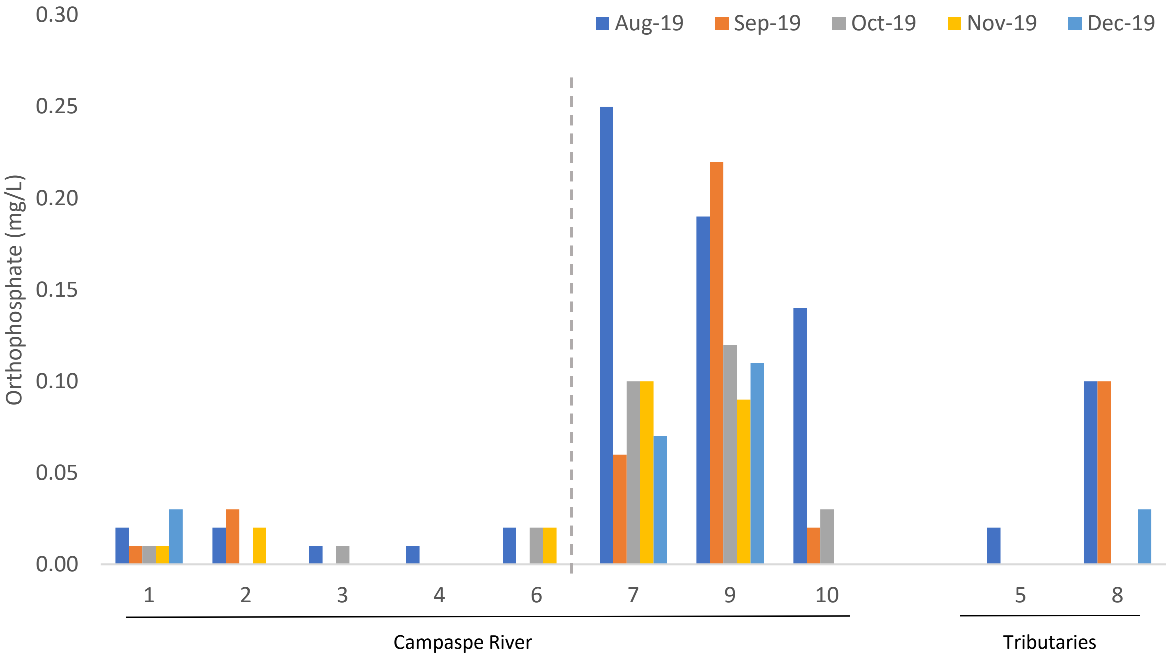

Figure 13. Monthly orthophosphate concentrations in surface waters during Year 2 monitoring from August to December 2019. The vertical dashed line indicates Kyneton WRP discharge point between Sites 6 and 7. Where concentrations were below the limit of reporting these have been presented as zero.

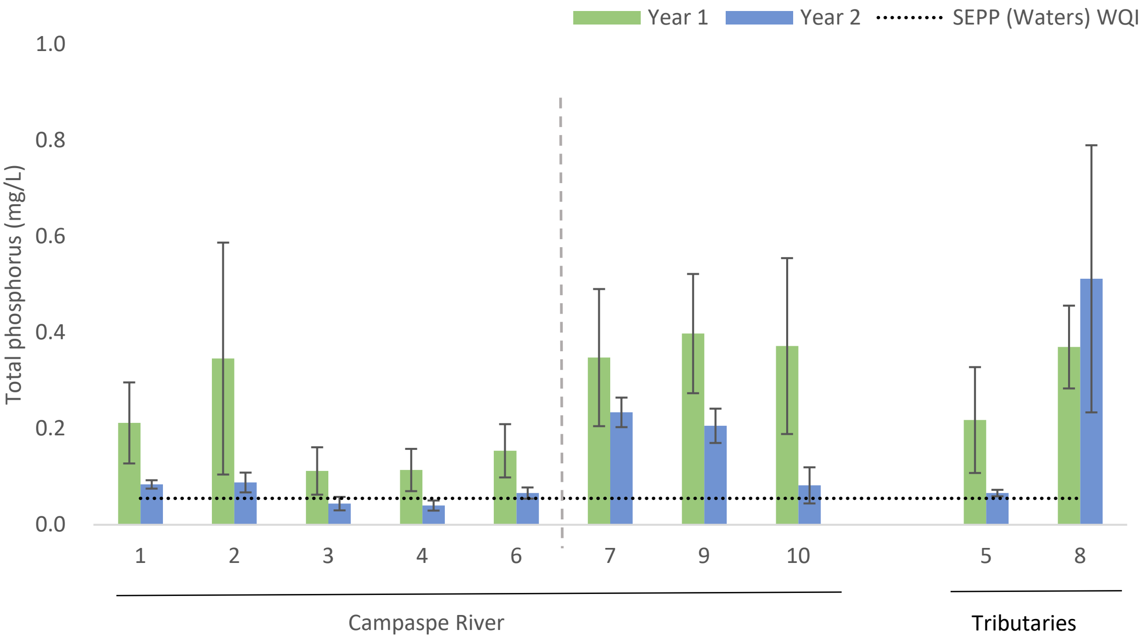

Figure 14. Mean total phosphorus concentrations in surface waters during Years 1 and 2 of monitoring. The vertical dotted line indicates Kyneton WRP discharge between Sites 6 and 7. The horizontal dotted line indicates SEPP (Waters) water quality indicator value.

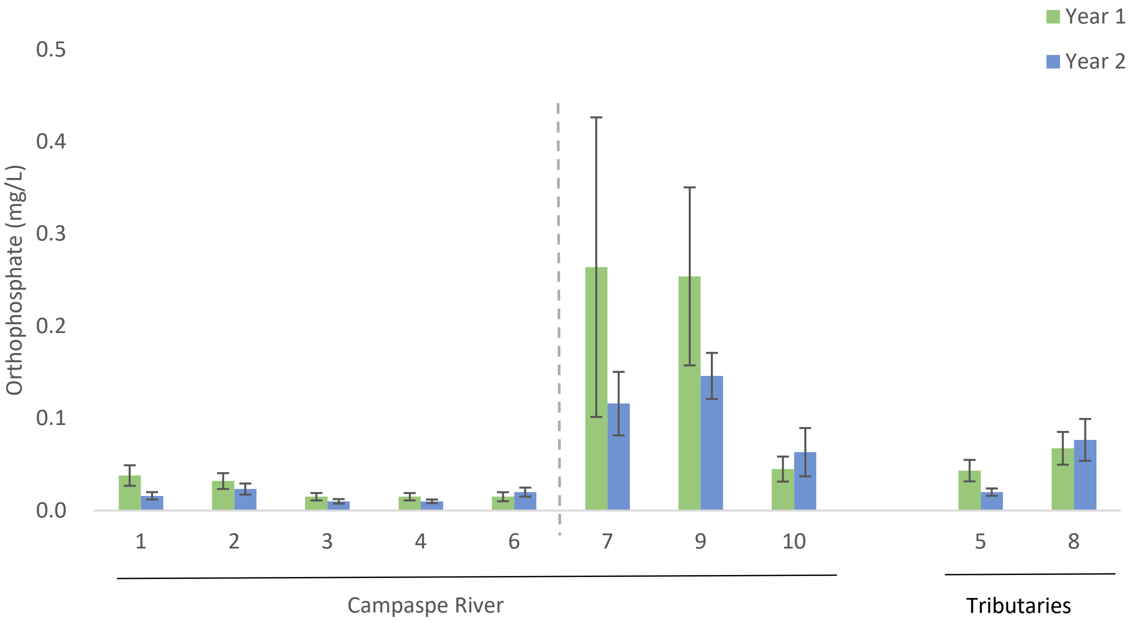

Figure 15. Mean orthophosphate concentrations in surface waters during Years 1 and 2 of monitoring. The vertical dotted line indicates Kyneton WRP discharge between Sites 6 and 7.

Table 5. Ratio of orthophosphate to total phosphorus in surface waters at study sites from August 2019 to December 2019. Where orthophosphate was below the limit of reporting this has been indicated with a dash.

Site #	Sampling Month				
	August 2019	September 2019	October 2019	November 2019	December 2019
Campaspe River					
1	0.22	0.14*	0.17*	0.09	0.33
2	0.25	0.50*	-	0.20	-
3	0.11	-	1.00*	-	-
4	0.13	-	-	-	-
6	0.29	-	0.25*	0.50*	-
Kyneton WRP discharge between Sites 6 and 7					
7	0.74	0.24	0.63*	0.43*	0.37*
9	0.73	0.69*	0.75*	0.64*	0.73
10	0.64	0.20*	0.60*	-	-
Tributaries					
5	0.22	-	-	-	-
8	0.42	0.33*	-	-	0.02

* Larger ratios of orthophosphate were found in year 2 relative to year 1 monitoring for the respective month and site.

Faecal Monitoring

Analysis for the presence of *Bacteroides* was used to determine the source of faecal contamination at sites during December 2018, and August, September and November 2019 (Table 6). 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). Of the 24 samples tested over the four sampling events, 13 (54.2%) samples indicated faecal contamination of human origin based on the detection of either the HF183 marker gene (12 (50%) samples), Lachno3 marker gene (13 (54.2%) samples) or both marker genes (10 (41.7%) samples). The concentrations of the HF183 marker in qPCR positive samples ranged from 3.46 to 5.18 gene copies (GC)/L, whereas the levels of the Lachno3 ranged from 3.55 to 6.19 GC/L. The concentrations of the HF183 and Lachno3 marker genes in several samples were above the gastrointestinal (GI) risk benchmark of 4.50 $\log_{10}$ GC/L (HF183) and 5.14 log10 GC/L (Ahmed *et al.* 2019). The prevalence of the ruminant marker was lower than the human-associated marker genes. Six of 24 (25%) samples were positive for the ruminant marker BacR, of which five were quantifiable. The concentrations of BacR marker gene in PCR measurable samples ranged from 3.24 to 6.21 GC/L of water (Table 6).

Like Year 1 results, *E. coli* levels in Year 2 were mostly below the SEPP (Waters) trigger values for a Class A/B rating indicating the water quality was suitable for primary contact and secondary recreation, and suitable for livestock drinking and application to pasture (with conditions)[2]. The exceptions were Sites 2, 7 and 8. Levels of *E. coli* exceeded 100 organisms per 100 mL at Site 2 in September 2019 and Site 7 in November 2019 (Figure 16). This is considered equivalent quality to Class C recycled water (not suitable for primary contact or livestock drinking; suitable for secondary

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[2] SEPP Waters objectives are a guideline only, as required sample numbers for comparison not met.

contact recreation). *Bacteroides* results indicate that faecal contamination at Site 2 was from both human and ruminant origins, whereas faecal contamination at Site 7 was predominantly ruminant origin, with an unquantifiable signal of human origin (Table 6). At Site 8, *E. coli* levels exceeded SEPP (Waters) trigger values for a Class D rating (551-5500 organisms/100 mL) in both August and September 2019, indicating the water was not suitable for primary contact or livestock drinking, but suitable for secondary contact recreation (Figure 16). Site 8 also exceeded 2400 organisms per 100 mL in July 2019 during the first year of monitoring. Furthermore, the *Bacteroides* results indicate that the faecal contamination present at Site 8 during August 2019 was predominantly of human origin, with an unquantifiable signal of ruminant origin (Table 6). In contrast, the origin of faecal contamination at this site in September 2019 was from both human and ruminant sources. The origins of faecal contamination in July 2019 are unknown as gene markers from the samples were not assessed.

Table 6. 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.

Site		Date	Human markers (GC/L)		Ruminant marker (GC/L)
			HF183	Lachno3	BacR
Campaspe River	2	Dec-18	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
Campaspe River	2	Aug-19	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	+
Campaspe River	2	Sep-19	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
Campaspe River	2	Nov-19	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

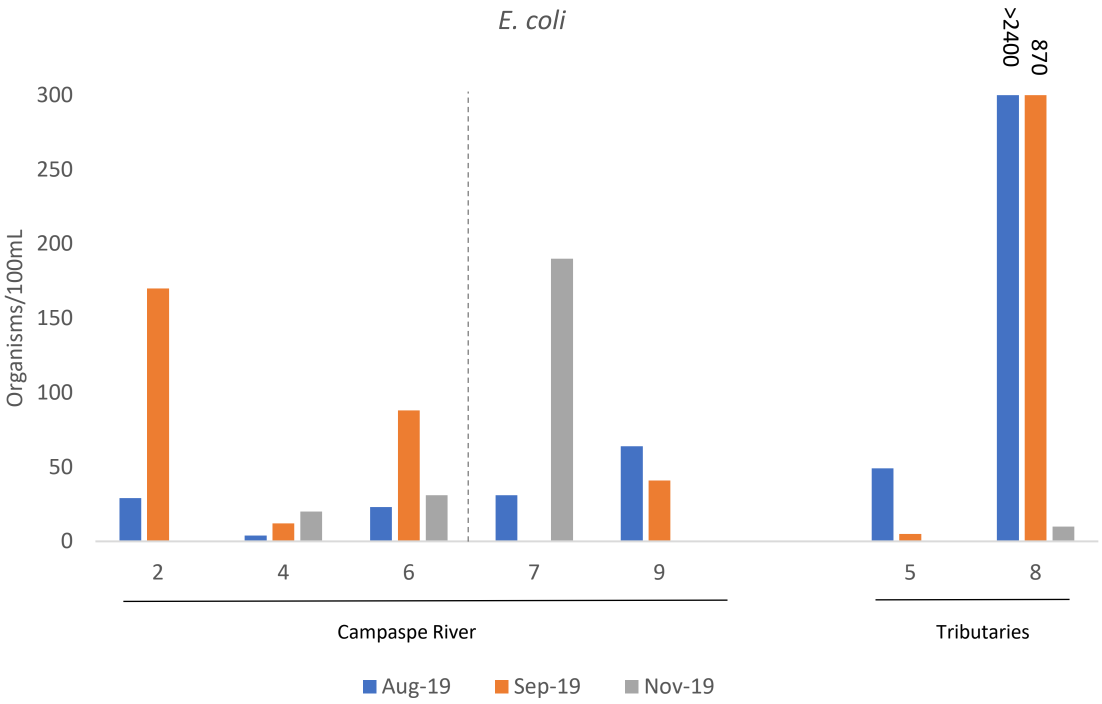

Figure 16: *E. coli* across six sites during August 2019, September 2019 and November 2019. The dashed line indicates Kyneton WRP discharge point between Sites 6 and 7. Where results were below the limit of reporting this has been presented as zero.

Aquatic Ecology

Macroinvertebrate Survey

SIGNAL2 scores and the number of families present were calculated to indicate waterway condition and assess whether the waterways met the biological standards outlined in the SEPP (Waters) objectives. The Campaspe River is in the central foothills and coastal plains zone and requires a SIGNAL2 score of ≥3.4 and 20 families (calculated for edge habitat in spring) to meet SEPP (Waters) objectives. These guidelines, however, are intended for permanently flowing streams. The upper Campaspe River, and the tributaries of Snipes Creek and Post Office Creek, are all ephemeral, and long-lived macroinvertebrates, which require permanent waters, are less likely to occur in these habitats.

SIGNAL2 scores for Sites 1, 5, 6, 7 and 9 were below the SEPP (Waters) objectives, as were the numbers of families at Sites 1, 2, 5, 7, 8, 9 and 10 (Table 7). Diversity was lowest at Site 10 (10 families) and greatest in the middle reaches of the river at Sites 3 (27 families), 4 (24 families) and 6 (23 families). Family level diversity was lower in Year 2 than in Year 1 at Sites 1, 2, 5, 7, 9 and 10 (Table 7). During the previous year of monitoring, 21 macroinvertebrate families were found at Site 10, including 8 EPT (Ephemeroptera (mayflies), Plecoptera (stoneflies), Trichoptera (caddisflies) families. Despite the lower numbers of families present in Year 2 SIGNAL2 scores were similar between years.

Site 10 had the highest SIGNAL2 score (4.80) due to the high number of Gripopterygidae (Plectoptera, stoneflies, SIGNAL grade 8) present. This is a considerable increase in the SIGNAL2 score for edge habitat from Year 1 of monitoring (3.60). In contrast, Site 5, Post Office Creek, had the lowest SIGNAL2 score (2.85) and only 13 families, and supported tolerant species such as Physidae (Gastropoda, snails, SIGNAL grade 1) and Oligochaeta (worms, SIGNAL grade 2). This is a decrease in SIGNAL2 score from 3.1 in Year 1 of monitoring. A reduction in SIGNAL2 scores from Year 1 monitoring was also observed for Sites 3 and 9 (Table 7).

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/or poor habitat. Although there are no SEPP (Waters) objectives for EPT taxa in edge habitats, the number of EPT families has been calculated for general guidance. EPT family diversity was greatest at Sites 3 and 4 (5 families) and was generally greater upstream of Site 7 at Old Station Rd. Few sensitive taxa were found at Sites 5, 7, 8, and 9 (1 family). The number of EPT families at Sites 3, 4, 5, 9 and 10 decreased from the previous monitoring period (Table 7).

All sites showed some variation in the taxonomic composition of macroinvertebrate assemblages across the two years. The degree of change was marginal for most sites, except for Site 10 which showed a large change in species composition (Figure 17, Table 8). In general, species composition among sites was most similar among sites located along the Campaspe River. The similarity between these sites was not indicative of their geographic position along the river or distance from one another. However, Sites 5 and 8, located along tributaries, showed some separation in their faunal assemblages from those in the main river (Figure 17).

Table 7. Macroinvertebrate biological indices at each site sampled during September 2019.

Site	SIGNAL2	Number of families	Number of EPT families
1	**3.17**	**19***	2
2	3.80	**15***	3
3	3.73*	27	5*
4	3.77	24	5*
6	**3.38**	23	4
Kyneton WRP discharge between sites 6 and 7			
7	**3.31**	**16***	1
9	**3.23***	**14***	1*
10	4.80	**10***	3*
Tributaries			
5	**2.85***	**13***	1*
8	3.67	**12**	1
SEPP (Waters) Objectives^			
Edge	3.4	20	N/A

* Reduction from Year 1 monitoring

^ SEPP (Waters) Objectives are used as a guideline only as only one season was sampled (Spring)

Values presented in **bold** are below State Environment Protection Policy (Waters) biological objectives

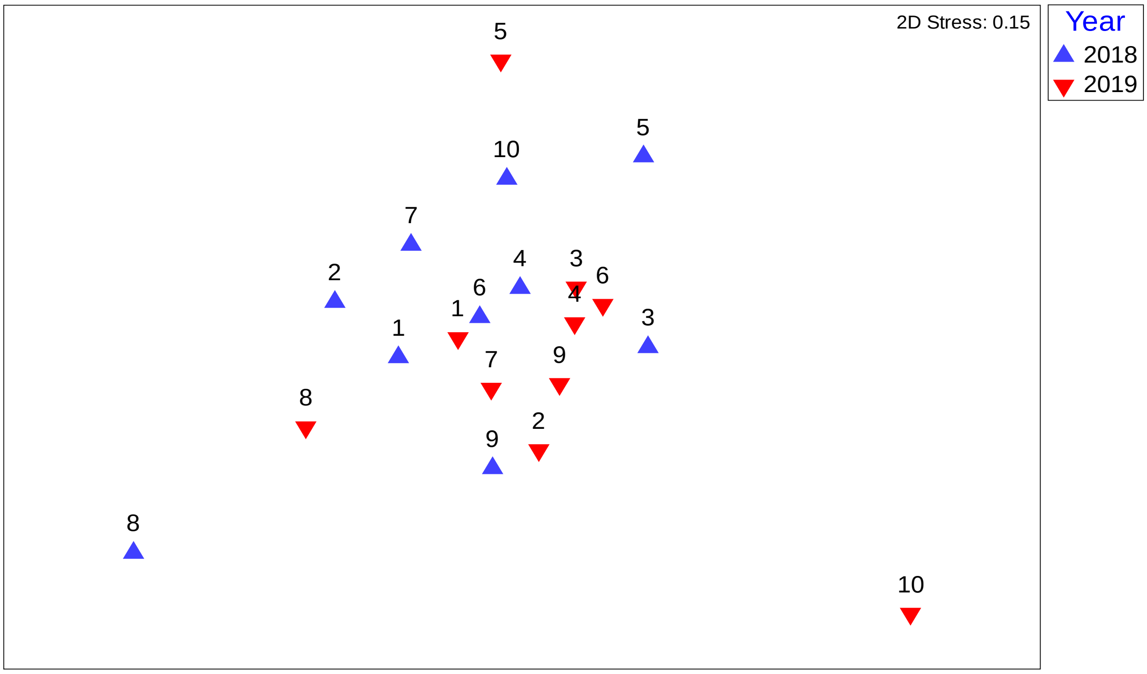

Figure 17. Non-metric multidimensional scaling ordination based on the presence or absence of macroinvertebrate taxa and the Bray-Curtis dissimilarity index. Points close together are similar in composition whereas points further apart have greater variation in their taxonomic composition.

Table 8. Changes in the abundance of the top 10 most common taxa between Years 1 and 2.

Site	Ceinidae (amphipod)	Oligochaeta (worm)	Orthocladiinae (non-biting midge)	Ceratopogonidae (sandfly)	Chironominae (non-biting midge)	Simuliidae (blackfly)	Physidae (snail)	Corixidae (water boatman)	Gripopterygidae (stonefly)	Leptophlebiidae (mayfly)	Dytiscidae (Larva)(beetle)	Coenagrionidae (dragonfly)
Campaspe River												
1	-	-	↑	-	-	NP	-	↑	NP	-	-	↓
2	↑	-	-	-	↓	NP	↑	↓*	↑*	↑*	-	↓*
3	↓	↑*	-	↓	↓	-	↑	-	↑	↑	↓*	-
4	-	-	↓	-	↓	NP	-	-	-	↓	-	-
6	-	-	-	↓*	-	↑*	-	↓	-	↓*	-	-
Kyneton WRP discharge between Sites 6 and 7												
7	-	↑	-	-	↓*	-	-	-	↑*	NP	-	-
9	↑	↑	↑	-	↓	↑	↓	↑*	↑	↓*	-	↓*
10	NP	↑	-	↓*	↓	↑*	↓*	↓	↑*	↓*	↓*	↓*
Tributaries												
5	↑*	↑	-	↓*	↓	NP	↑	↓	NP	NP	NP	↓
8	NP	-	↑*	-	-	NP	NP	NP	NP	NP	-	↑*

- No change between years; ↑* Not present in 2018; ↑ Increase in abundance in 2019; ↓* Not present in 2019; ↓ Decrease in abundance in 2019; NP taxa not observed at site

Nutrient Bioavailability

Visual Assessments of Macrophyte and Algal Growth

Visual assessments of macrophyte and filamentous algal growth were undertaken to assess the level of nuisance algal and plant growth. Aquatic macrophytes are important structural and biological components of streams. Healthy macrophyte assemblages (i.e. not excessive) support ecosystem processes in degraded streams (Paice et al. 2017). In general, macrophyte cover was greater at sites along the Campaspe River, compared with the tributary sites. Mean monthly macrophyte cover (Figure 18) was lowest at Sites 10 (1.6%) and 8 (4.3%), and highest at Sites 1, 2 and 4 (83.3%, 85.0% and 77.94%). Mean macrophyte cover was greater in Year 2 of monitoring at Sites 1, 2, 4, 5, 7 and 9 (Figure 19). Reaches with poor macrophyte coverage typically have lower macroinvertebrate abundance and family richness (as seen at Sites 5 and 8) than those with healthy macrophyte communities (Paice et al., 2017). In contrast, high macrophyte cover may arise from elevated nutrient availability and may be a nuisance.

Filamentous algae are considered to have reached nuisance levels at 30% coverage of the stream bed (Biggs & Kilroy 2000). Site 10 presented with the lowest average monthly filamentous algal cover (20.2%), while all other sites had filamentous algal cover above nuisance levels. Sites 5 and 3 had the highest levels of nuisance filamentous algal cover (67.3% and 64.0%) (Figure 20). Differences in the length of filamentous algae were also noted, as longer filamentous algae typically reflect waters with a greater availability of nutrients. Short filamentous algae were dominant across all sites but were proportionately greatest at Sites 3 (58.6%) and 6 (56.7%). Long filamentous algae were present in low proportions but were most common from September to November 2019. Compared with Year 1 of monitoring, there has been a notable decline in both medium and long filamentous algae across all sites (Figure 21) suggesting a reduction in nutrient availability. Overall, filamentous algal cover had decreased at Sites 1, 2, 3, 4 and 10 between Years 1 and 2 (Figure 21), which is possibly related to reductions in nutrients, but could also be related to flow, light availability and water temperatures.

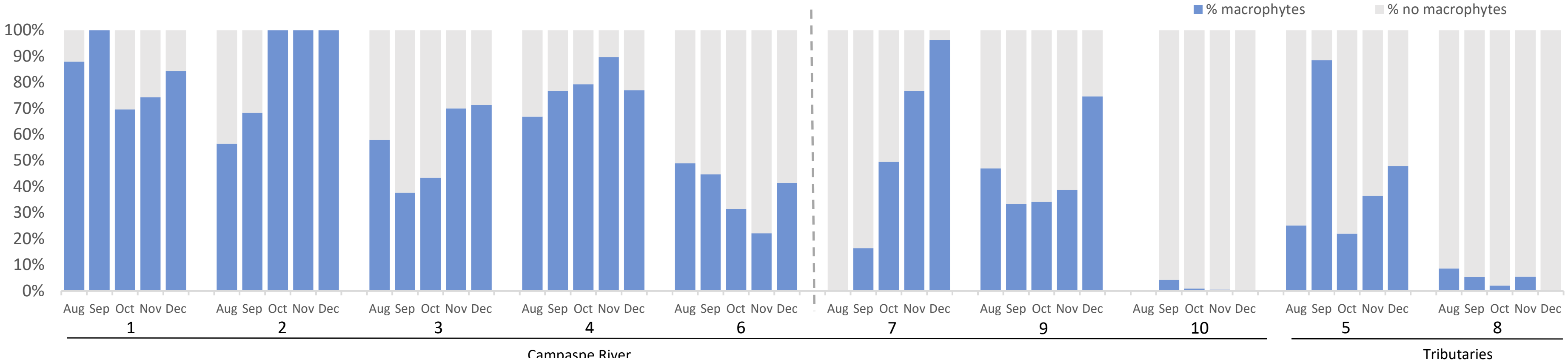

Figure 18. Monthly mean macrophyte cover at monitoring sites. The grey dashed line indicates Kyneton WRP discharge point between Sites 6 and 7.

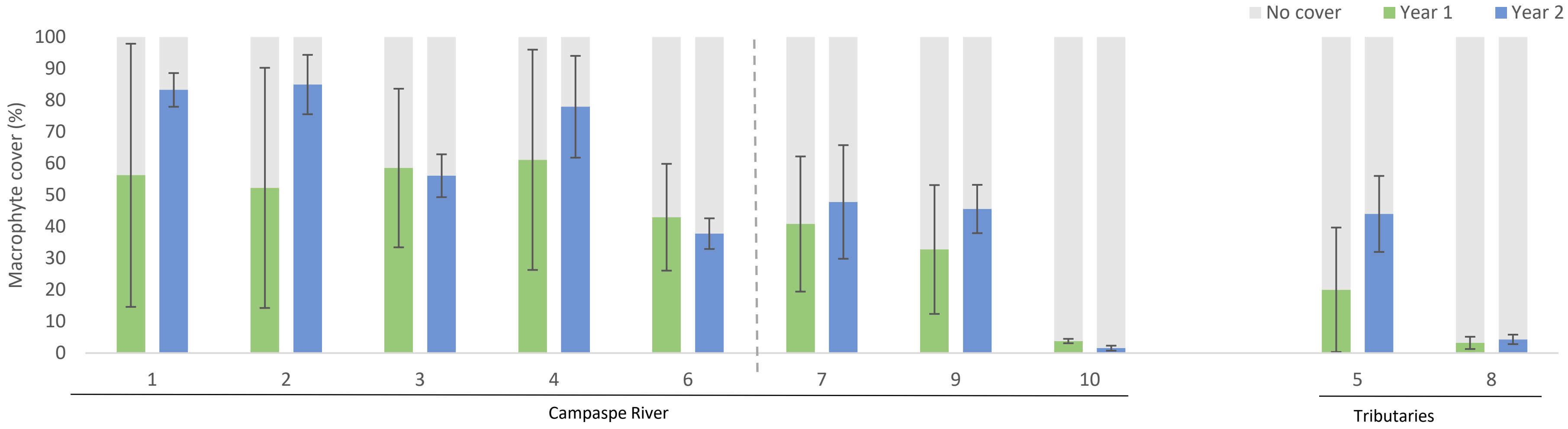

Figure 19. Mean macrophyte cover at sites in Years 1 and 2 of monitoring. The grey dashed line indicates Kyneton WRP discharge point between Sites 6 and 7.

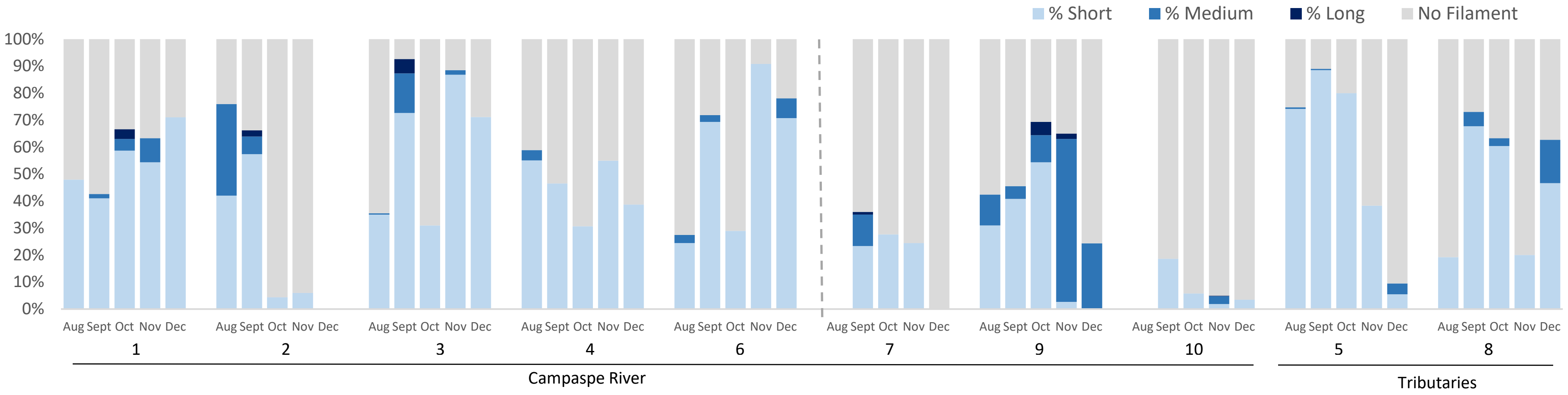

Figure 20: Monthly mean filamentous algal cover within sites. The grey dashed line indicates Kyneton WRP discharge point between Sites 6 and 7. Filament classifications are as follows; short = <2 cm, medium = 2-10 cm, and long = >10 cm.

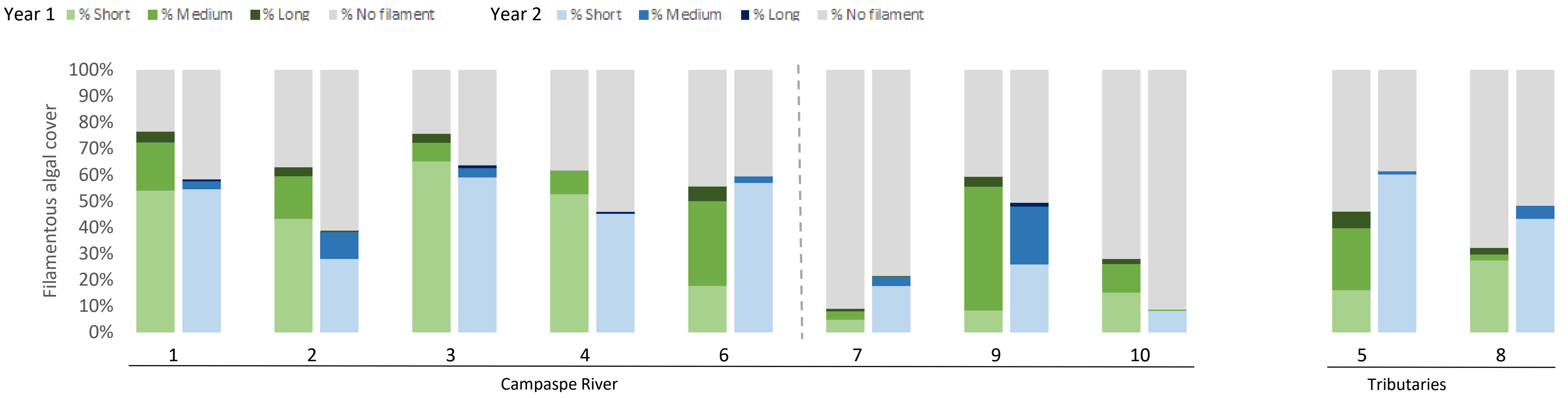

Figure 21. Mean filamentous algal cover in Years 1 and 2 of monitoring. The grey dashed line indicates Kyneton WRP discharge point between Sites 6 and 7. Filament classifications are as follows; short = <2 cm, medium = 2-10 cm, and long = >10 cm.

Artificial Substrates

Artificial substrate sampling was used to measure biomass production, where the precise effects of water quality need to be assessed, but where the natural substrates cannot be sampled. Algae are important primary produces in aquatic ecosystems, and provide an indication of ecosystem health, and qualitative and quantitative food availability for higher trophic levels. Algal biomass production and relative community composition were measured from artificial substrates in September and November 2019. Chlorophyll-a and ash free dry mass (AFDM) were used as measures of algal biomass production. Chlorophyll-a provides an indication of the autotrophic component of algal biofilms, whereas AFDM provides a measure of the combined autotrophic, heterotrophic and detrital carbon found in biofilms. From these measures an autotrophic index (AI) was calculated from the ratio of AFDM:chlorophyll-a. This 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 that are impacted by sources of organic pollution.

A significant difference in chlorophyll-a concentrations was observed between sites in both September (Kruskal-Wallis, H=20.44, df=9, p=0.015) and November 2019 (Kruskal-Wallis, H=27.24, df=9, p=0.001)(Figure 22). Chlorophyll-a concentrations at Sites 5, 7, 8, and 9 were greater than those at Sites 1, 2, 3 and 4 in September 2019, whereas in November 2019 chlorophyll-a concentrations at Sites 2, 4 and 5 were greater than those at all other sites.

Greater chlorophyll-a concentrations were observed in November 2019 at Campaspe River Sites 1, 2, 3, 4 and 10 compared to September 2019. While at Campaspe River Sites 6, 7 and 9, and tributary Sites 5 and 8, greater concentrations were observed in September as compared to November 2019 (Figure 22). Higher chlorophyll-a indicates a higher level of autotrophic biomass (e.g., more algae) and may reflect greater nutrient availability or recent rainfall.

Chlorophyll-a concentrations across Sites 2, 3, 4, 6, 7, 8 and 9 were comparable to Year 1 results, chlorophyll-a concentrations had decreased substantially at Sites 1 and 5, and to a lesser extent at Site 10. Sites 6, 7 and 9 showed an increase in chlorophyll-a relative to year 1 indicating a greater number of autotrophs at these sites relative to Year 1.

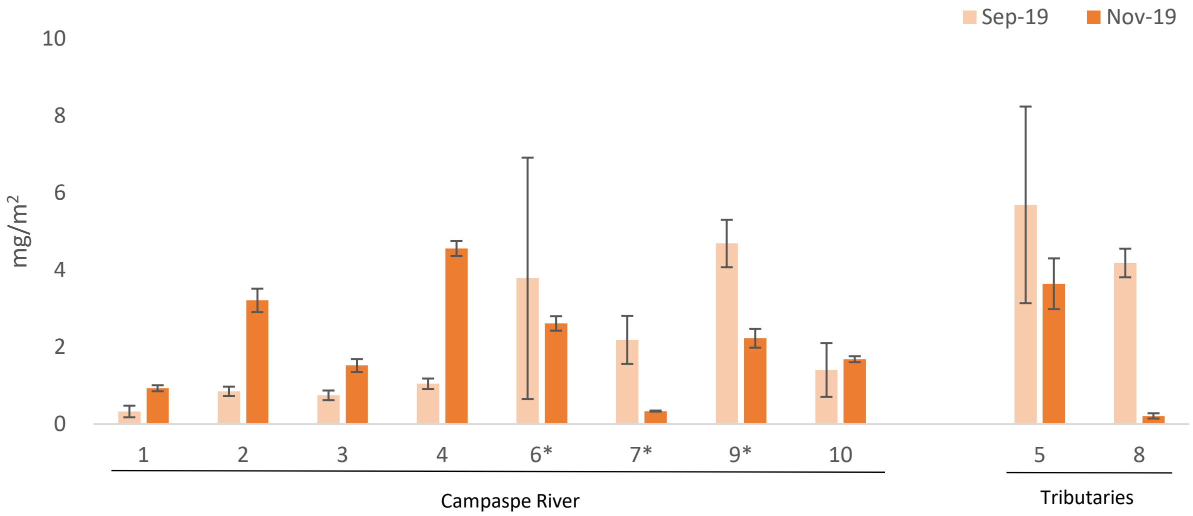

Figure 22: Chlorophyll-a (±SE) of biofilms on artificial substrates deployed across sites during September and November 2019. The dashed line indicates Kyneton WRP discharge point between Sites 6 and 7. Sites that had greater chlorophyll-a concentrations in Year 2 compared with Year 1 are denoted with *.

A significant difference in the AFDM between sites was observed in November 2019 (Kruskal-Wallis, H=26.33, df=9, p=0.002), but not during September 2019 (Kruskal-Wallis, H=15.85, df=9, p=0.070). Seasonal variation was observed at Sites 1, 3, 5, 6, 7, 8 and 10 (Figure 23). AFDM was higher in September 2019 at Sites 3, 5 7, and 8, but was higher in November 2019 at Sites 1, 6 and 10. AFDM showed little variation at Sites 2, 4, and 9 between sampling months. AFDM was particularly elevated at Site 5 in September 2019, and far exceeded values observed at other sites during Years 1 and 2 of monitoring. Overall mean AFDM was greater in Year 2 of monitoring relative to Year 1 at Sites 1, 4, 5, 6, 7 and 9 indicating an increase in the amount of autotrophic, heterotrophic and detrital carbon found in biofilms.

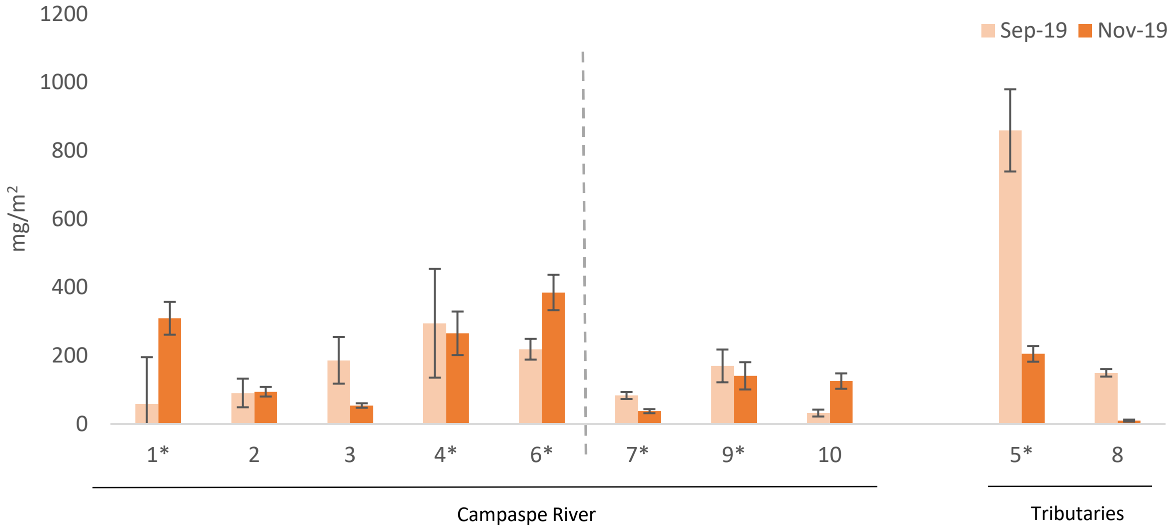

Figure 23: Mean ash-free dry mass (AFDM) (±SE) of artificial substrates deployed across sites during September and November 2019. Dashed line indicates Kyneton WRP discharge point between Sites 6 and 7. Sites that had greater AFDM in Year 2 compared with Year 1 are denoted with *.

The autotrophic index (AI) provides a measure of the autotrophic-heterotrophic balance of the biofilm community. Values over 400 typically indicate biofilm communities with reduced algal assemblages, and a greater relative abundance of heterotrophic organisms, such as bacteria and fungi. Biofilm communities with this balance of autotrophic to heterotrophic organisms are typically indicative of organic pollution. A mean AI value of more than 400 was found only at Site 1 during September 2019. Mean AI values at all other sites were below this threshold, although did exceed the threshold on some of the deployed substrates at Sites 3 and 5 (Figure 24). Mean AI values were lower across all sites in Year 2 relative to Year 1, and this suggests biofilms are comprised of greater quantities of algal communities, and lower quantities of heterotrophic organisms or amorphous organic material.

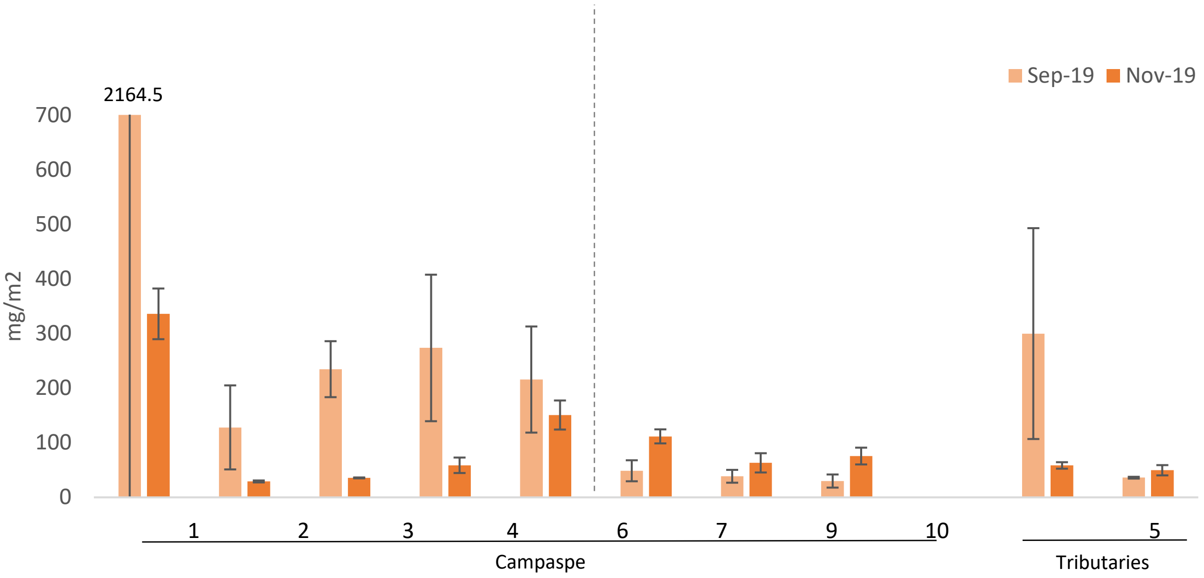

Figure 24: Autotropic index (±SE) of artificial substrates deployed across sites during September and November 2019. The dashed line indicates Kyneton WRP discharge point between Sites 6 and 7. Mean autotrophic index had decreased at all sites relative to Year 1.

The community structure of cyanobacteria, chlorophytes (green algae) and diatoms is an important indicator of ecosystem health (particularly food quality) and is sensitive to ambient environmental changes (Zhang et al., 2020). Communities dominated by cyanobacteria can indicate nutrient enrichment of surface waters, whereas strong diatom communities provide a high-quality food source for aquatic invertebrates.

Communities in September 2019 were generally comprised of relatively equal proportions of cyanobacteria and diatoms, whereas in November 2019 generally fewer diatoms and more chlorophytes were present (Figure 26). The greater proportion of chlorophytes indicated higher amounts of green algae. The proportion of cyanobacteria found on artificial substrates was relatively consistent between sites and seasons, except for Site 1, where the proportion of cyanobacteria was considerably greater in November 2019. Chlorophytes were notably absent from Sites 2, 4 and 5 in September 2019, and Sites 1, 3, 5 and 7 in November 2019 (Figure 25), and this may indicate depleted N:P ratio in surface waters.

Compared with Year 1, mean relative community composition appeared more heterogenous across sites in Year 2 (Figure 26), as chlorophytes were noticeably absent from sites during July 2018). Compared with November 2018 (Year 1), mean community composition of Year 2 supported relatively more cyanobacteria at Sites 1, 8, 9 and 10; more chlorophytes at Sites 2, 3, 4, 6, 7, 8 and 9; and more diatoms at Sites 1, 5, 6, 7, 8 and 10.

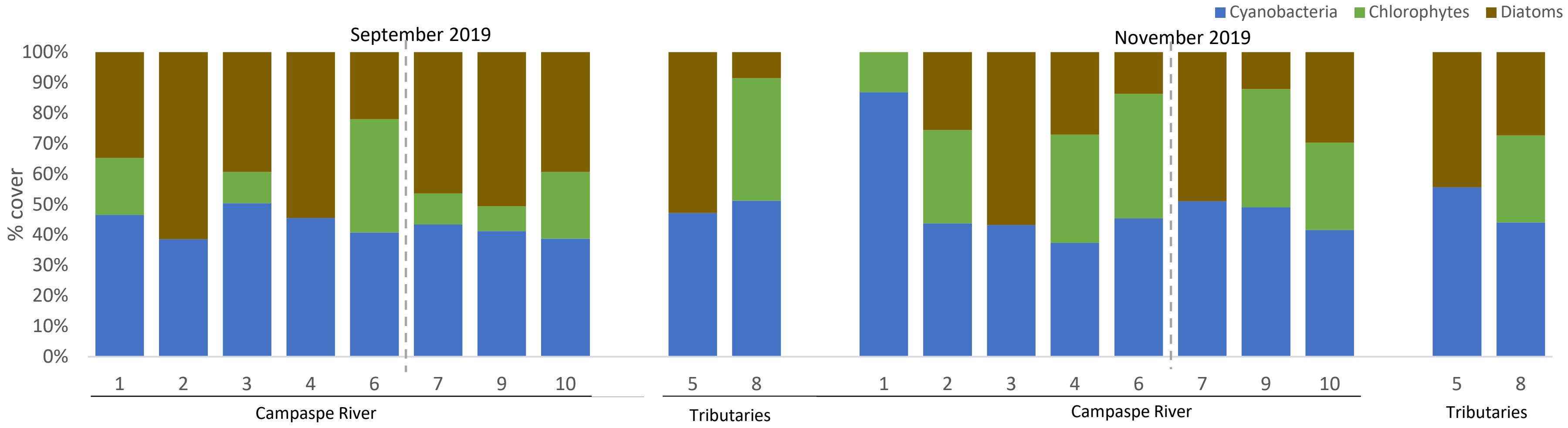

Figure 25: Relative composition of Cyanobacteria, Chlorophytes and diatoms, as determined using a Phyto-PAM, on artificial substrates deployed across sites in September and November 2019. The dashed lines indicate Kyneton WRP discharge point between Sites 6 and 7.

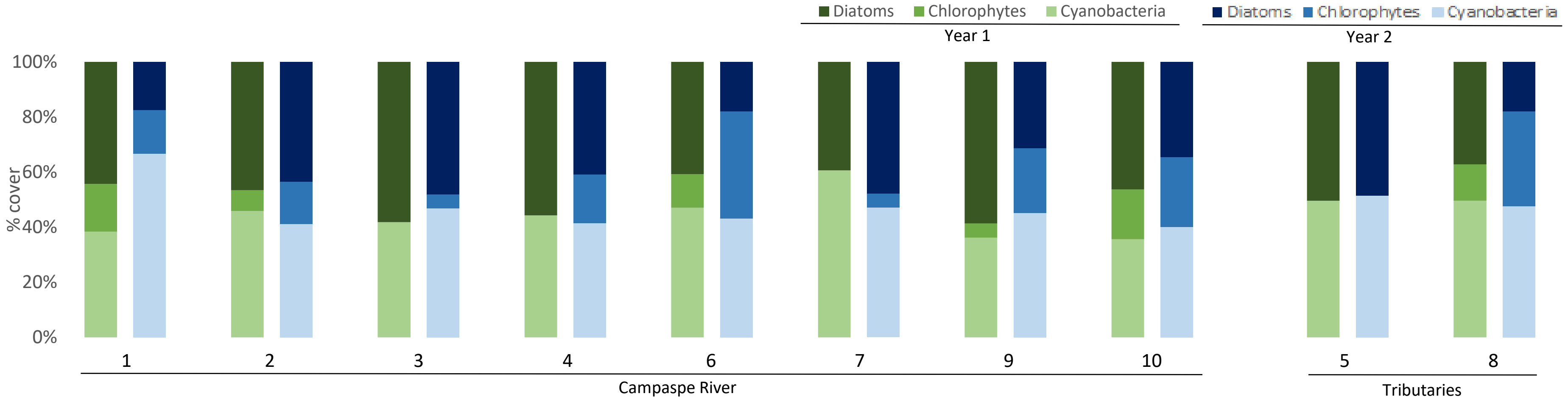

Figure 26: Mean relative composition of algal groups on artificial substrates in Years 1 and 2 of monitoring. Chlorophytes were absent from all sites during July 2018 making the variability of algal communities appear more consistent across sites.

Ecotoxicology

Water Toxicity - Snails

The ecotoxicity of surface waters were tested on aquatic snails to determine whether contaminants present at each site were causing any ecological impairment. Mud snail survival and reproductive success were used as measures of ecological impairment.

Mud snail survival did not differ across sites (Kruskal-Wallis, H=11.58, df=9, p=0.24) (Figure 27), and ranged between 94 to 98% for all sites, except at Site 7, indicating no significant impairment on survival. Average survival was lowest at Site 7 for the second year in a row, albeit survival had improved in Year 2 of monitoring compared with Year 1 (Year 1 survival 60%, Year 2 survival 79%).

The impact of water quality on the reproductive success of the mud snail was determined by the number of embryos present. Low numbers indicate possible disruption of endocrine function. No significant difference was found between sites (Kruskal-Wallis, H=8.47, df=9, p=0.49) (Figure 28). Embryonic production was greatest at Site 6 (55%) and lowest at Site 8 (41%). Similar results were observed during Year 1 monitoring, with embryo production ranging from 37% to 65%.

Overall, ecotoxicology results indicate surface waters were not impacting on invertebrate health, perhaps with the exception of Site 7.

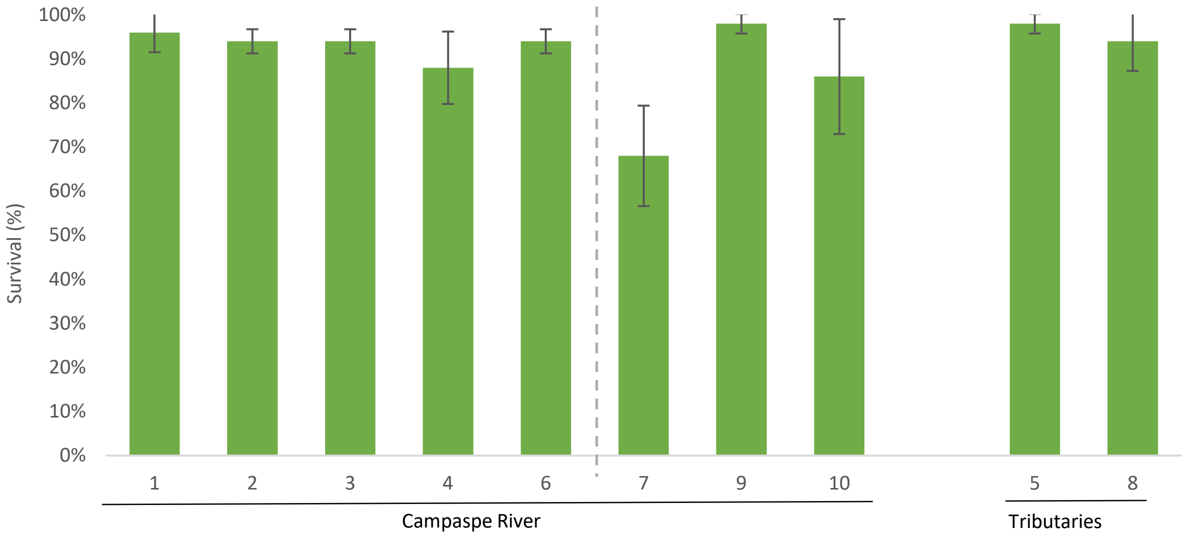

Figure 27: Mean survival (±SE) of mud snails, *P. antipodarum*, across sites during October 2019. Blue dashed line indicates Kyneton WRP discharge point between Sites 6 and 7.

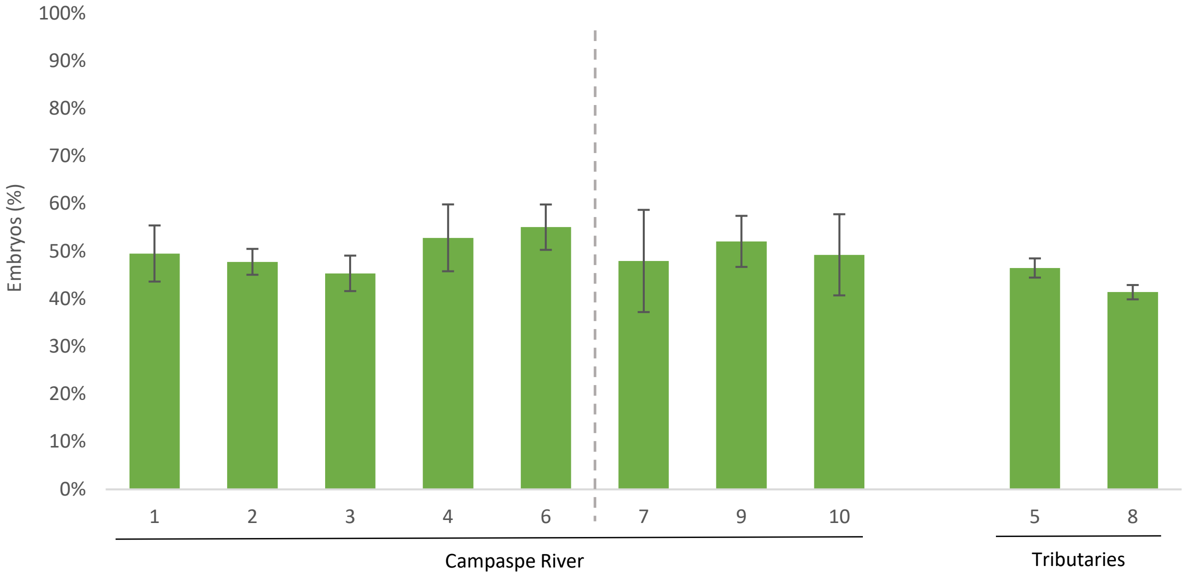

Figure 28: Mean percentage (±SE) of mud snails, *P. antipodarum*, with embryos present across sites in October 2019. The dashed line indicates Kyneton WRP discharge point between Sites 6 and 7.

Water Toxicity - Algae

The effects of surface water nutrient availability and toxicity on flora were determined from algal balls left *in situ* at sites during September and November 2019. Exposed algal balls were compared to the control to determine if exposure to site conditions inhibited or encouraged algal growth. Positive values indicate growth inhibition; values of 0-20% indicate minimal impact, 20-50% indicate moderate impact and >50% indicate a large impact. Negative values indicate increases in biomass, suggesting that site conditions are encouraging growth and thus indicate nutrient enrichment.

Algal biomass in algal balls was measured in September and November 2019. Growth inhibition ranging from 18 to 29% was observed at Sites 4, 6 and 10 in September 2019 (Figure 29), while increases in algal growth were observed at Sites 2, 3, 5, 8 and 9. In November 2019, growth inhibition ranged from 5 to 69% for Sites 1, 2, 3, 4, 5, 6, 9 and 10. A substantial increase in algal growth was observed at Site 7 (361%), along with a small increase Site 8 (Figure 29). This suggests Site 7 is enriched with nutrients.

Algal growth was stimulated at Sites 5, 7, and 8 in Year 1 of monitoring, however, in Year 2, algal growth was considerably reduced or inhibited at Sites 5 and 8, whereas much greater algal growth was observed at Site 7. Relatively high concentrations of the herbicides triclopyr and 2,4-D were detected in surface waters and sediments at Sites 5 and 8 and may be adversely affecting algal health.

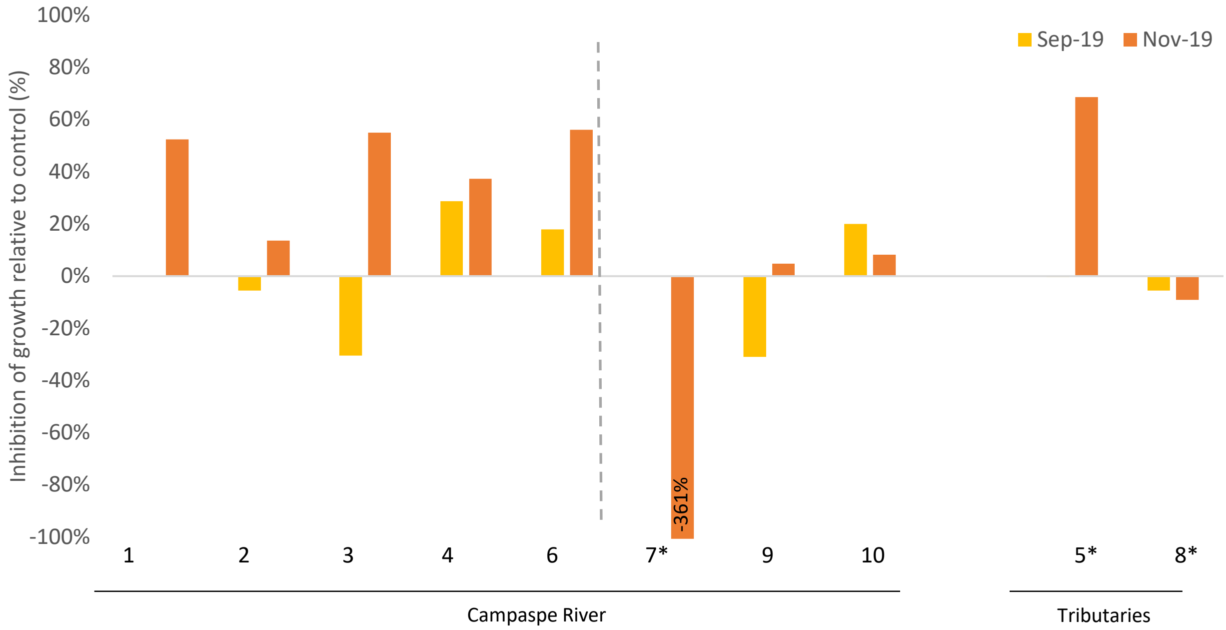

Figure 29: Percent inhibition of algal growth (relative to site control) across sites during September and November 2019. The dashed line indicates Kyneton WRP discharge point between Sites 6 and 7. Sites where algal growth was stimulated in Year 1 are indicated with *.

Sediment Chemistry

The results for the heavy metals, petroleum hydrocarbons and pesticides analysed from the ten sites are presented in Table 9. ANZECC/ARMCANZ Sediment Quality Guidelines (2000) were exceeded for lead (Site 5, 83 mg/kg), zinc (Site 5, 836 mg/kg), mercury (Site 9, 1.2 mg/kg), and nickel (all sites, 23-48 mg/kg). The metals exceeded, and sites at which they were exceeded, are consistent with results from Year 1 of monitoring. The level of mercury reported at Site 9 in November 2019 (1.2 mg/kg) was almost double the result from July 2019 (0.7 mg/kg) and is eight times the designated guideline value of 0.15 mg/kg. The concentration of zinc at Site 5 was more than four times the guideline value but had not changed significantly from previous monitoring.

Petroleum hydrocarbons were above ANZECC/ARMCANZ Sediment Quality Guidelines (2000) at all sites but were particularly elevated at Sites 8 and 10 (3730 mg/kg and 3060 mg/kg). The lowest concentration of petroleum hydrocarbons was recorded at Site 7 (560 mg/kg). Concentrations at all other sites ranged between 1000-2200 mg/kg (Table 9). Compared to July 2019, this is a substantial increase in the concentration of petroleum hydrocarbon in sediments.

Two synthetic pyrethroid insecticides, bifenthrin and permethrin, commonly used in commercial and household pest control, were detected in sediments from Site 5 at concentrations of 0.016 and 0.0111 mg/Kg, respectively (Table 9).

Other pollutants

Polar Organic Chemical Integrated Samplers (POCIS) deployed at each site (Table 10) indicated varying levels of pharmaceuticals and herbicides present in surface waters.

The pharmaceutical carbamazepine, an anticonvulsant medication used primarily in the treatment of epilepsy and neuropathic pain, was detected at six sites (Sites 1, 2, 3, 7, 9 and 10). While cholesterol, a type of lipid biosynthesized by animal cells, was detected at Campaspe River Sites 6 and 7 (Table 10).

The herbicides, triclopyr and 2,4-D, were detected in surface waters (Table 10). Triclopyr, a systematic herbicide used to control broad-leaved weeds and woody plants such as blackberry, was detected at all sites. While 2,4-D, a systematic acid-based herbicide which selectively kills most broadleaf weeds, was detected at three sites in the Campaspe River (Sites 3, 4 and 6) and the two tributary sites (5 and 8). Triclopyr was also detected during Year 1 monitoring at all sites, while this was the first detection of 2,4-D.

Table 9. Heavy metals, petroleum hydrocarbons, total organic carbon and pesticides detected in sediments across sites in November 2019.

Site #	Site Code	Heavy Metals (mg/Kg)															Petroleum Hydrocarbons (mg/kg)	Pesticides (mg/Kg)		Total Organic Carbon (%)
		Arsenic	Barium	Beryllium	Boron	Cadmium	Chromium	Cobalt	Copper	Lead	Manganese	Mercury	Nickel	Selenium	Vanadium	Zinc	C10 - C36 Fraction (sum)	Bifenthrin	Permethrin	
Campaspe River																				
1	1741	<5	170	1	<50	<1	40	10	16	18	249	<0.1	**23**	<5	30	100	**2190**	<0.01	<0.01	5.5
2	1742	<5	180	1	<50	<1	45	18	30	37	460	<0.1	**27**	<5	38	135	**1300**	<0.01	<0.01	4.25
3	1743	<5	180	1	<50	<1	69	20	27	19	470	<0.1	**44**	<5	54	146	**1020**	<0.01	<0.01	4
4	1744	7	250	1	<50	<1	59	35	27	29	1980	<0.1	**43**	<5	55	166	**1270**	<0.01	<0.01	5.03
6	1746	<5	170	1	<50	<1	66	27	37	22	508	<0.1	**38**	<5	57	169	**1480**	<0.01	<0.01	6.01
Kyneton WRP discharge between Sites 6 and 7																				
7	1747	<5	190	1	<50	<1	65	14	21	22	192	<0.1	**28**	<5	46	132	**560**	<0.01	<0.01	3.91
9	1749	16	280	1	<50	<1	66	42	27	28	1990	**1.2**	**48**	<5	61	86	**1010**	<0.01	<0.01	3.99
10	621	8	180	1	<50	<1	41	16	21	25	479	<0.1	**33**	<5	38	96	**3060**	<0.01	<0.01	5.62
Tributaries																				
5	1745	11	280	<1	<50	<1	55	25	53	**83**	427	0.1	**45**	<5	49	**836**	**2050**	0.016	0.011	4.39
8	1748	19	840	<1	<50	1	37	206	17	8	13400	<0.1	**45**	<5	67	65	**3730**	<0.01	<0.01	6.95
Trigger Value*		20	-	-	-	1.5	80	-	65	50	-	0.15	21	-	-	200	280			

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

Values **bold** if exceedance of trigger value

Values showing < are at the limit of detection

Table 10. Pharmaceuticals, personal care products and pesticides detected in surface waters using POCIS passive samplers (µg/0.2 g sorbent) across sites during November 2019.

Site	Pharmaceuticals and Personal Care Products		Herbicides	
	Carbamazepine	Cholesterol	2,4-D	Triclopyr
Campaspe River				
1	1.67	<10	<5	16.78
2	2.38	<10	<5	6.84
3	1.71	<10	9.55	14.19
4	<0.5	<10	10.97	9.33
6	<0.5	19.8	8.35	28.93
Kyneton WRP discharge between Sites 6 and 7				
7	38.33	84.6	<5	14.5
9	14.25	<10	<5	17.84
10	4.42	<10	<5	<2.5
Tributaries				
5	<0.5	<10	44.39	86.65
8	<0.5	<10	6.7	10.28

Discussion

Over the last few decades improvements in stream frontage management (SFM), mainly in the form of rehabilitation measures, such as the removal of exotic vegetation, riparian planting/fencing and improved stock management, have become commonly applied methods for attempting to improve water quality and reduce the export of pollutants to waterways (Hughes and Quinn 2014; McKergow et al 2016). However, the benefits of SFM on water quality and biodiversity have rarely been assessed (Quinn et al 2009; Hughes and Quinn 2014). This program monitors the effects on ambient water quality and biodiversity of implementing stream frontage management works (SFMW) on a 13 km stretch of the Upper Campaspe River and Post Office Creek around Kyneton over a 5-year period. The monitoring program is focused on measuring changes in ambient water quality, particularly nutrients, indicators of faecal contamination and physico-chemistry; together with measures of instream plant growth and macroinvertebrate diversity. Additionally, the program aims to understand whether other factors may be influencing water quality and instream health through assessments of water and sediment chemistry, and ecotoxicology.

Stream frontage management works along the Upper Campaspe River and Post Office Creek had mostly been completed at all sites funded by Coliban Water by the end of December 2019 (end of Year 2 monitoring). The main SFMW implemented included the installation of permanent fencing to exclude livestock from the river; removal of woody weeds, predominately willows, from along riverbanks and re-vegetation in riparian areas with native plants. The principle focus of these works is a reduction in the generation and delivery to the river of runoff of sediment, nutrients and pathogens from stock faeces. The first two years of monitoring show that elevated levels of nutrients occur across the study area, particularly in the two sites in the upper reaches of the Campaspe River at Carlsruhe; in the lower reaches of the River, from Sites 7 to 10; and in the two tributaries of Snipes and Post Office Creeks. Poor water clarity, as measured by turbidity, was also evident, particularly in the two tributary sites, although it also occurs, especially following higher rainfall, in the main River. Willows can increase instream nutrient concentrations and reduce water quality through inputs of organic matter during autumn and winter when they abscise their leaves (Lester et al., 1994). In addition, disturbance caused by willow removal may temporarily increase nutrients and sediment inputs through the mobilisation of organic matter and nutrients in sediments; the impacts of willow removal on nutrient levels can be seen for up to five years (Wagenhoff & Young, 2013).

Unrestricted stock access to waterways is a factor well known to contribute nutrients and sediments, and also faecal contamination to waterways (Biggs 2000; Shearman and Wilcock 2011; Hughes and Quinn 2014; McKergow et al 2016). Stock typically graze pasture to the water’s edge, resulting in the breakdown of the banks, and with no physical barrier to runoff, faeces, urine and nutrients on pastures enters waterways more freely when it rains (Shearman and Wilcocks 2011). When faecal matter is found in water, it's indicative of the potential for harmful pathogens to be also present (Ministry for the Environment, 2001; Shearman and Wilcock 2011). Elevated levels of *E. coli* were observed in Year 1 and 2 of monitoring at several sites, however, *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 in the Campaspe River and tributaries, markers for the presence of *Bacteroides*, a bacterium that inhabit the digestive tracts of animals, were applied to samples from a subset of sites. The markers indicated that faecal contamination at Campaspe River Sites 2, 4, 7 and 9 and tributary Site 8 (Snipes Creek) are in part attributable to stock.

Other sources of elevated nutrients and poor water clarity in waterways include urban runoff and wastewater, from both septic leakages and treated discharges. Inputs from these sources are observed at Campaspe River sites, including Old Station Road, Kyneton, Boundary Road, Langley, and the two sites around Carlsruhe, as well as Post Office Creek and Snipes Creek. Continued monitoring over the next three years will provide a greater understanding of the benefits that the completed stream frontage management works that seek to restrict stock access and remove exotic willows from along the River will have on nutrient inputs and water clarity in the Upper Campaspe.

Aquatic macrophytes and algae process stream nutrients and provide habitat and food resources for aquatic biota. However, 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). Macrophyte and algal abundance is generally strongly correlated with light and nutrient availability (Biggs 2000; Rutherford and Cuddy 2005). Shade from riparian canopy plays an important role in reducing water temperatures and controlling nuisance plant and algal growth (Hughes and Quinn, 2014; McKergow et al., 2016). Monitoring of macrophyte and filamentous algal abundance across the study sites indicated a higher macrophyte abundance (>50% cover) at sites with less riparian shade cover (<10% shading), and greater abundance of long filamentous algal cover (>30%) at sites with increased shade (>30% shading) and higher concentrations of available nutrients, particularly orthophosphates. Over time, a better balance in macrophyte abundance is likely to occur in these upper study reaches as new plantings grow and the level of shade increases.

For downstream reaches, particularly Sites 7-9, lower macrophyte abundance and greater filamentous algal growth is likely related to elevated nutrient levels and the higher shading observed across these sites (>40% riparian shade cover). Too much shade by riparian vegetation can result in reduced nutrient uptake by in-stream macrophytes, tipping the balance between nutrient uptake and export (Hughes and Quinn, 2014; McKergow et al., 2016). Campaspe River Site 7, at Old Station Road, is an example of where this is likely to be occurring. Heavy shading of the stream by willows has likely contributed to a reduced abundance of rooted instream macrophytes and lower biofilm biomass. This results in little instream processing of nutrients and greater export to downstream reaches, which is reflected in the excessive filamentous algal growth observed at Site 9 at Boundary Road. A similar issue is also observed in Snipes Creek (Site 8), where there is also a lack of instream macrophytes and biofilms, likely in part due to elevated turbidity and poor riparian cover, which is dominated by willows. These sites present great opportunities for improvement if stream frontage management activities were to be undertaken.

Assessments of macroinvertebrate communities provide a picture of ecological health in the River, with increased richness and diversity indicating better water quality and better ecological condition. In general, the Campaspe River is in a moderate condition, with good habitat available for macroinvertebrate communities at several sites; however, some sites, particularly Sites 7-9, and the tributaries, show a trend of poorer water quality, as shown by lower macroinvertebrate diversity and richness. To a certain extent, the moderate condition is related to the river being an ephemeral system, and, thus, without a permanent water source, macroinvertebrates must recolonise sites each year. Moreover, for sites in poorer condition, it is likely due to the effects of agricultural, and urban and industrial land uses on water quality and habitat, such as nutrient runoff, lack of quality riparian vegetation, lack of instream plants and algae for habitat and food, and aquatic pollution. For instance, willows are known to restrict macroinvertebrate diversity (Lester et al., 1994; McInerney et al., 2016), as a result of increased nutrient concentrations, and a lack of a continual supply of organic matter of appropriate quality as a food source. In time, as revegetated areas establish, and we are likely to see improved instream habitat and food resources, and improvements in macroinvertebrate diversity and richness will likely also occur, notably in the upper sites around Carlsruhe.

To achieve overall improvements in river water quality and biodiversity, and to protect the values of the Campaspe River, an understanding of all factors impacting waterway health across the catchment is required. Monitoring of several pollutants, other than nutrients and faecal contaminants in surface waters and sediments, paired with toxicological assessments, provides us with an understanding of different pressures influencing waterway health. Several additional pressures were detected across the study area, including the presence of a range of pollutants associated with wastewater inputs, urban, industrial and agricultural runoff.

Bacteroides markers, HF183 and *Lachno3,* indicated impacts from wastewater, both treated and untreated, which were evident 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 River and tributaries.

Urban, industrial and agricultural runoff can result in the contribution of various pollutants to waterways, particularly heavy metals, hydrocarbons and pesticides. The detection of heavy metals and hydrocarbons is 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 and lead have been detected at concentrations of concern for aquatic life. Additionally, hydrocarbon concentrations were elevated across several sites, particularly those directly surrounded by heavy traffic roads and rail tracks.

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

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

Data collected during the second year of monitoring has provided information from which to assess the short-term benefits of the stream frontage management program to the Campaspe River and two associated tributaries, and potential issues that remain. Benefits of the SFMP will continue to be seen over the next few years as initial impacts from willow removal dissipate and riparian vegetation becomes more established.

Immediate benefits have started to present at some sites. Nutrient concentration at Sites 3, 4 and 6 have reduced, improving the aquatic environment for macroinvertebrates. At sites further downstream, the condition of sites remains consistent with data collected during Year 1 baseline surveys. Sites 5, 7 and 8, in particular, show signs of poor ecological health due to ongoing stock access to the River, poor riparian and instream habitat condition, the presence of toxicants and impacts from treated wastewater, including elevated nutrients and faecal contamination. Both planned wastewater discharges and septic systems may be contributing to the condition of these sites.

Continued improvements and longer-term benefits of the SFMP are expected to present as the riparian vegetation becomes more established, providing a more optimal microclimate and improving habitat quality and food availability.

Future Sampling

The five-year monitoring and assessment program is due for completion in 2022. Sampling for Year 3 is expected to be completed between August to December 2020, with results available in April 2021. 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