Coliban Water Monitoring Program

Monitoring Program for Assessing the Benefits of Environmental Offsets on the Condition of the Campaspe River: Year 1 (2018-2019)

Technical Report #22
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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 or 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
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

The North Central Catchment Management Authority is running a stream frontage management program (SFMP) that aims to improve the ecological condition of the Campaspe River by improving riparian vegetation and providing stock exclusion fencing. As part of an environmental offset program, Coliban Water is providing additional funding to this program so that a larger area of the river can be improved. The Aquatic Environmental Stress Research Group (AQUEST), from RMIT University, was commissioned by Coliban Water to assess the environmental benefits of the SFMP, especially near Kyneton, on the ecological condition of the Campaspe River. This report presents the monitoring and assessment program developed by AQUEST to determine the short-term benefits (within 5 years) of this program on water quality and the aquatic ecosystems in the Campaspe River. Expected short-term benefits include reduced faecal contamination and nutrient pollution resulting from the removal of livestock, especially cattle, from the river corridor, and the creation of better riparian habitat via the removal of exotic willows and revegetation with indigenous species. Longer term benefits would also occur as the riparian vegetation becomes more established and provides shade, snags and food for aquatic animals.

Water quality, aquatic ecology, nutrient bioavailability and ecotoxicology were surveyed at eight sites along the Campaspe River, from Carlsruhe to Redesdale, as well as one site on Post Office Creek within the urban area of Kyneton, and one site on the lower reach of Snipes Creek. Sampling was constrained to winter and spring when the Campaspe River was flowing. Water quality was measured at all sites on five occasions (September, October, November and December 2018, and July 2019). The aquatic ecosystem was assessed through a macroinvertebrate survey undertaken at all sites on one occasion (October 2018).

A variety of assessments were conducted to get a general idea of water quality and whether contaminants are affecting aquatic ecosystems. Nutrient bioavailability was examined through visual assessments, water quality monitoring, the use of artificial substrates and algal passive samplers to assess algal growth rates. Six sites were also surveyed on three occasions for faecal contamination (stock and human sources). Sediments were collected and analysed for heavy metals, hydrocarbons and pesticides on one occasion, and pharmaceuticals, pesticides and personal care products were surveyed in surface waters during July 2019. Surface waters were tested using ecotoxicity tests on aquatic snails to determine whether pollutants were causing ecological impairment.

Based on the results to date, macroinvertebrate diversity was considered quite good for an ephemeral river system, with a few sites having lower diversity, corresponding to those with elevated nutrients, poor habitat availability due to excessive willow growth and/or the occurrence of other contaminants. Similarly, ecotoxicology results with snails indicated generally that surface waters were not impacting on invertebrate health, with the exception of Post Office Creek and the Campaspe River at Old Station Rd. The waters through the catchment were enriched with nutrients and this often led to excessive algal and macrophyte growth.

Snipes Creek, Post Office Creek and the Campaspe River at Old Station Rd were in poor condition compared to the rest for the study area. The major contributors to poor water quality are urban, and possibly industrial runoff from Kyneton, wastewater inputs, including agricultural runoff, treated discharges from the Kyneton Water Reclamation Plant, and possibly septic tank systems.

We predict that there will be substantial improvements in the physical and ecological condition of the Campaspe River with the expanded SFMP and there are also opportunities for improvement by reducing major pollution sources.

Introduction

Extending from the Great Dividing Range in the south, to Murray River in the north, and covering an area of approximately 4,000 square kilometres, the Campaspe River (the River) is part of the southern Murray-Darling Basin (MDBA, 2012). The River is approximately 220 km in length and flows north from near Woodend to converge with the Murray River at Echuca (Figure 1). The main tributaries of the River are the Coliban River, and several creeks, including the McIvor, Pipers, Mount Pleasant, Forest and Axe Creek (Figure 1).

Prior to the 1850s (Victorian gold rush) the River would have been a low energy environment, experiencing seasonally variable flows, comprising of high flow in winter and spring, and low to no flow in summer and autumn. Since then, agricultural and urban development, native vegetation clearing, and river regulation has altered the flow regime, with the construction of reservoirs and weirs (for potable water supply and irrigation) significantly reducing flows (NCCMA, 2014). Further, the construction and operation of Lake Eppalock has altered the flow patterns of the River (VEWH, 2019).

Nowadays, the River supports many economic, social and ecological values in the region, including water supply, drought refuge, primary production, lifestyle/recreational activities, and is home to numerous flora and fauna species. Specifically, the River supports iconic River Red Gum communities, threatened fauna species (e.g. swift parrot and squirrel glider) and native fish populations (e.g. Murray Cod and Golden Perch), as a result of its direct connection to the Murray River (NCCMA, 2014).

An audit of the health of the River, undertaken as part of a wider assessment of ecological health in the Murray-Darling Basin (Sustainable Rivers Audit 2012), reported fish as very poor, macroinvertebrates, physical form and hydrology as moderate, and riverine vegetation as extremely poor (MDBA, 2012). These ratings resulted in the River system being rated overall as very poor (MDBA, 2012). With the highest water demand on the River coming from agriculture, and a proportion of raw water supplied to the Goornong township (Coliban Water, 2019), the continued health of the River is vital.

Stream frontage management projects are an option for improving the condition of rivers and creeks. These projects aim to increase bed/bank stability, reduce nutrient and sediment inputs, and improve aquatic/terrestrial habitat and water quality through a variety of initiatives. Since 2012, the North Central Catchment Management Authority has been running a stream frontage management project (SFMP) “Caring for the Campaspe” along the Campaspe River system to enhance the waterway health and biodiversity of the river. As part of an environmental offset program, Coliban Water is providing additional funding to continue this program, with a further fourteen kilometres of land along the Campaspe River and Post Office Creek seeking to benefit from environmental improvement works.

Twenty-one hectares of river frontage will be revegetated, using native trees and shrubs, while thirteen kilometres of fencing will be installed to keep livestock out of the waterway. In the short term, the SFMP 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, snags and food for aquatic animals.

To assess the environmental benefits of the SFMP, especially near Kyneton, on the ecological condition of the Campaspe River, the Aquatic Environmental Stress Research Group (AQUEST) from RMIT University was commissioned by Coliban Water to undertake a 5-year monitoring program. The program will utilise assessments of water quality, together with aquatic ecology surveys and toxicology techniques to investigate short term improvements to water quality and biodiversity in the Campaspe River, from Carlsruhe to Redesdale, along with two associated tributaries. This report presents results for the first year of the monitoring and assessment program.

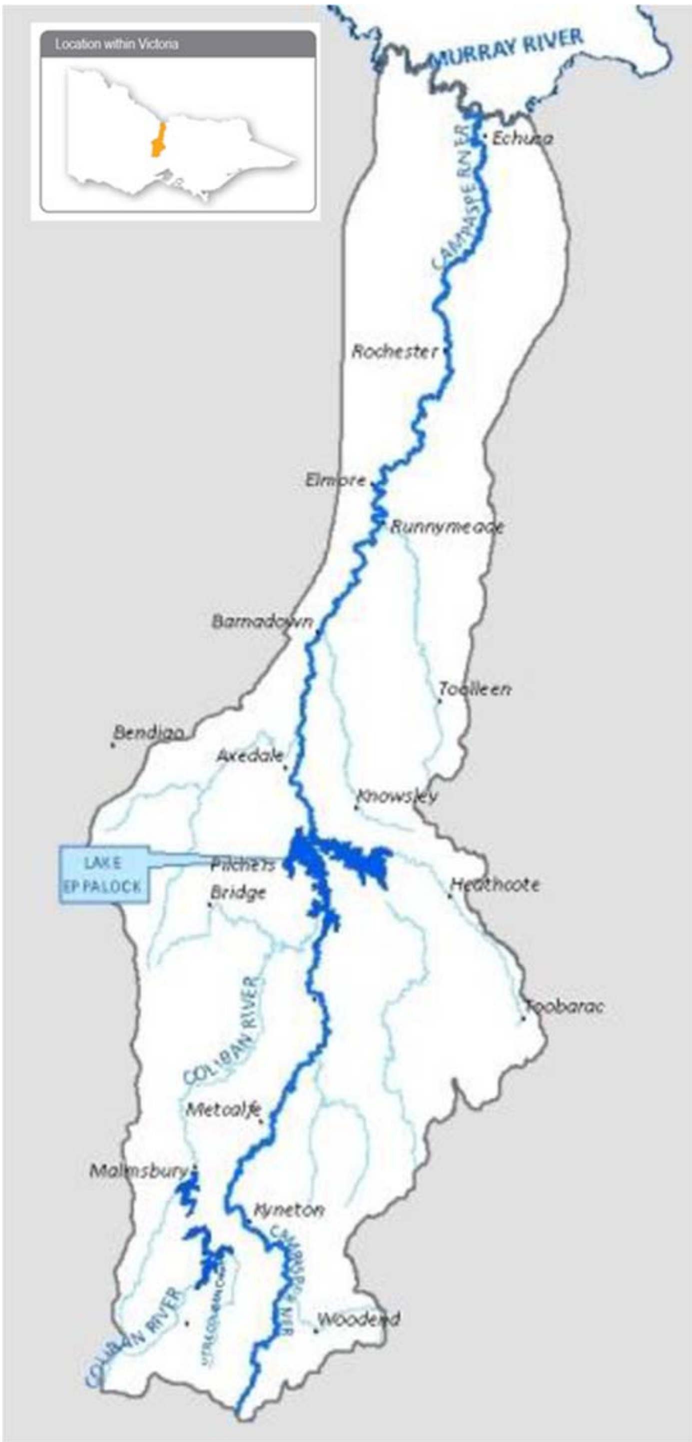


Figure 1: Campaspe River System (NCCMA, 2015)

Study Objectives

The objectives of the five-year monitoring and assessment program are to investigate the success of stream frontage management projects on improving the health of the Campaspe River. Over the five-year period, the program will assess whether environmental offsets (riparian revegetation and fencing) will lead to:

- reduced 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),
- reduced faecal contamination from cattle during base flows (as indicated by *E. coli* and a specific biomarker of cattle faeces),
- improvements in the ecological health of the river, as indicated by aquatic macroinvertebrates and in situ toxicology assessments.

Methods

Study Area

The 5-year monitoring and assessment program is focused on the Upper Campaspe River from Carlsruhe to Redesdale. Ten sites (Table 1 and Figure 2), including eight along the Campaspe River and one in each of Post Office Creek and Snipes Creek, were selected to assess the benefits of the SFMW.

Samples will be collected annually from all ten sites at five time points to allow for assessment of seasonal trends. During the first-year of monitoring surveys were conducted in September, October, November and December 2018, and during July 2019.

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.
7*	37°12’31.9” S 144°25’25.7” E	Campaspe River	Old Station Rd.
8	37°10’57.4” S 144°27’16.7” E	Snipes Creek	Barbower Rd.
9	37°10’07.2” S 144°27’49.6” E	Campaspe River	Boundary Rd.
10	37°00’57.4” S 144°32’28.2” E	Campaspe River	Redesdale

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

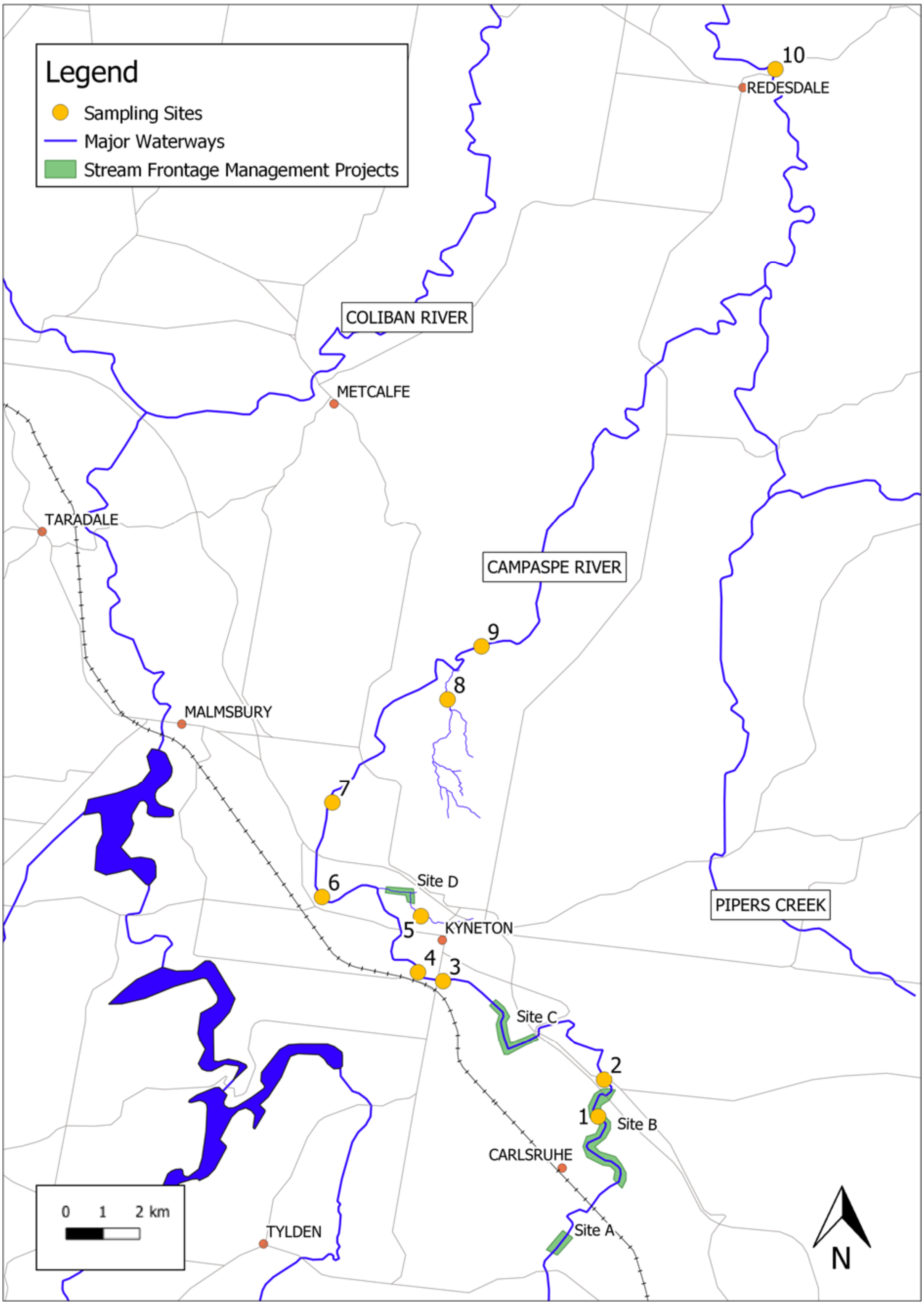


Figure 2: Locations of monitoring sites within the Campaspe River system and SFMW.

Summary Descriptions of Survey Sites

Sites 1 and 2 were in the upper reach of the study area around the rural township of Carlsruhe (Figure 2). Stream frontage management works (SFMW) site A was upstream of both study sites 1 and 2. while SFMW site B was upstream of study site 2 and surrounded study site 1. However, in this first year of sampling SFMW sites A and B had not been undertaken. Land-use surrounding the sites is predominantly farming and rural residential. The riparian zone at Site 1 ranges between 1-3m wide and consists mostly of grasses and tussocks and several willow trees. At Site 2, the riparian zone ranges between 4 and 20 m width and consists of grasses, tussocks, Typha and native trees (Plate 1 a, b, c and d). Both sites support a diversity of instream macrophytes including *Myriophyllium* sp., *Valliseria* sp. *Nitella* sp., *Alisma* sp., *Triglochin* sp. and *Phragmites* sp.

Sites 3 and 4 are in the township of Kyneton, downstream of SFMW site C (Figure 2). There have also been SFMW undertaken around these sites as part of the NCCMA's "Caring for Campaspe Project.". Land-use surrounding Site 3 is predominantly urban, while Site 4 is a mix of urban parkland and farming, with stock having access to the river on the southern side. The riparian zones at range between 2-6m and 2-7m at Sites 3 and 4, respectively. Riparian vegetation surrounding the sites is predominately grass and tussocks along with native tree plantings. Site 4 also has a mix of larger trees (Plate 1 e, f, g and h). A variety of macrophytes colonise the river at these sites including *Triglochin* sp., *Myriophyllium* sp., *Potamogeton* Sp., *Vallisaria* Sp., *Elodea* sp. or *Egeria* sp., and several sedges.

Site 5 is situated in Post Office Creek, which is a small urban creek in the Kyneton township (Figure 2). The site is situated upstream of SFMW site D. Land-use surrounding the site is predominately urban residential and industrial. The riparian zone ranges in width from 1-4m and consists mostly of grasses and tussocks, with some small shrubs and larger trees (Plate 2 a and b). Several macrophytes occur within the shallows of the creek's banks including *Alisma* sp., *Lemna* sp., *Rumex* sp., sedges and rushes.

Sites 6 and 7 are in the middle reaches of the study area, being downstream of the Kyneton township and SFMW including willow removal and revegetation works that were conducted as part of the NCCMA's "Caring for Campaspe Project." (Figure 2). The Kyneton WRP discharge is situated approximately 2km downstream of Site 6 ad 800m upstream of Site 7. Land-use is predominantly rural residential and farming. The riparian zone for Site 6 is approximately 3-7m wide and consists mostly of grasses, tussocks and native tree plantings, while at Site 7 is between 1-3m wide. At Site 7 willows dominate the riparian and instream area, with some small grasses and tussocks also present (Plate 1i, j, k and l). Stock have access to the northern side of the river at Site 7. Several macrophyte species occur along the shallows of the banks and within the river channel at Site 6, including *Callitriche* Sp., *Lemna* sp. and *Myriophyllium* Sp. While at Site 7, *Azolla* sp. and *Lemna* sp. dominate over the water surface, with small amounts of *Callitriche* Sp. around the willow tree roots.

Site 8 is situated in Snipes Creek, a small creek which enters the Campaspe downstream of Site 7 (Figure 2). Land-use surrounding the site is farming. The riparian zone is between 1-2m in width and consists of grass and tussocks with several willows. Stock have unrestricted access to the creek at this site (Plate 2 c and d). Small amounts of *Persicaria* Sp., *Vallisaria* Sp., and *Rumex* sp.occur in the creek.

Site 9 is downstream of the confluence of Snipes Creek with the Campaspe River (Figure 2). The surrounding land-use is farming. The riparian zone is approximately 20m in width and consists of native trees, shrubs, grasses and tussocks. Stock have access to the river on at least one bank (Plate 1 m and n). *The river has various macrophytes within the channel including Persicaria* Sp., *Vallisineria* *sp.*, several sedges, *Azolla sp.*, *Lemna* sp., *Crassula* Sp., *Alisma* Sp., *Callitriche* Sp., *Triglochin* sp., and *Typha* sp.

Site 10 is the most downstream site in the study area, located just downstream of the rural township of Redesdale (Figure 2) and is a routine monitoring site for DEWLP. Land-use surrounding the site is predominately farming and rural township. The riparian zone is approximately 30m in width and consists mostly of native trees, shrub, grasses and sedges (Plate 1 o and p). Small patches of macrophytes including *Azolla* sp., *Lemna* sp., sedges and *Typha* sp. occur in the river channel.

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.

Water Quality

Physico-Chemistry and Nutrients

Water temperature, dissolved oxygen (% saturation), pH, electrical conductivity and turbidity were assessed at each site during each survey. Water samples were collected from approximately 0.2 m below the water surface in the centre of the waterbody and sent to ALS Global for nutrient analyses. Nitrogen was tested for via ammonia as N (NH_4-N), total nitrogen (TN), total Kjeldahl Nitrogen (TKN), nitrate and nitrite (NOx). Phosphorus was tested for via orthophosphate (OP) and total phosphorus (TP). Water quality measurements were compared to Victorian guideline values for the protection of aquatic ecosystems (State Environment Protection Policy (Waters) and Australian and New Zealand Water Quality Guidelines 2000).

Faecal Monitoring

Faecal monitoring was undertaken at six sites (Sites 2, 4, 5, 6, 7, and 8) on three occasions (September 2018, December 2018 and July 2019), with grab water samples collected for the analysis of *E. coli* (key indicator of faecal contamination). This indicator just indicates whether faecal pollution is present, but it does not provide any information about whether the origin of this pollution is from humans, cattle or other warm-blooded animals. Therefore, additional samples were collected on two occasions (December 2018 and July 2019) for assessment of faecal sourcing (e.g. human, bovine) using a *Bacteroides* assay. Results were then compared to guideline values (State Environment Protection Policy (Waters) and EPA Use of Reclaimed Water).

Aquatic Ecology

Macroinvertebrate Survey

Aquatic macroinvertebrates are widely used in Australia, and globally, to determine the overall health of aquatic ecosystems. They are affected by the quality of the instream and riparian habitat that is present, stream flows and by water and sediment pollution. All 10 sites were surveyed in

October 2018 using the Rapid Bioassessment (RBA) method. Collection and identification took place according to EPA Victoria guidelines (EPA Victoria, 2003a). Two categories of biological indices were used to assess the condition of the aquatic ecosystem, a measure of diversity (number of families) and biotic indices (the SIGNAL and EPT indices). The measure of diversity is calculated by summing the total number of 'families' of macroinvertebrates present at each site. The SIGNAL2 biotic index is the Stream Invertebrate Grade Number – Average Level (SIGNAL), an index of water pollution based on pollution tolerance sources as per Chessman (2003). The EPT biotic index is the total number of families in the insect orders Ephemeroptera (mayflies), Plecoptera (stoneflies) and Trichoptera (caddisflies). These taxa are known to be particularly sensitive to aquatic pollution. Biological indices were then compared to guideline values (State Environment Protection Policy (Waters)).

Nutrient Bioavailability

Visual Assessment

Visual assessments of macrophyte and algal growth were undertaken at each of the 10 sites at all time points to assess the availability of nutrients. Visual assessments of percentage cover of aquatic macrophytes and filamentous algae were recorded, with the length of filamentous algae also noted (short <2cm, medium 2-10cm, long >10cm). Trends in observations were compared between and within sites and seasons.

Artificial Substrates

Artificial substrates, thin disks suspended within the water column (Plate 3), were deployed at each of the 10 sites on two occasions (November 2018 and July 2019), for assessment of algal biofilm production. Artificial substrates were deployed for a 4-week period, allowing adequate time for biofilm colonisation. Following deployment, artificial substrates were analysed for biofilm biomass (biomass of algae, cyanobacteria, heterotrophic microbes and detritus), photosynthetic health (photosynthetic efficiency) and community composition.

Plate 3: Artificial substrate Samplers (A) and (B) during deployment

In-situ Algal Growth

In-situ algal growth measurements were undertaken to further examine nutrient bioavailability and the toxicity of surface waters to floral species. Algal balls, containing algae immobilised in alginate beads, were deployed in specially made exposure or, control, cages at each site (Plate 4 and 5). "Exposure" cages consist of tubes with mesh sides which allow the algal balls to be exposed to site waters, while "control" cages are fully sealed, and algal balls were exposed to an optimal growth medium. Algal cages were deployed on two occasions (November 2018 and July 2019), each time for a 10-day period. Following deployment, algal health was assessed by examining growth (biomass) over the deployment period, relative to the site control to eliminate, to the extent possible, differences in abiotic site conditions.

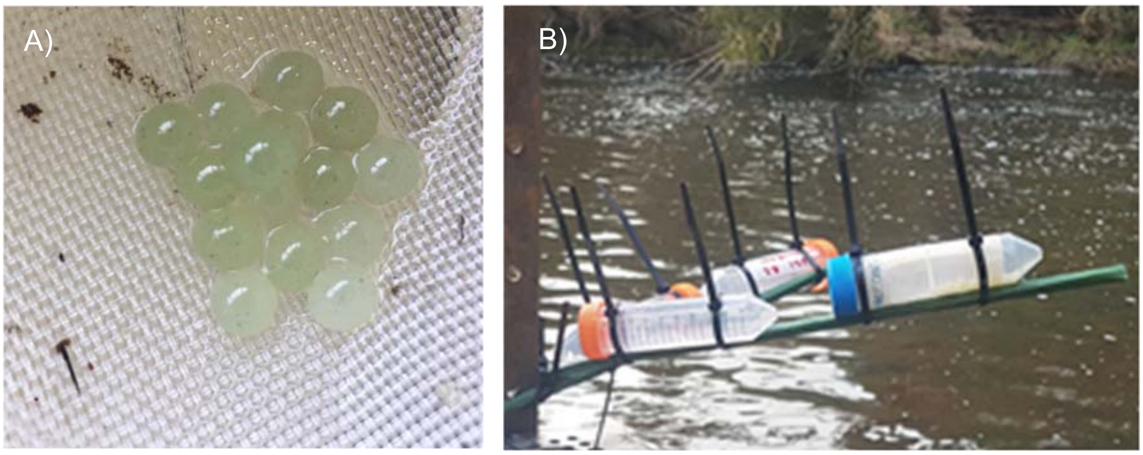


Plate 4: In situ Algal Balls to assess algal growth (A) and in cages ready for deployment (B).

Ecotoxicology

Water Toxicity

The effects of surface water toxicity on aquatic fauna were examined through the survival and reproductive ability of the mud snail, *Potamopyrgus antipodarum (Plate 5)*. Mud snails, a commonly used indicator organism, were deployed in cages for a 4-week period at each of the 10 sites during November 2018. Following deployment, survival was recorded, with reproductive success (to determine effects of endocrine disrupting chemicals) determined by the number of embryos present.

Sediment Chemistry

Sediment samples (<63µm fraction) were collected at each of the 10 sites on one occasion (July 2019) for analysis for heavy metals, petroleum hydrocarbons and multi-residue pesticides (see Appendix 1 for full list). 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

Testing for the presence of other pollutants, including personal care products (PPCP), pharmaceuticals, herbicides, insecticides and pesticides occurred in surface waters (see Appendix 2 for full list), involving the deployment of Polar Organic Chemical Integrated Samplers (POCIS) (Plate 5 and 6). POCIS passive samplers were deployed at each of the 10 sites on one occasion (July 2019) for a 4-week period.

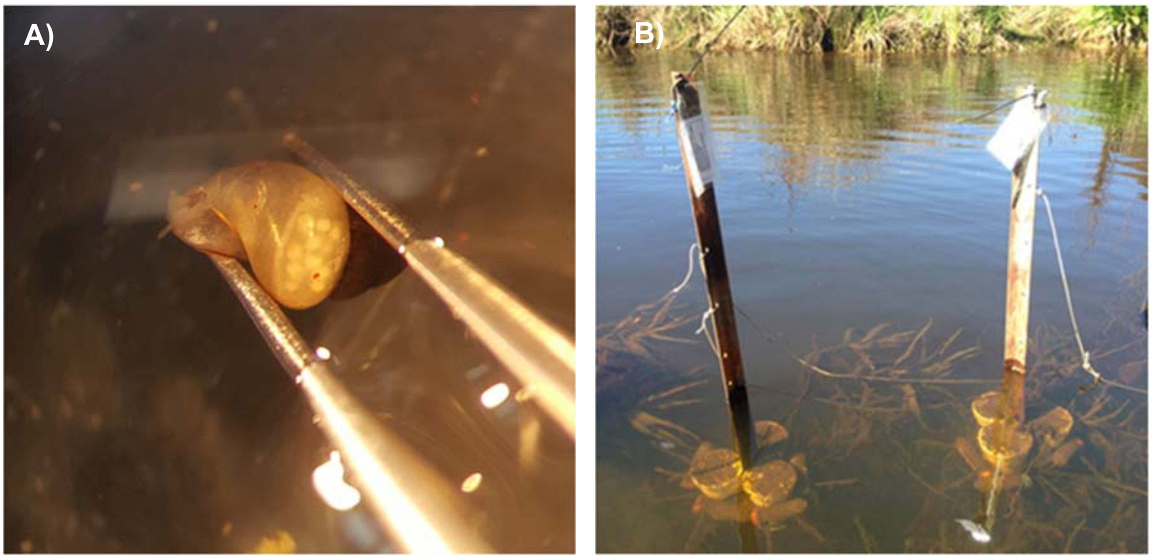


Plate 5: Embryos present in mud snail (*P. antipodarum*) (A) and Mud snails, algal balls and POCIS passive samplers during deployment (B).

Plate 6: POCIS passive samplers following retrieval

Results

Rainfall

Highest rainfall (125 mm) occurred during June 2019 (Figure 3). Above average rains fell during December 2018 (63mm in total) and the lowest rainfall (2.8 mm) occurred in April 2019 (Figure 3). Sampling effort reflected rainfall conditions, with access being difficult during December 2018 and July 2019, as sites were too deep to use waders and/or too turbid to conduct visual assessments for macrophytes and filamentous algae at Sites 1, 3, 5, 6, 8 ,9 and 10.

Summary descriptions of the 10 study sites and associated water quality data averaged across the sampling periods are detailed below.

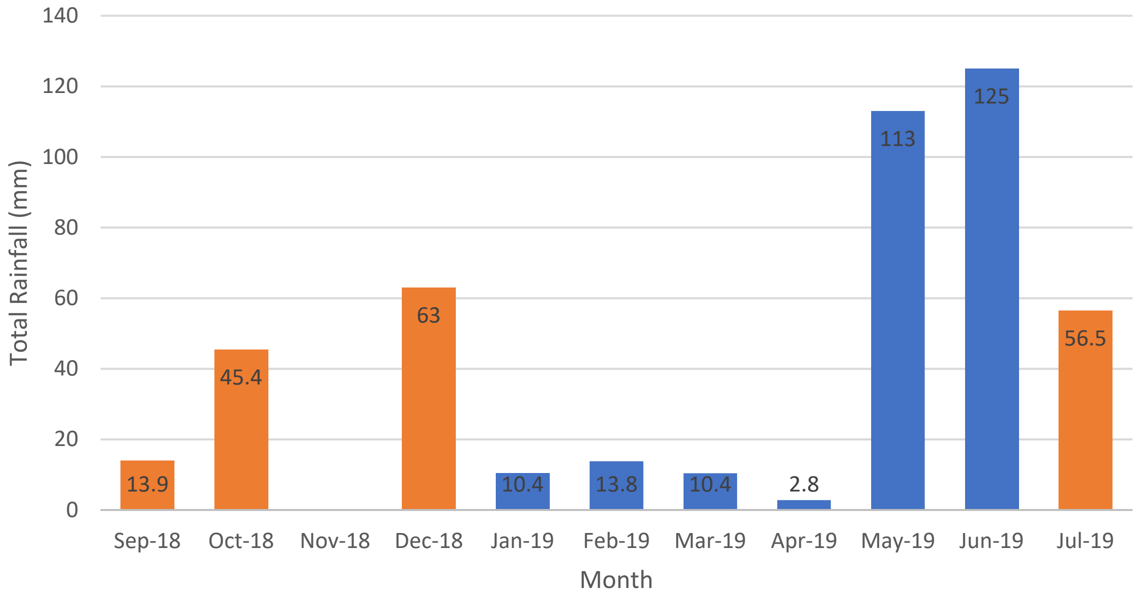


Figure 3: Rainfall at Kyneton over the study period. Orange bars indicate months sampling occurred. Number in bar is the total rainfall for the month (mm) (source: BOM 2019).

Catchment Water Quality

Physico- Chemistry

Surface water temperatures varied by season; however, were generally similar amongst sites, with the highest temperature (21.4°C) recorded at Site 6 in November 2018 and the lowest (7.3°C) at Site 4 in July 2019 (Table 2).

Dissolved oxygen levels were highest at Site 10 in October 2018 (107.1%), with the lowest level recorded at Site 1 during September 2018 (31%). Values at all sites were within the SEPP Waters trigger value (Table 2).

Comparisons between sites for pH levels showed generally consistent levels, with differences across sites of 0.61 units, with means ranging between 7.56 and 8.17 units. Maximum values at all sites exceeded the SEPP Waters trigger value (8.0 units) across all time points, with average levels at Site 9 also exceeding values throughout Year 1 (Table 2).

Electrical conductivity was consistent across sites, except for Site 8, where mean levels were twice as high. However, all sites were well below SEPP Waters trigger values (≤2000 µs/cm) (Table 2).

Turbidity was site dependent, with the highest differences between sampling time points observed at Site 6 (3.39 to 26.2 NTU) and Site 7 (3.26 and 24 NTU). Maximum levels at Sites 6, 7 and 8 exceeded the SEPP Waters trigger value (15 NTU), with the average level over the five time points at Site 8 slightly exceeding the trigger value (15.78 NTU) (Table 2).

Nutrients

Mean nitrogen concentrations at each site are presented in Figure 4 (note: values less than the limit of detection were halved for graphical representation). Total nitrogen (addition of NOx and Organic N) differed across sites, with concentrations ranging from 0.2 to 5.0 mg/L, with the highest concentration recorded at Site 8 in December 2018. Ammonia concentrations also differed between sites (ranging from 0.01 to 4.2 mg/L), with the highest level also recorded at Site 8 in December 2018. Total nitrogen values at all sites, except Site 2, exceeded the SEPP Waters environmental quality indicator (≤1.05 mg/L) on at least one occasion, with most exceedances observed in December 2018[1]. Ammonia values at Site 8 exceeded the ANZECC/ARMCANZ (2000) trigger value (0.9 mg/L at pH 8.0) on two occasions (October 2018 and December 2018).

Mean total phosphorus and orthophosphate concentrations at each site are presented in Figure 5. Total phosphorus concentrations ranged from 0.02 to 1.31 mg/L, with the highest concentration recorded at Site 2 in October 2018. Total phosphorus values at all sites exceeded the SEPP Waters environmental quality indicator (≤0.055 mg/L) on at least one occasion[1].

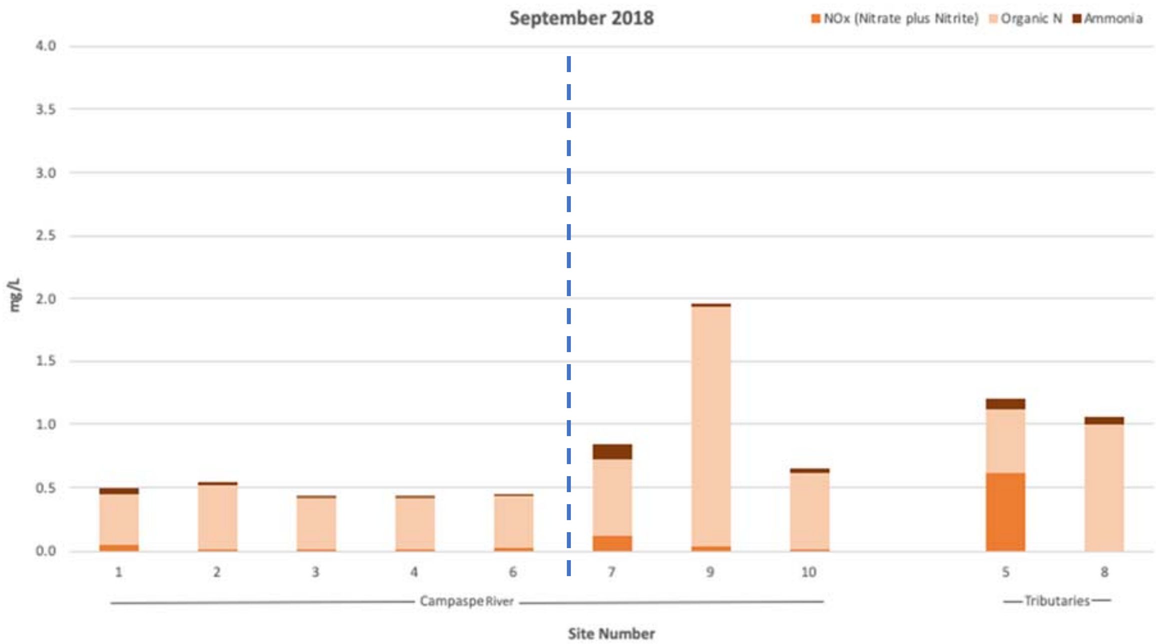
[1] SEPP Waters objectives are a guideline only, as required sample numbers for comparison not met.

Table 2: Mean, minimum and maximum temperatures (°C), dissolved Oxygen (% saturation), pH, electrical conductivity (µs/cm) and turbidity (NTU) measured across sites during year 1 monitoring.

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	13.9	8.3	17.5	60.1	31	80.9	7.65	7.19	**8.1**	400	341	494	7.79	3.28	11.1
2	13.5	7.7	17.8	55.5	34	75	7.59	6.79	**8.29**	383.8	328	464	6.25	2.56	9
3	14.2	8.3	17.7	85.3	62.4	101.4	7.73	6.62	**8.85**	459.6	346	670	4.98	2.61	8.94
4	13.2	7.3	16.2	87	85	89	7.76	6.97	**8.81**	433.6	343	670	4.68	2.57	8.1
6	15.5	8	21.4	71.8	50	89.7	7.56	6.68	**8.51**	412.4	321	510	9.93	3.39	**26.2**
Kyneton WRP discharge between sites 6 and 7															
7	13.9	8.6	17.4	66.1	46.5	85	8.03	7.64	**8.68**	430.8	317	536	10.13	3.26	**24**
9	14.1	8	19.2	79.8	52	102.5	**8.17**	7.28	**9.65**	491.6	321	645	6.35	3.57	10.8
10	15.8	8.8	19.4	82.3	46.7	107.1	7.73	6.97	**9.01**	591	428	802	7.65	4.4	13.9
Tributaries															
5	13.6	8.6	19	67	56	76.8	7.7	7.44	**8.54**	556.8	386	834	10.09	7.76	14.2
8	14.9	9.5	20.1	66.3	50.1	83.9	7.67	6.95	**8.46**	1173	722	1447	**15.78**	12.1	**22.7**
Trigger Values*	**-**			**max 130***			**6.8-8.0***			≤2000*			≤15*		

*Trigger value from State Environment Protection Policy (Waters) (SEPP Waters)
Values in **bold** if exceedance of trigger value

(a)



(b)

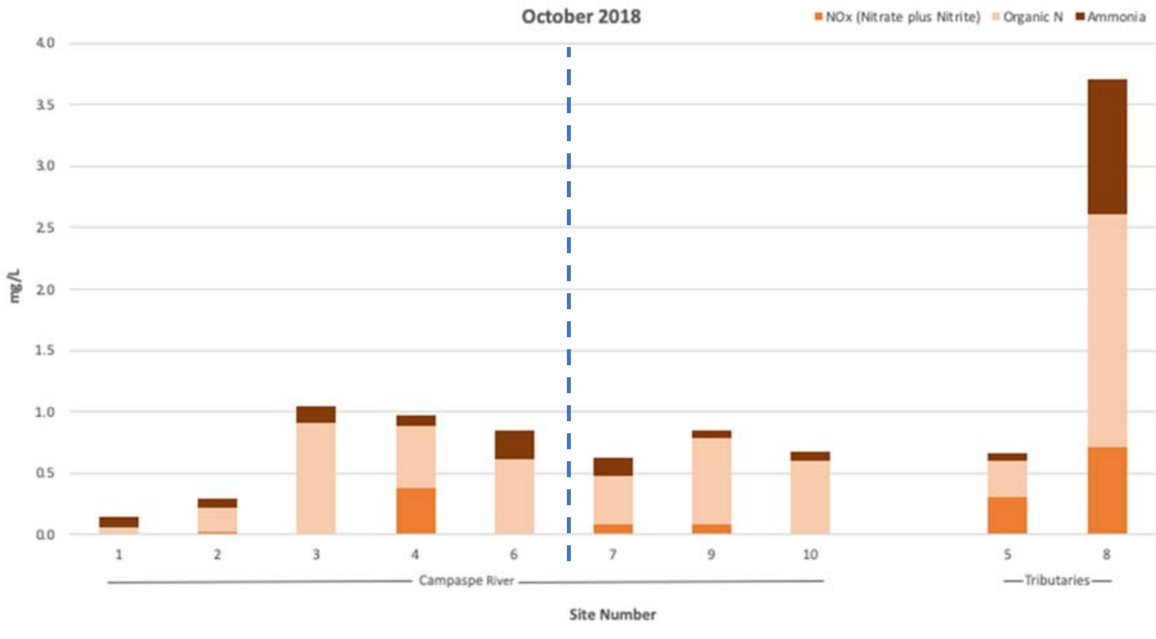
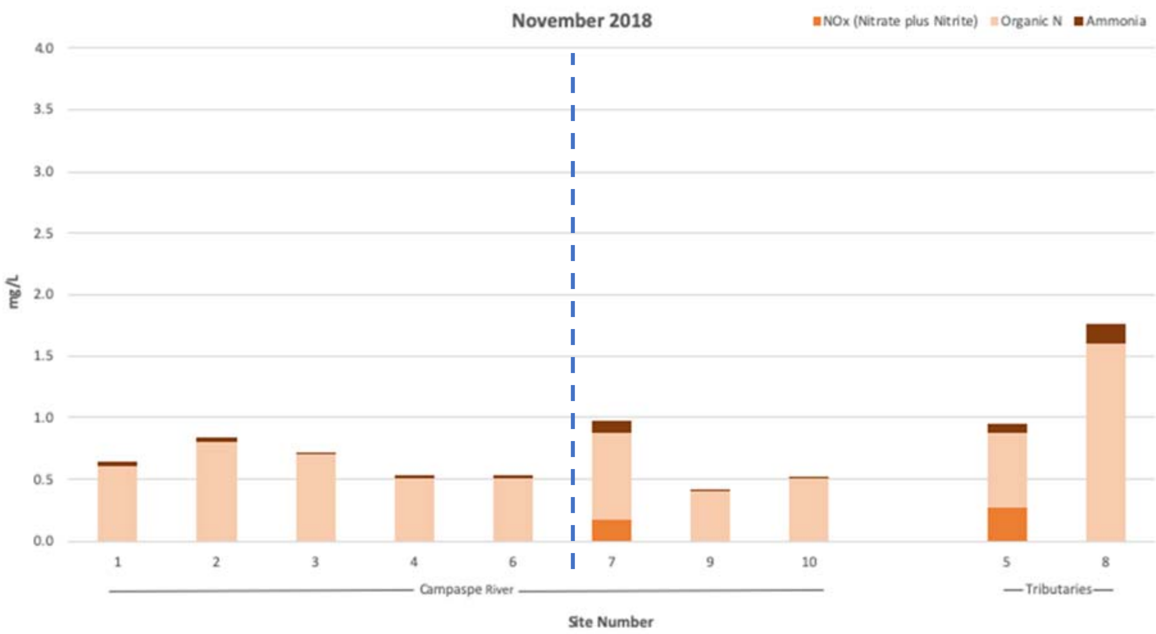


Figure 4: Mean nitrogen concentrations in surface waters during (a) September 2018, (b) October 2018, (c) November 2018, (d) December 2018 and (e) July 2019. Blue dashed line indicates Kyneton WRP discharge point between Sites 6 and 7.

(c)



(d)

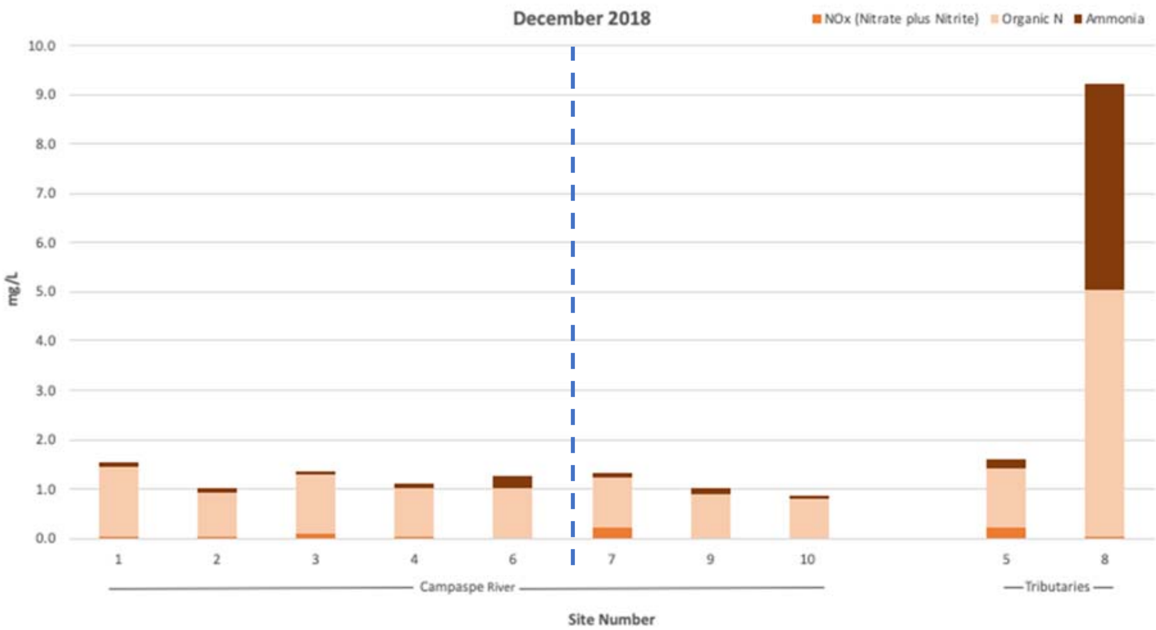


Figure 4: Mean nitrogen concentrations in surface waters during (a) September 2018, (b) October 2018, (c) November 2018, (d) December 2018 and (e) July 2019. Blue dashed line indicates Kyneton WRP discharge point between Sites 6 and 7.

(e)

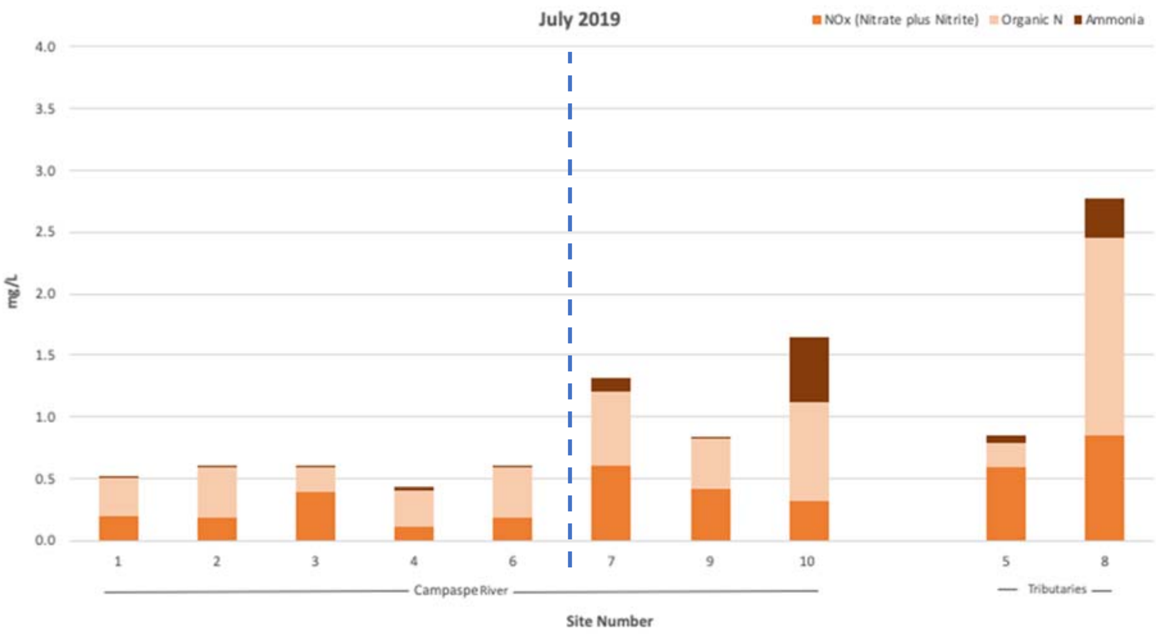


Figure 4: Mean nitrogen concentrations in surface waters during (a) September 2018, (b) October 2018, (c) November 2018, (d) December 2018 and (e) July 2019. Blue dashed line indicates Kyneton WRP discharge point between Sites 6 and 7.

(a)

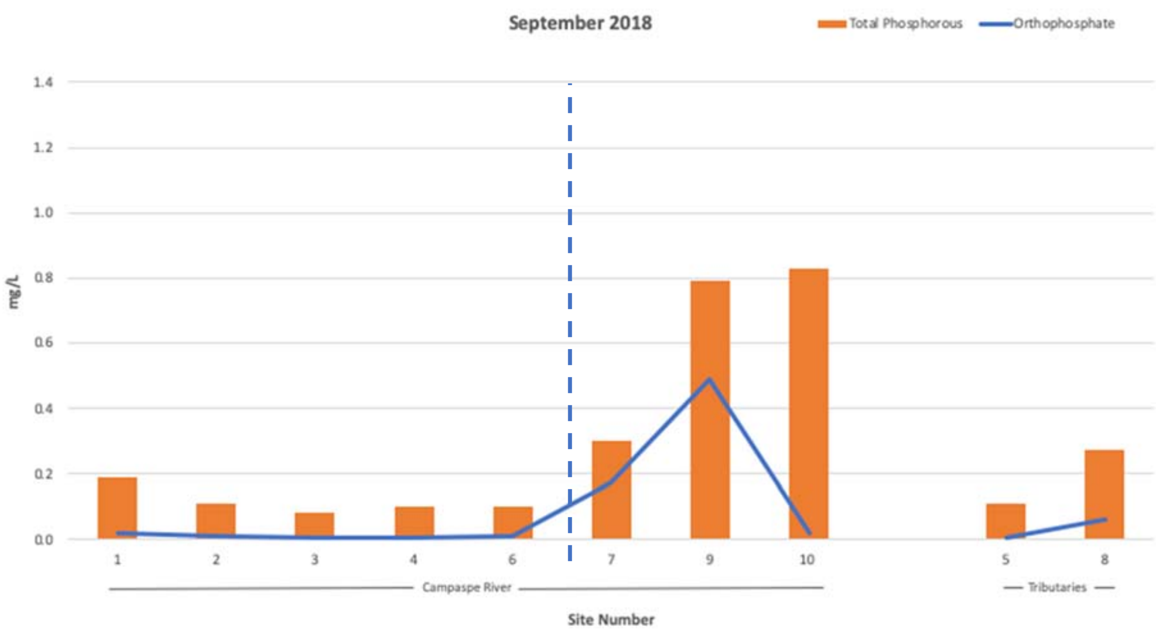
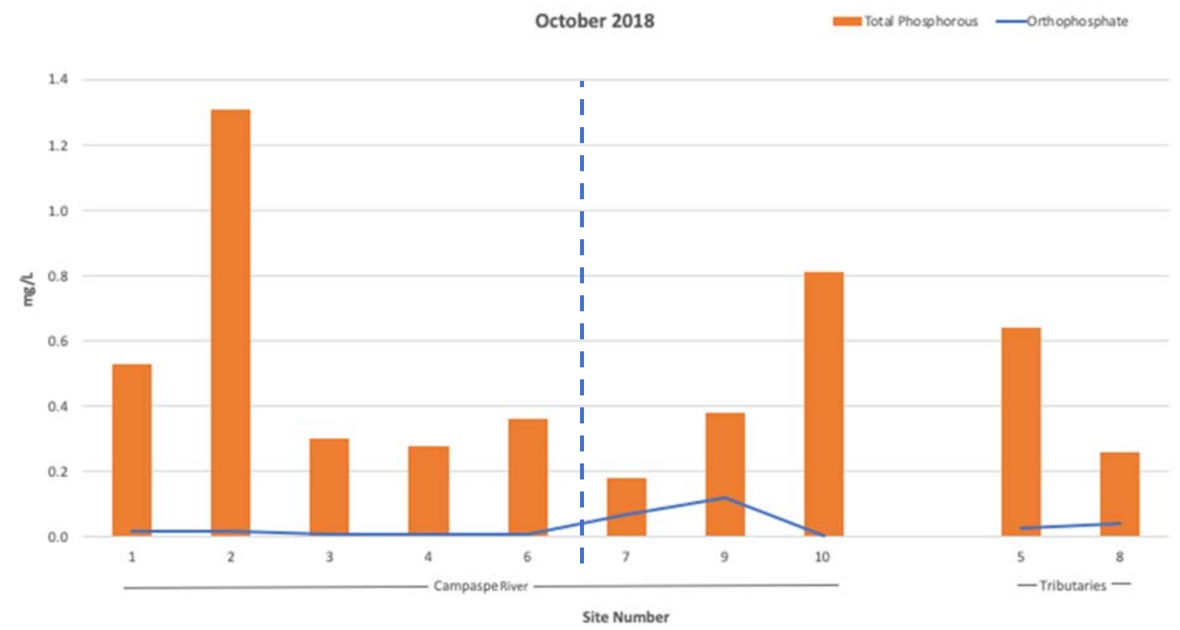


Figure 5: Mean phosphorous concentrations in surface waters during (a) September 2018, (b) October 2018, (c) November 2018, (d) December 2018 and (e) July 2019. Blue dashed line indicates Kyneton WRP discharge point between Sites 6 and 7.

(b)



(c)

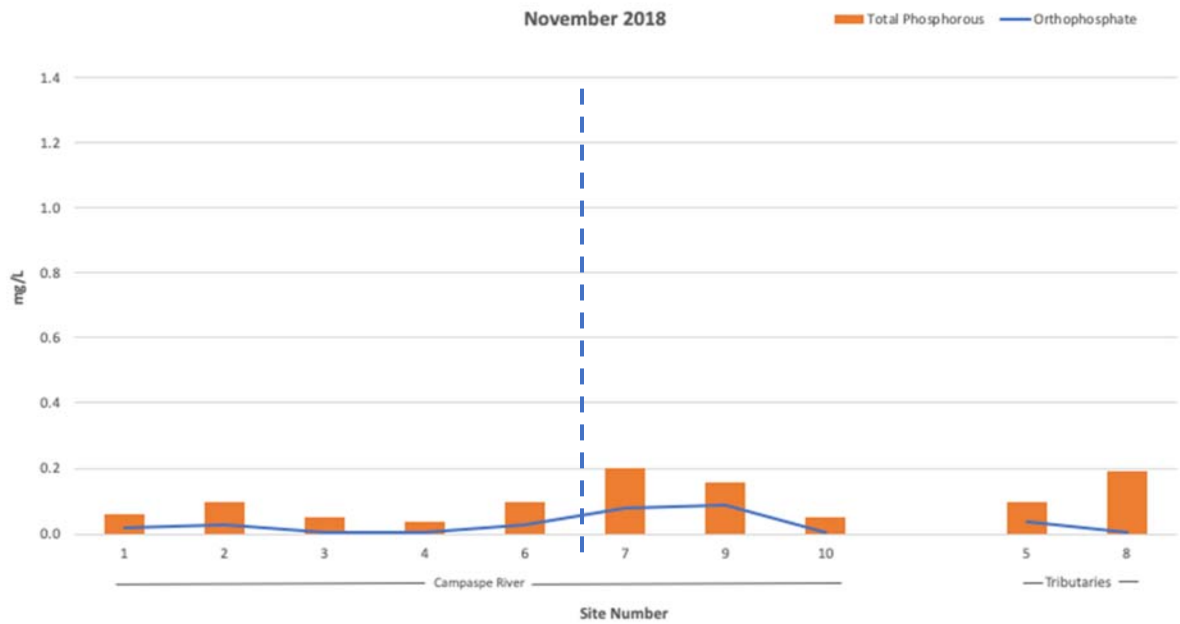
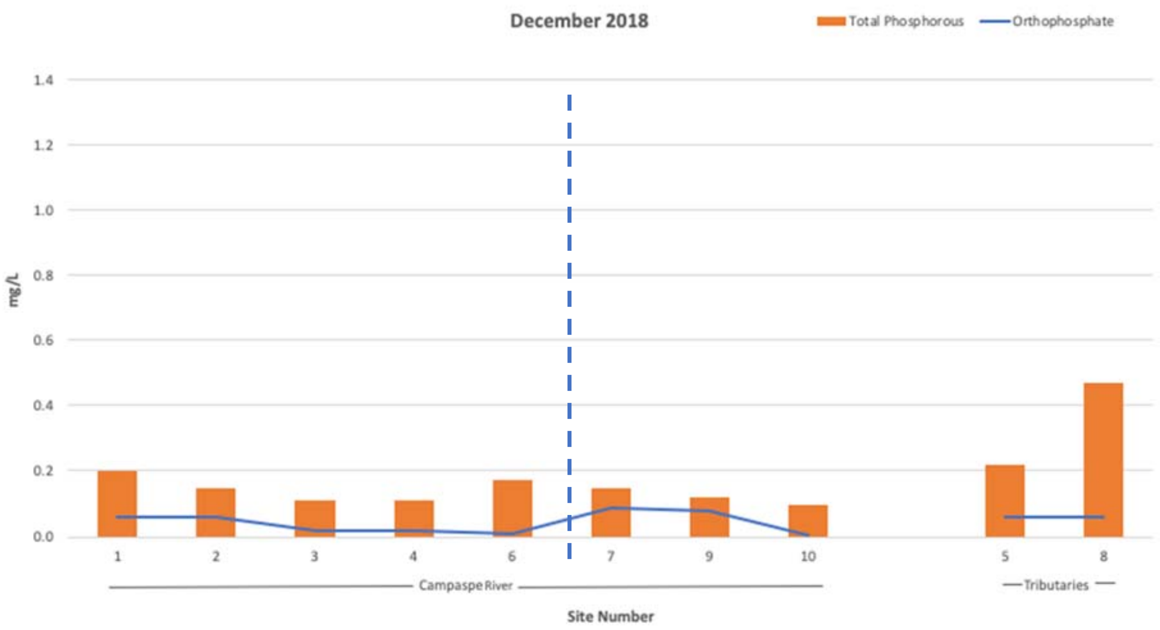


Figure 5: Mean phosphorous concentrations in surface waters during (a) September 2018, (b) October 2018, (c) November 2018, (d) December 2018 and (e) July 2019. Blue dashed line indicates Kyneton WRP discharge point between Sites 6 and 7.

(d)



(e)

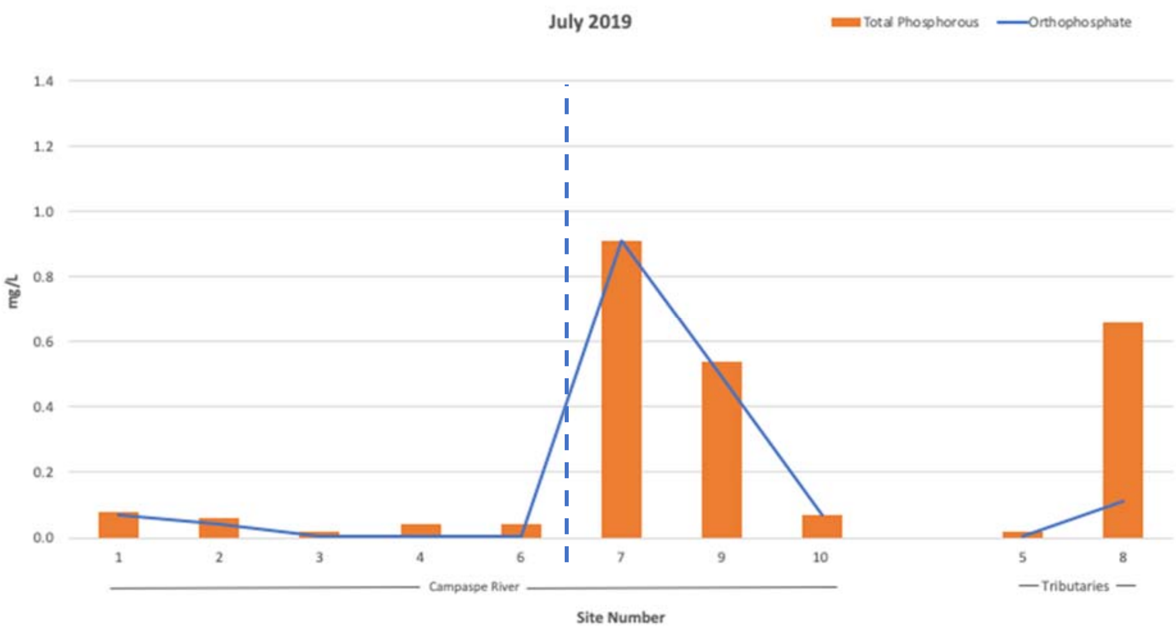


Figure 5: Mean phosphorous concentrations in surface waters during (a) September 2018, (b) October 2018, (c) November 2018, (d) December 2018 and (e) July 2019. Blue dashed line indicates Kyneton WRP discharge point between Sites 6 and 7.

The ratio of orthophosphate to total phosphorus (Table 3) provides an indication of the bioavailability of phosphorus, and showed relatively low proportions across Sites 1, 2, 3, 4 and 6 (except for Site 1 in July 2019). Higher proportions were observed at Sites 7, 9 and 10 in July 2019, indicating a rich source of phosphorous at these sites during this time point.

Table 3: Ratio of orthophosphate to total phosphorus in surface waters at each site during all time points

Site #	September 2018	October 2018	November 2018	December 2018	July 2019
Campaspe River					
1	0.11	0.04	0.33	0.30	0.88
2	0.09	0.02	0.30	0.40	0.67
3	0.06	0.03	0.10	0.18	0.25
4	0.05	0.04	0.13	0.18	0.13
6	0.10	0.03	0.30	0.06	0.13
Kyneton WRP discharge between sites 6 and 7					
7	0.57	0.39	0.40	0.60	1.00
9	0.62	0.32	0.56	0.67	0.91
10	0.02	0.01	0.10	0.05	1.00
Tributaries					
5	0.05	0.05	0.40	0.27	0.25
8	0.22	0.15	0.03	0.13	0.17

Faecal Monitoring

E. coli levels varied spatially and temporally (Figure 6), however were mostly below the SEPP Waters trigger values indicating suitability for primary contact and secondary recreation[2]. In addition, most levels were <100 organisms per 100 mL, indicating that the river water was of an equivalent quality as Class B recycled water, which is suitable for livestock drinking and application to pasture (with conditions) (EPA Victoria, 2003b). On three occasions (once in September 2018, at Site 8, and twice in December 2018, at Sites 5 and 7) levels exceeded 100 organisms per 100mL meaning the river water was of an equivalent quality as Class C recycled water (not suitable for livestock drinking). An exception to the above occurred at Site 8 in July 2019 where levels exceeded guideline values, requiring further investigation. Results of the Bacteroides assay undertaken in Year 1 will be available in Year 2.

#

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

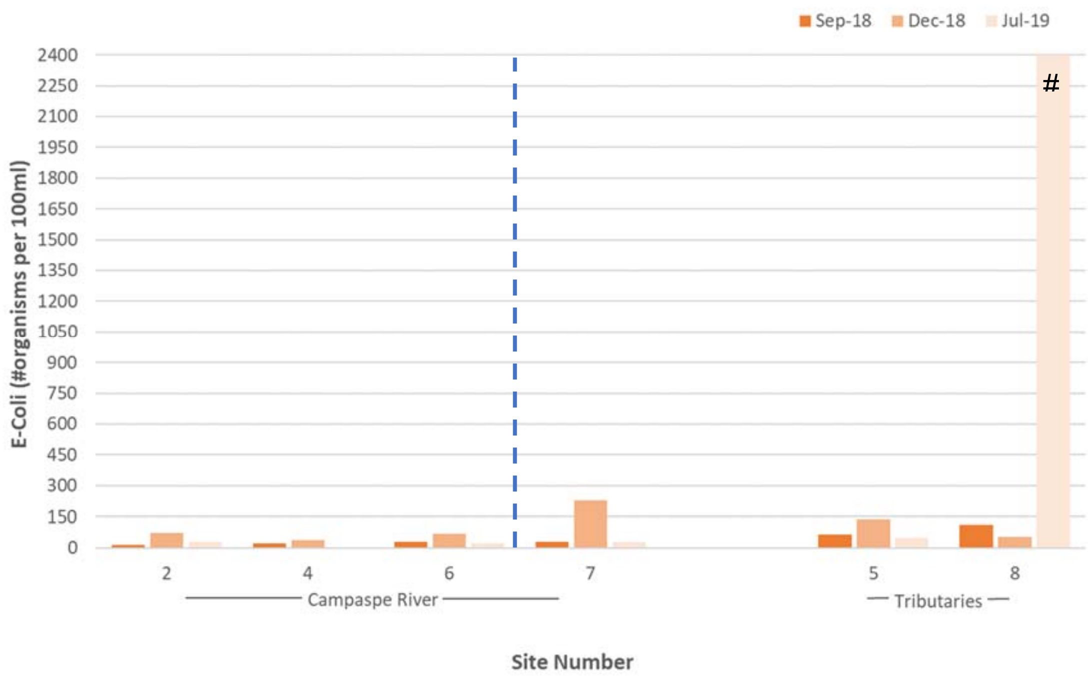


Figure 6: *E. coli* across six sites during September 2018, December 2018 and July 2019 (note: # value exceeds 2400 organisms per 100ml). Blue dashed line indicates Kyneton WRP discharge point between Sites 6 and 7.

Aquatic Ecology

Macroinvertebrate Survey

Macroinvertebrate diversity and biotic indices differed across sites (Table 4), with most sites below SEPP Waters objectives (Sites 5, 6, 7, 8, 9 and 10). Samples were collected predominantly from edge habitats (areas along the creek bank with little to no flow) due to the absence of riffle habitats (areas with broken water with rapid current).

The lowest diversity occurred at Site 8 (10 families), with the highest observed at Site 4 (24 families). SIGNAL2 scores were below SEPP Waters objectives at half the sites (Sites 1, 5, 6, 7 and 8), with the highest score recorded at Site 3 (4.05) and the lowest at Site 8 (<2.8). Generally, the higher the SIGNAL 2 score, the higher the quality of the site for macroinvertebrates. EPT scores provide an indication of the presence of sensitive taxa at a site, with generally, the lower the number the more degraded the site. Lowest levels were observed at Site 8 (1), with the highest levels recorded at Site 10 (riffle - 8) and Sites 3 and 4 (edge - 6).

It should be noted that these guidelines are intended for permanently flowing streams, not ephemeral streams, like the upper Campaspe River or Snipes Creek. Long living macroinvertebrates requiring permanent waters would be less likely to be present in ephemeral waters.

Table 4: Macroinvertebrate biological indices at each site sampled during October 2018.

Site #	Type	Diversity	Biotic Indices	
		Number of Families	SIGNAL2	EPT
Campaspe River				
1	Edge	20	**3**	2
2	Edge	20	3.4	3
3	Edge	20	4	6
4	Edge	24	3.5	6
6	Edge	**15**	**3.1**	2
Kyneton WRP discharge between sites 6 and 7				
7	Edge	**18**	**2.9**	0
9	Edge	**16**	3.6	3
10	Edge	**19**	3.6	5
10^	Riffle	21	4.8	8
Tributaries				
5	Edge	**16**	**3.1**	3
8	Edge	**10**	**2.9**	1
SEPP Waters Objectives				
Edge*		20	3.4	N/A
Riffle*		16	4.5	5

* Only one season was sampled (Spring), therefore these objectives are a guideline only
^Riffle habitat was only present at Site 10 thus it was the only site sampled for this habitat type
N/A – value not applicable
Values **bold** if below State Environment Protection Policy (Waters) biological objectives

Nutrient Bioavailability

Visual Assessment

Macrophyte growth differed across sites (Figure 7), with differences between time points also observed. Higher percent cover was seen at the Campaspe River sites (except for Site 10) compared to the tributaries, with the highest cover at Site 2 and the lowest at Site 8.

Differences between sites and time points for filamentous algal growth were observed (Figure 8), with the lowest percent cover noted at Site 7. Differences in the length of filamentous algae were also noted, with a higher proportion of short filaments (<2cm) observed at Sites 1, 2, 3 and 4, and a higher proportion of medium filaments (2-10cm) at Site 9. Site 1 was not measured in July 2019 due to access issues.

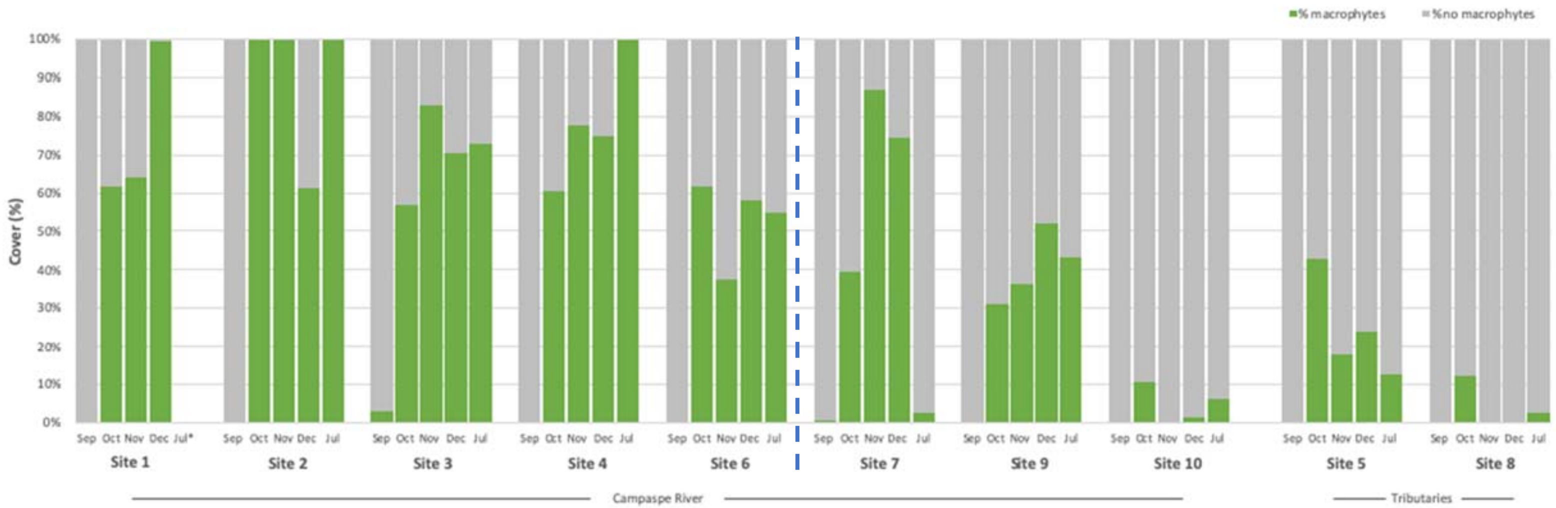


Figure 7: Monthly mean macrophyte cover within sites. Blue dashed line indicates Kyneton WRP discharge point between Sites 6 and 7.

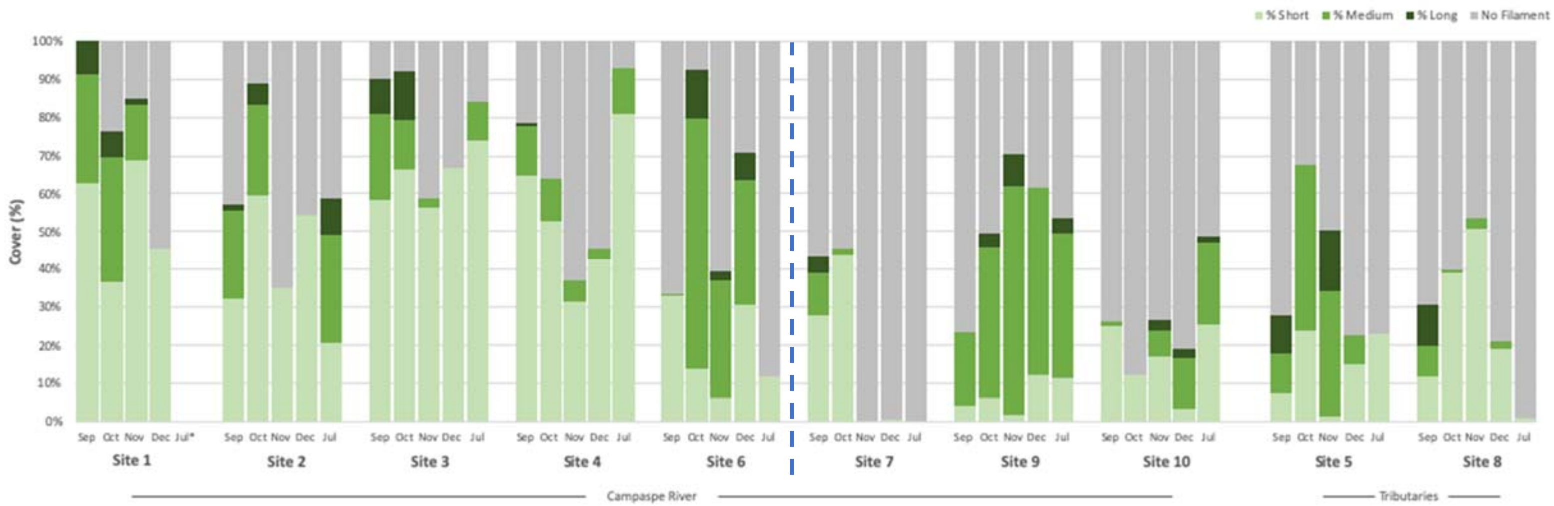


Figure 8: Monthly mean algal filament cover within sites. Blue dashed line indicates Kyneton WRP discharge point between Sites 6 and 7.

Artificial Substrates

Biofilm biomass on artificial substrates was measured across sites (Site 2 not measured in July 2019 due to access issues) following deployment in November 2018 and July 2019 (Figures 9, 10 and 11). Two standard measures of biomass were used, chlorophyll-a (indication of autotrophic organisms) and ash-free dry mass (AFDM) (measure of total amount of organic material, including autotrophic, heterotrophic and detrital carbon). From these measures, the autotrophic index (AI) (measure of the autotrophic-heterotrophic balance of the community present) is calculated as the ratio of AFDM to chlorophyll-a.

Significant differences in chlorophyll-a concentrations between sites were observed in November 2018 (Kruskal-Wallis, H= 25.324, DF= 9, *p*=0.003) and July 2019 (Kruskal-Wallis, H= 21.894, DF= 8, *p*=0.005) (Figure 8). Differences between time points within a site were seen across sites, except at Sites 3 and 7. Highest concentrations of chlorophyll-a were seen in July 2019, at Sites 1 and 5, indicating predominantly higher levels of autotrophic biomass.

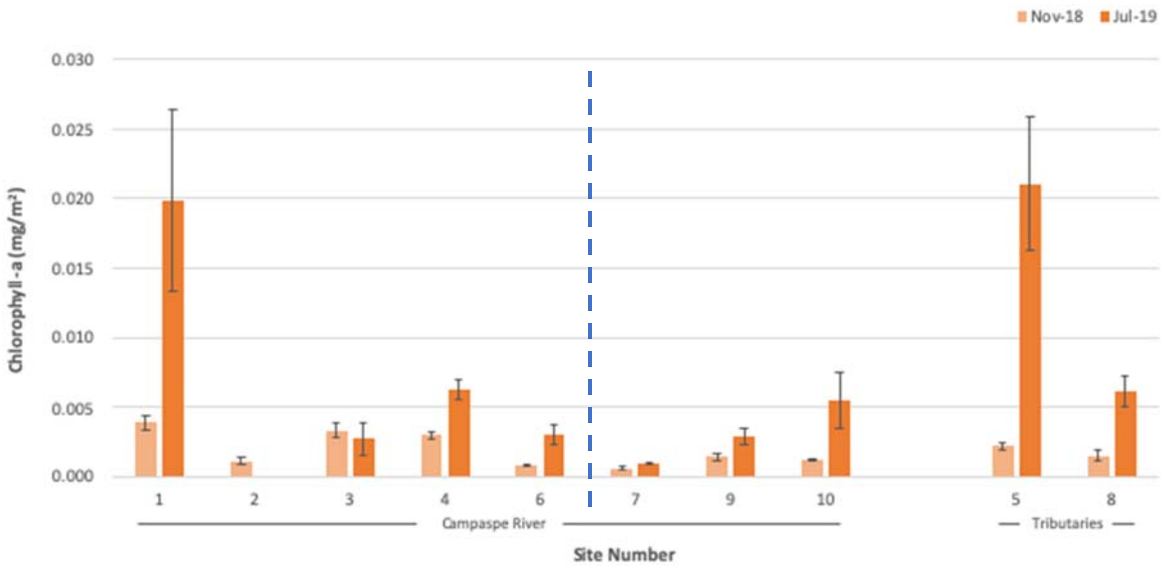


Figure 9: Chlorophyll-a (±SE) of biofilms on artificial substrates deployed across sites during November 2018 and July 2019. Blue dashed line indicates Kyneton WRP discharge point between Sites 6 and 7.

Significant differences in AFDM between sites were observed in both November 2018 (Kruskal-Wallis, H= 18.399, DF= 9, *p*=0.031) and July 2019 (Kruskal-Wallis, H= 15.909, DF= 8, *p*=0.044) (Figure 9). Generally, higher average values were seen in November 2018 across all sites, indicating higher levels of biomass when compared to July 2019.

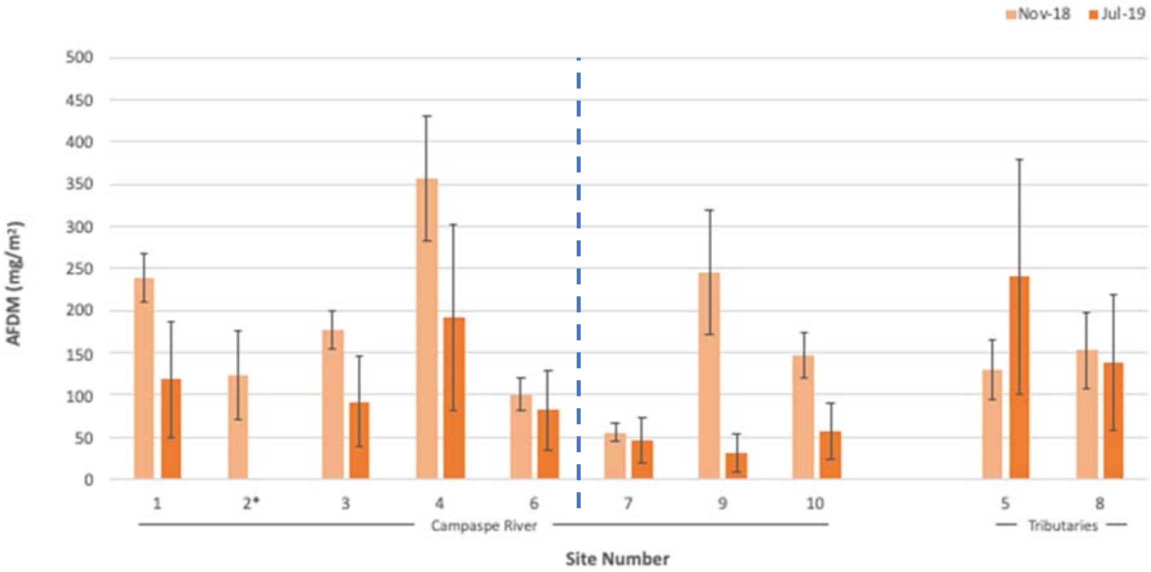


Figure 10: Ash-free dry mass (AFDM) (±SE) of artificial substrates deployed across sites during November 2018 and July 2019. Blue dashed line indicates Kyneton WRP discharge point between Sites 6 and 7.

There were no significant differences in autotrophic index (AI) between time points (November 2018 - Kruskal-Wallis, H= 12.880, DF= 9, p=0.168 and July 2019 - Kruskal-Wallis, H= 15.328, DF= 8, p=0.053); however, differences within sites were evident, with higher values observed in November 2018 (Figure 11). AI values between 50-100 are characteristic of non-polluted conditions, with values greater than 400 indicating communities impacted by organic pollution (e.g. decreasing occurrence of algal communities). All Sites in November 2018 exceeded 400, with Sites 1, 4, 5 and 8 exceeding in July 2019.

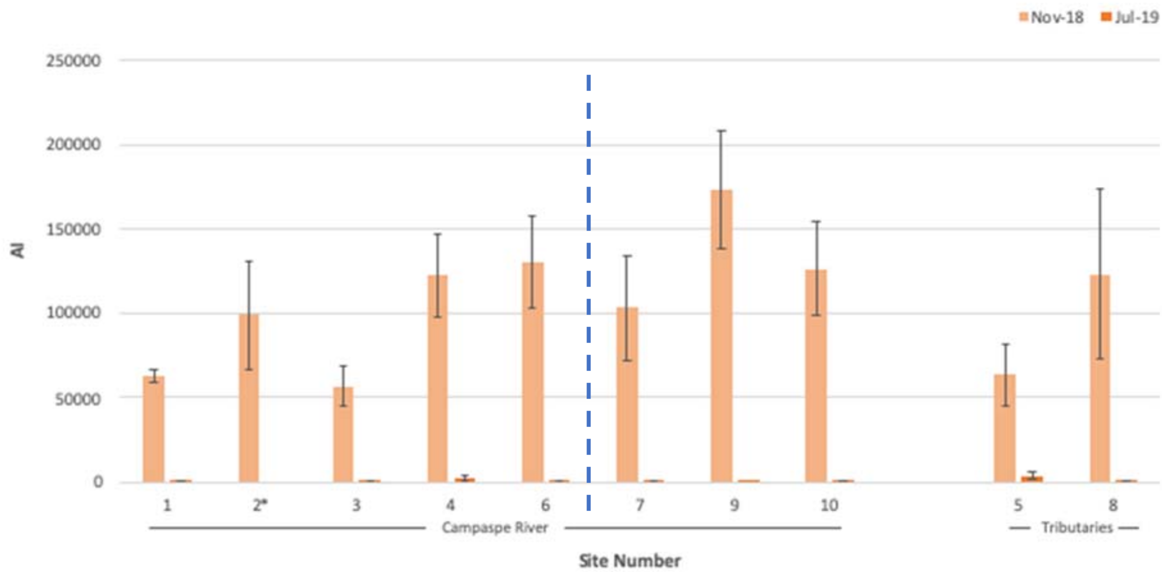


Figure 11: Autotropic index (AI) (±SE) of artificial substrates deployed across sites during November 2018 and July 2019. Blue dashed line indicates Kyneton WRP discharge point between Sites 6 and 7.

In addition, the photosynthetic health (photosynthetic efficiency) and community composition of the biofilms, observed on the artificial substrates, was also measured (Figures 12 and 13). Photosynthetic efficiency (Y) differed significantly between sites in November 2018 (Kruskal-Wallis, H= 22.172, DF= 9, *p*=0.008) and July 2019 (Kruskal-Wallis, H= 22.951, DF= 8, *p*=0.003) (Figure 10), with differences between time points within a site seen at Sites 3, 4, 5 and 8. Within sites, lower values were observed in November 2018 at Sites 3, 4 and 5, with the opposite observed at Site 8, indicating a reduction in photosynthetic health.

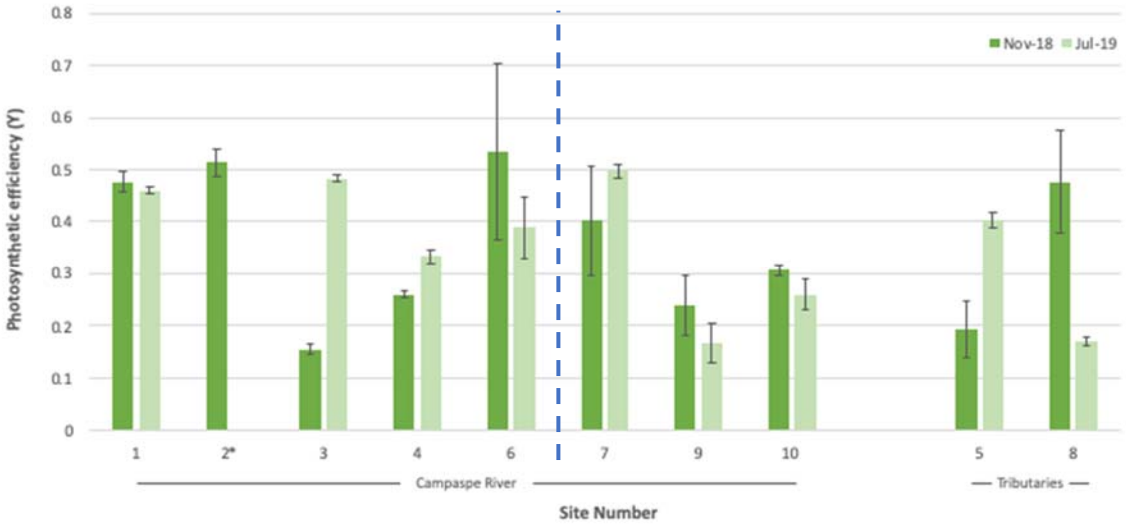


Figure 12: Mean photosynthetic efficiency (Fv/Fm) (±SE) of biofilms on artificial substrates deployed across sites during November 2018 and July 2019. Blue dashed line indicates Kyneton WRP discharge point between Sites 6 and 7.

Relative community composition differed across sites (Figure 13), with communities predominately comprised of cyanobacteria in November 2018 and diatoms in July 2019. Chlorophytes were present in November 2018, but absent from all sites in July 2019, indicating a reduction in green algae, possibly linked to the sampling time point (e.g. sampling occurred immediately following the rainfall event in June 2019 with chlorophytes requiring time to establish into the system).

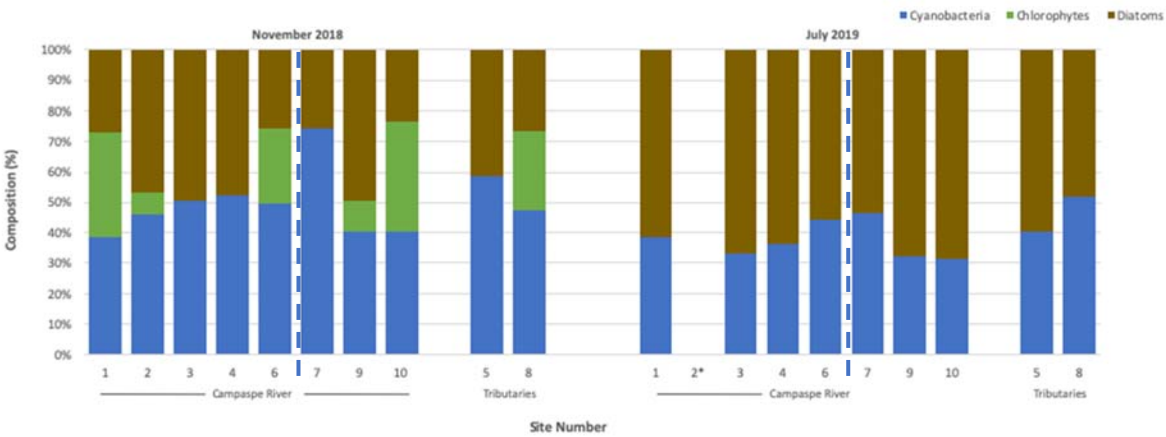


Figure 13: Relative composition of cyanobacteria, chlorophytes and diatoms, as determined using a Phyto-PAM, on artificial substrates deployed across sites during November 2018 and July 2019. Blue dashed lines indicate Kyneton WRP discharge point between Sites 6 and 7.

In-situ Algal Growth

Differences in algal growth (biomass) between control and exposed algal balls were examined at each site during November 2018 and July 2019 (Site 2 was not measured in July 2019 due to access issues) (Figure 14). Exposed algal balls were compared to the control to determine if exposure to site conditions inhibited or encouraged growth. Positive values indicate inhibition, with 0-20% minimal impact, 20-50% moderate impact and >50% significant impact. Negative values indicate increases in biomass, suggesting that site conditions (e.g. high levels of nutrients) are encouraging growth. In November 2018, percent inhibition relative to the control ranged from 9 to 56%, with increases in growth observed at Sites 5, 7 and 8. In July 2019, percent inhibition relative to the control ranged from 0 to 28%, with increases in growth observed at Sites 5, 7 and 8.

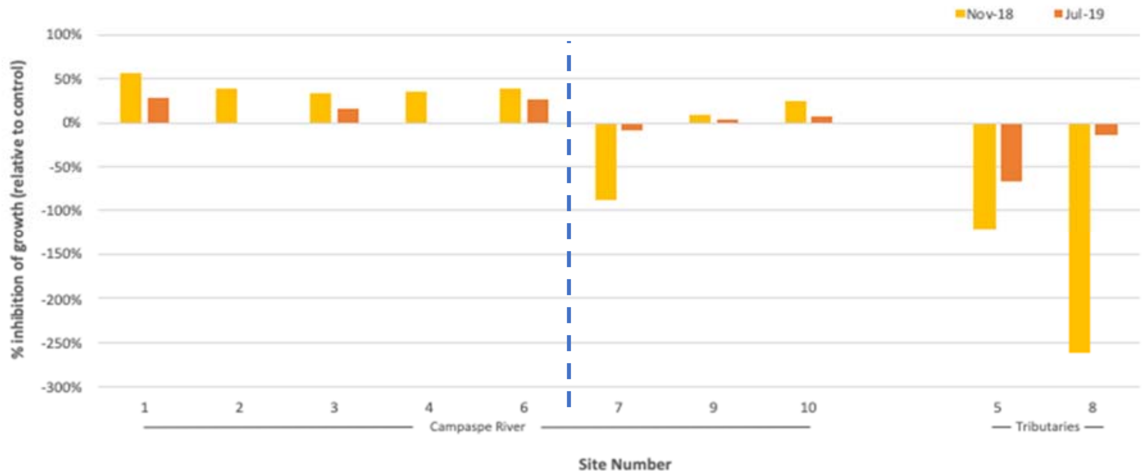


Figure 14: Percent inhibition of algal growth (relative to site control) across sites during November 2018 and July 2019. Blue dashed line indicates Kyneton WRP discharge point between Sites 6 and 7.

Ecotoxicology

Water Toxicity

Survival of the mud snail, *P. antipodarum* (Figure 15), did not differ across sites (Kruskal-Wallis, H= 12.188, DF= 9, p=0.203). Average survival was highest at Site 6 (100%), and lowest at Sites 5 and 7 (both 60%), with the reference sites (Sites 1 and 2) 78% and 88%, respectively.

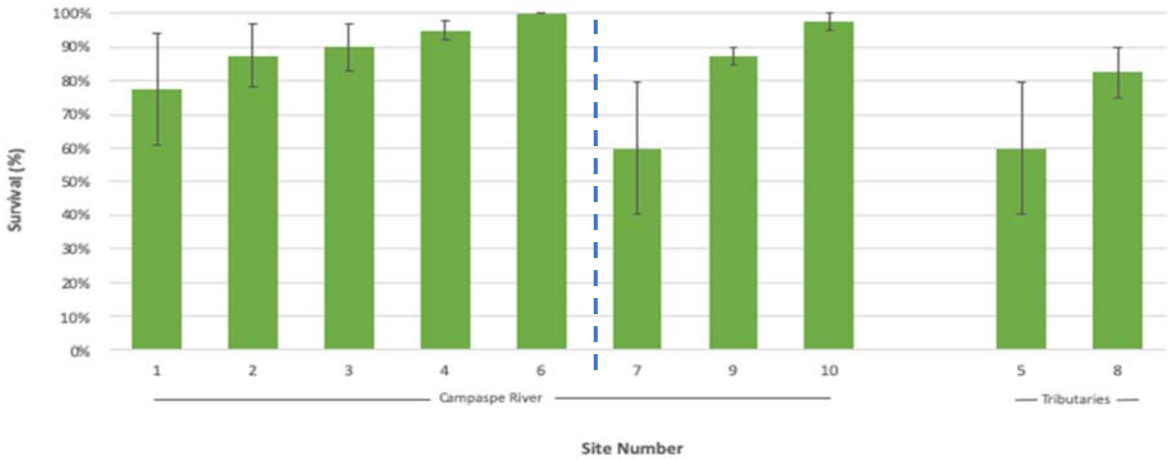


Figure 15: Mean survival (±SE) of mud snails, *P. antipodarum*, across sites during November 2018. Blue dashed line indicates Kyneton WRP discharge point between Sites 6 and 7.

Reproductive success of the mud snail, *P. antipodarum* (Figure 16), was determined by the number of embryos present, with lower numbers indicating the possibility of impacts from endocrine disrupting chemicals. No significant differences across sites were observed (Kruskal-Wallis, H= 9.327, DF= 9, *p*=0.408), with the highest value recorded at Site 1 (65% of mud snails had embryos) and the lowest value at Site 8 (37%).

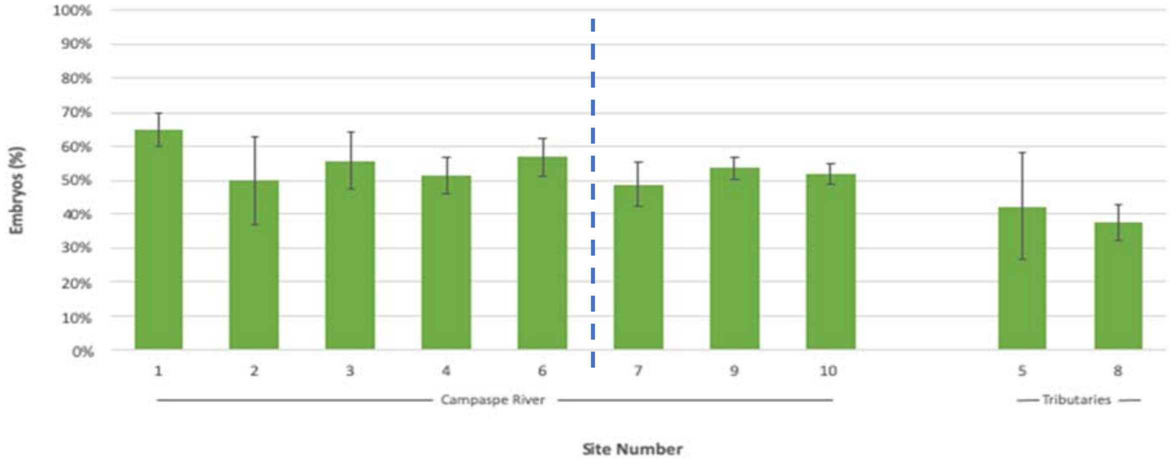


Figure 16: Mean percent (±SE) of mud snails, *P. antipodarum*, with embryos present across sites during November 2018. Blue dashed line indicates Kyneton WRP discharge point between Sites 6 and 7.

Sediment Chemistry

Sediment samples collected at eight sites (Table 5) were analysed for heavy metals, petroleum hydrocarbons and pesticides. Exceedances of the ANZECC/ARMCANZ trigger values were recorded for lead, mercury, nickel and zinc. At Site 5, exceedances for lead, nickel and zinc (more than four times the recommended level) were observed, with exceedances for mercury and nickel observed at Site 9. All sites sampled recorded exceedances in nickel, with values at Sites 3, 4, 5 and 9 more than double the recommended level. Levels of petroleum hydrocarbons at Site 3, 4, 5, 6, 9 and 10 were above the ANZECC/ARMCANZ trigger value. No pesticides were recorded within the sediments collected.

Other pollutants

Polar Organic Chemical Integrated Samplers (POCIS) deployed at each site (Table 6) indicated varying levels of surface water pollutants, including, pharmaceuticals, herbicides and insecticides, were present at sites.

A total of five herbicides were recorded across the sites, Simazine (a selective herbicide used to control broad-leaved weeds and annual grasses), Atrazine (a selective herbicide used to control broad-leaved weeds and annual grasses), Diuron (a broad spectrum residual herbicide and algaecide used to control broad-leaved weeds and grass weeds), MCPA (a selective herbicide used to control broad-leaved weeds) and Triclopyr (a selective herbicide used to control broad-leaved weeds and woody plants, such as blackberry). Simazine was recorded at Sites 4, 5, 6 and 10. Atrazine was recorded at Site 10, with Diuron recorded at Sites 5 to 10. MCPA was recorded at Sites 6 and 9, with Triclopyr recorded at all sites.

Two insecticides, Imidacloprid (noenicitinoid used to control insects in agricultural and domestic situations) and Carbaryl (used to control insects in agricultural and domestic situations, and commercial and industrial areas) were recorded. Imidacloprid was recorded at all sites, except Site 4 and Site 8. Carbaryl was recorded at Sites 4, 5, 7 and 10.

In terms of pharmaceuticals, the antidepressant Venlafaxine was recorded at Site 8 and Site 9, with no indication at other sites.

Table 5: Heavy metals, petroleum hydrocarbons and pesticides detected in sediments across sites in July 2019

Site #	Heavy Metals (mg/Kg)															Petroleum Hydrocarbons (mg/kg)	Pesticides (µg/Kg)
	Arsenic	Barium	Beryllium	Boron	Cadmium	Chromium	Cobalt	Copper	Lead	Manganese	Mercury	Nickel	Selenium	Vanadium	Zinc	C10 - C36 Fraction (sum)	
Campaspe River																	
3	5	210	1	<50	<1	73	26	28	19	777	<0.1	**48**	<5	62	171	**770**	All recorded as <0.1
4	6	200	1	<50	<1	62	28	29	35	986	<0.1	**46**	<5	55	183	**550**	
6	<5	170	1	<50	<1	64	25	34	20	402	<0.1	**38**	<5	53	173	**690**	
Kyneton WRP discharge between sites 6 and 7																	
7	<5	150	<1	<50	<1	54	12	18	19	252	<0.1	**24**	<5	39	120	210	All recorded as <0.1
9	11	220	1	<50	<1	71	31	25	17	1460	**0.7**	**48**	<5	61	81	**330**	
10	11	190	1	<50	<1	46	19	23	31	674	<0.1	**36**	<5	40	111	**490**	
Tributaries																	
5	10	260	1	<50	<1	56	24	51	**87**	451	0.1	**44**	<5	50	**849**	**960**	All recorded as <0.1
8	<5	310	1	<50	<1	54	43	24	12	1300	<0.1	**35**	<5	67	95	210	
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 6: Pharmaceuticals, herbicides and insecticides detected in surface waters using POCIS passive samplers (µg/0.2 g sorbent) across sites during July 2019

Site #	**Pharmaceuticals**	**Herbicides**					**Insecticides**	
	Venlafaxine	Simazine	Atrazine	Diuron	MCPA	Triclopyr	Imidacloprid	Carbaryl
Campaspe River								
1	<1	<1	<1	<1	<1	8.6	1.3	<1
2	<1	<1	<1	<1	<1	9.5	0.8	<1
3	<1	<1	<1	<1	<1	8	1.1	<1
4	<1	1.8	<1	<1	<1	7.3	<1	1.8
6	<1	1.8	<1	1.8	12.6	11.2	1.3	<1
Kyneton WRP discharge between Sites 6 and 7								
7	<1	<1	<1	1.7	<1	7.5	3.2	1.6
9	4.6	<1	<1	1.9	7.5	7.5	6	<1
10	<1	3.5	1.54	1.7	<1	6.1	1.9	3.5
Tributaries								
5	<1	1.7	<1	4.8	<1	5.7	3.2	1.7
8	3.7	<1	<1	1.7	<1	15.1	<1	<1

Values showing < are at the limit of detection

Discussion

This first year of monitoring provides an initial assessment of the condition of the upper Campaspe River, and two associated tributaries between Carlsruhe and Redesdale, and provides a baseline to assess improvements to instream water quality and aquatic biodiversity from stream frontage management works involving willow removal, revegetation and restricting access of stock to the river. The monitoring program applied multiple techniques, including an assessment of ambient surface water and sediment chemistry, in situ water toxicology, macroinvertebrate diversity, and nutrient availability surveys assessing plant and algal productivity, to assess instream health of the River. As the Campaspe River and associated tributaries within the study area are ephemeral for long periods of the year, meaning much of the study reach is dry during summer and autumn, the monitoring program was concentrated around periods when the river is connected and flowing, which for Year 1 monitoring was from September 2018 to December 2018, and in July 2019.

A major focus of the monitoring program is to measure changes in nutrient concentrations and reductions in faecal contamination in the Campaspe River following revegetation works and subsequent restriction of stock access to the river. The ambient surface water chemistry indicated that the levels of nutrients, faecal contamination and general physico-chemistry (pH, turbidity) varies spatially and temporally across the study area. Elevated levels of nutrients (Total Nitrogen (TN), Total Phosphorus (TP), Ammonia (NH_3)), turbidity and *E. coli* were generally related to periods of higher rainfall (i.e. October 2018, December 2018 and July 2019). The Campaspe River Sites 7 and 9, and the two tributaries (Sites 5 and 8), often had considerably higher levels of nutrients (TN, TP, NH_3), turbidity and/or *E. coli*[3], compared to other sites, with levels often exceeding SEPP guideline values.

Elevated levels of nutrients, turbidity and *E. coli* can be the result of agricultural and urban runoff, direct stock access to streams, leaking septic tank systems and/or treated wastewater discharges. It is likely all these contribute to the elevated levels observed in the study area. For instance, at Campaspe Sites 7-10 the higher ratios of orthophosphate to total phosphorus suggest sites are being influenced by wastewater inputs, whether this be from septic tank systems or a point discharge cannot be determined. In contrast, the low phosphorus ratio at Snipes Creek (site 8) suggests inputs are not usually wastewater related. There is a lack of riparian vegetation and free access to the creek for stock at Site 8, which could be contributing to the elevated nutrients and *E. coli* readings. Interestingly, Site 8 had elevated electrical conductivity compared to all other sites in the study area. This could be an indication of groundwater influence.

Post Office Creek provides an example of the influence of urban runoff on instream water quality. This site is situated in Kyneton and would receive runoff from the surrounding residential and upstream industrial areas, which are the likely contributors to the elevated levels of zinc, petroleum hydrocarbons, nutrients, turbidity and *E. coli*. As this study progresses we will gain a better understanding of how the SFMP benefits the instream health of the Campaspe River and associated tributaries via influencing processes such as nutrient and faecal transport. It is worth noting that *E. coli* is a general measure for faecal contamination which cannot be used to determine whether the contamination is from human sources or other animals. An assessment of the source of faecal contamination (human or bovine) was undertaken; however, results are not yet available for this report and will be provided in the next annual report. This data will provide a better understanding of the sources contributing to elevated faecal levels.

The SFMP works over time will enhance the amenity value of the Campaspe River and provide habitat for wildlife. However, it is unclear what benefits they will have on aquatic ecosystem health in terms of

[3]*E. coli* only measured at 6 sites.

nutrient and faecal reductions, or whether this may reduce the risk of excessive plant and algal growth. Thus, macroinvertebrate monitoring and *in situ* invertebrate assays were conducted to assess the benefits of SFMW to biodiversity. While surveys of plant and algal communities were conducted to assess benefits to reducing excessive algal and plant growth. Specialised algal passive sampler assays were deployed to assess the bioavailability of nutrients and river productivity.

Initial assessments of macroinvertebrate communities indicate that there is good habitat available, providing for healthy communities at numerous sites within the study area. The Campaspe River system, within the study area, is ephemeral, meaning that for parts of the year (often summer and autumn) much of the river is dry, with some pools providing refugia for aquatic life. This intermittency in water availability tends to support macroinvertebrates that can rapidly colonise the river once it commences flowing, as well as macroinvertebrates that can survive stagnant conditions. Longer living macroinvertebrates tend to not be observed. That said, the two upstream sites (1 and 2), and the sites in the township (3 and 4) had a relatively good diversity of macroinvertebrates. This is likely a reflection of several factors including the more stable water levels at Sites 3 and 4 over the year due to the presence of weirs upstream and downstream; restricted access of stock to the waterway (Sites 1, 3, 4); better riparian cover; availability of habitat and generally best water quality across the study area.

Poorest macroinvertebrate diversity was observed in the mid sections of the study area (Sites 6, 7 and 9) and in the two tributaries (Sites 5 and 8). This is likely a reflection of poorer water quality and riparian habitat (particularly Sites 6, 7 and 8). For instance, Site 7 had some of the poorest water quality, in terms of elevated nutrients. This site is choked with willows and stock have clear access to the waterway. In particular, willows provide poor habitat for macroinvertebrates, with a lack of continual supply of organic matter of appropriate quality.

An assessment of surface water toxicity to the freshwater snail, *Potamopyrgus antipodarum*, indicated that, in general, water quality was not significantly impacting on snail health. Slightly higher mortality was observed in the two tributaries, compared to the Campaspe River. This could reflect poorer water quality at these sites.

Directly assessing the algal and macrophyte cover in the river, together with the algal passive sampler assays and assessments of biofilm growth on artificial substrates allowed for the assessment of potential for excessive algal growth, river productivity and provided an indication of nutrient availability. Aquatic macrophytes cover generally exceeded 30% of the area at most sites, with exceptions being Sites 5, 8 and 10. Aquatic macrophytes provide benefits for invertebrates, fish and other aquatic biota, in the form of food, habitat and nutrient removal; however, excessive growth can cause eutrophication and a loss of oxygen.

There were differences in the types of macrophytes occurring at various sites, the main being upstream sites (Sites 1-4) were dominated by submerged macrophytes, such as *Myriophyllium* species, while downstream sites had an increasing presence of floating macrophytes, such as *Azolla* and *Lemna* species (particularly Site 7, but also seen at Sites 9 and 10). These latter species are often dominant in nutrient rich, low flowing systems, and would tend to become more prevalent when the river ceases to flow. Heavy filamentous algal growth occurred across the study area, however, upstream sites (1-4) were dominated generally by shorter filamentous algae, while the Campaspe River downstream sites and the site in Post Office Creek were generally dominated by medium to long filamentous algae. These results are likely reflective of higher nutrient concentrations in the more downstream sites. Biofilm growth on artificial substrates and the algal passive sampler assays indicated a bioavailability of nutrients for the promotion of algal growth, with higher growth observed where and when nutrients were higher e.g.: higher algal growth in July, with higher nutrient levels, compared to November, and stimulation of algal growth at Campaspe River Site 7, and in Post Office Creek and Snipes Creek, where the highest nutrient levels were generally observed.

The values of the Campaspe River may be affected by pollutants other than nutrients and faecal contaminants, such as petroleum hydrocarbons, pharmaceuticals, pesticides and heavy metals. Understanding the significance of these pollutants helps understand whether there are other important pollution issues that need to be considered as impacting on instream water quality and biodiversity. An assessment of toxicants, other than nutrients, at the sites, in sediments and waters, indicated that there are several other pollutants present across the catchment at levels that may be adversely impacting on stream biodiversity and water quality. Elevated levels of hydrocarbons, heavy metals, pharmaceuticals and pesticides were detected across the study sites.

Hydrocarbons and heavy metals are common pollutants related to urbanisation, with roads and road transport, industrial activities (e.g.: metal recyclers, old mining) and housing (e.g.: zinc roofing) a source of these compounds. However, some may also come from natural sources in the environment. Hydrocarbons were detected at elevated levels generally at sites located close to large or busy roads or at sites that would directly receive road runoff (Sites 3, 5 and 10).

Elevated concentrations of zinc, lead and mercury were detected at some sites, while nickel occurred at concentrations exceeding ANZG trigger values at all sites. The source of nickel is most likely natural within the study area, being sourced from basaltic rock. Zinc and lead were detected at concentrations in Post Office Creek that could pose a risk to resident instream biota. This tributary is situated within the town of Kyneton and would receive runoff from the surrounding urban and industrial area, which is the most likely source of these two heavy metals. Both metals are related to road transport, and zinc also enters runoff from materials in residential and industrial buildings. Mercury was detected at Site 9 in the Campaspe River at concentrations exceeding environmental trigger values (ANZG). It is likely this is a legacy issue from gold mining. Mercury is persistent in sediments potentially lasting many years after its use.

Pesticides are used in both urban and agricultural situations to control unwanted pests, such as weeds, insects and fungi. Pesticides can enter waterways via various pathways, including surface runoff during irrigation and/or rainfall, aerial deposition during application (spray drift), and infiltration via groundwater. Several herbicides and two insecticides were detected across the study area, with up to 6 different pesticides detected at any one site. The herbicides detected included diuron, simazine, atrazine, MCPA and triclopyr. All these herbicides are registered for use in numerous agricultural and non-agricultural situations. The most frequently detected, occurring at all sites, was triclopyr. This herbicide is used for control of broadleaf weeds such as nettles, docks and blackberries. It is also commonly used in turf, landscaping and lawn care. Diuron was detected at the second highest frequency, at 6 sites, including all sites downstream and inclusive of Site 6 in the Campaspe River and the two tributaries. Diuron is most commonly used in agricultural situations, such as on grass and vegetable crops, but also in non-agricultural situations for fallow areas, bore drains, ditches and irrigation channel weed control. Two insecticides, imidacloprid and carbaryl, were detected. Imidacloprid occurred at all sites, excluding Sites 8 and 4. Imidacloprid is a highly water-soluble insecticide, thus can be transported easily in surface runoff, but also via groundwater. It is commonly used in domestic situations for control of insects, such as fleas, in domestic animals, for flea and worm control, and in agricultural situations as a seed dressing, or on fruit, vegetable and cropping situations. Carbaryl occurred at Sites 4, 7 and 10 in the Campaspe River and at the Post Office Creek site. Carbaryl is commonly used to control insects in home garden and domestic situations, on ornamentals, lawns, fruit and vegetables, and around commercial and public buildings.

Pharmaceuticals are used in various applications, including humans, and domestic and stock animals. Their occurrence in the environment is generally related to wastewater inputs, either from treated discharges, septic tank systems or animal waste runoff. Venlafaxine, an antidepressant, was the only pharmaceutical detected, which occurred at Site 9 in the Campaspe River and Snipes Creek. Venlafaxine is used in humans, indicating most likely a treated wastewater discharge or septic tank system leakage. Septic tanks generally remove solid wastes and partly treat effluent before it is discharged onto properties. The performance differs based on type, age, maintenance, soil type, slope and size of property. Runoff from unsewered residential properties can have a considerable impact on stream water quality. Further investigation would be needed to determine exact sources. It did appear to be a localised incident, not occurring upstream or downstream of Campaspe River Site 9 at Boundary Rd.

Further investigation into the temporal and spatial occurrence of these 'other' pollutants would provide a greater understanding of potential risks posed to river health and provide a better understanding of their sources so that management actions can be identified, if needed.

Conclusions

The stream frontage management programs (SFMPs) are expected to provide an immediate benefit to the Campaspe River through reducing faecal contamination and nutrient inputs. The removal of willows and revegetation, while initially likely to cause some impact to the river via sediment runoff, over the years should result in improved river health, and as the native vegetation establishes and grows, it will provide habitat for wildlife (e.g. corridors for birdlife) and a source of leaf litter (food) for aquatic macroinvertebrates. In about 30 years' time, snags from fallen limbs and trees will provide instream habitat for aquatic animals. The SFMPs are also expected to benefit the Campaspe River through reducing nutrient transport from grazing lands and cattle faeces, which will offset any extra nutrients that may be delivered to the river from any non-compliant wastewater discharge. Year one monitoring results, of a 5-year monitoring program, provide an initial baseline of the state of the Campaspe River and associated tributaries in the study area and a benchmark to assess the benefits of SFMPs in improving instream water quality and biodiversity.

Our results show that there are spatial and temporal changes in water quality, with elevated nutrients, turbidity and levels of faecal contamination occurring across the study area, generally correlating with periods of higher rainfall. Monthly monitoring of macrophyte and filamentous algal cover, together with algal passive sampler assays, indicate nutrients are available for the promotion of macrophyte and algal growth. Higher levels of medium to long filamentous algae, and the occurrence of nutrient tolerant macrophytes, such as *Lemna* and *Azolla,* were generally observed at sites with elevated nutrients. Macroinvertebrate diversity was considered quite good for an ephemeral river system, with a few sites having lower diversity, corresponding to those with elevated nutrients, poor habitat availability, due to excessive willow growth, and/or the occurrence of other contaminants. Similarly, ecotoxicology results, using snails, indicated generally that surface waters were not impacting on invertebrate health, with the exception of Post Office and Snipes Creeks. An assessment of sediment and surface waters for pollutants, other than nutrients and faecal contaminants, has highlighted the catchment is influenced by runoff from urban and agricultural lands, with several heavy metals and hydrocarbons being detected in sediments at elevated levels, and several pesticides and a pharmaceutical detected in surface waters.

Overall, three sites were identified across several parameters monitored to be in poorer condition compared to the rest for the study area. These included Site 7 on the Campaspe River and the two tributaries. Several factors, including anthropogenic inputs from urban, agricultural and wastewater, together with poor riparian characteristics, appear to be influencing water quality. Monitoring over the next 5 years will provide a greater understanding of the key factors influencing water quality and biodiversity and how the stream frontage management works being undertaken benefit instream health.

Future Sampling

The five-year monitoring and assessment program is due for completion in 2022. The Year 2 sampling period occurred from August to December 2019 with results available in April 2020.

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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