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Upper Barwon River Investigation 2019-2021

- Final
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Contents

1. Introduction

Austral Research and Consulting (Austral) were contracted by Barwon Water to undertake an investigation into the sediment and water quality and macroinvertebrate condition of the upper Barwon River with regard to the extent of impact of low pH inflows from Boundary Creek.

The survey determined a baseline for ongoing monitoring of the Barwon River as part of a remediation plan required by a section 78 Ministerial Notice by:

- Assessing the spatial extent of surface water effects resulting for acidic discharge from Boundary Creek in the Barwon River,
- Determining if acidic discharge from Boundary Creek has affected sediment in the Barwon River and if so, the spatial extent and depth of accumulation, and;
- Assess the potential impact of acidic discharge from Big Swamp at Yeodene on the macroinvertebrate community structure in Boundary Creek and the Barwon River.

This report will provide an overall assessment of all the macroinvertebrate and water quality sampling undertaken to date as part of the remediation plan.

1.1. Background

Studies have confirmed that past water extractions from the Barwon Downs borefield by Barwon Water to boost Geelong's water supply in conjunction with a dry climate led to reductions in flows in lower Boundary Creek (Jacobs, 2017). This in turn caused Big Swamp to dry out activating naturally occurring acid sulfate soils that when rewetted have released acidic water into the lower reaches of Boundary Creek. Boundary Creek joins the Barwon River 3.7km downstream of Big Swamp.

2. Methods

A total of twelve sites were surveyed along East Barwon, West Barwon, and Barwon Rivers in addition to Boundary Creek (Figure 1).

- **Figure 1: Barwon River and Boundary Creek (base map from Waterwatch Victoria)**

2.1. Site Selection

Sites were selected in consultation with Barwon Water to best give an indication of the impact of water coming from Big Swamp on Boundary Creek and particularly the Barwon River. Two sites are on the East Barwon River, one site is on the West Barwon River, one site is on Boundary Creek and eight sites are on the mainstem Barwon River. They incorporate existing Waterwatch sites, upstream sites that are unimpacted by Boundary Creek (sites 1-4) and sites focused on any impacts from Boundary Creek (Table 1).

Site no.	Existing number	Site description	Lat	Long
1	CO_BAR004 (inact.)	East Barwon River @ Kents Road, Yaugher	-38.512196	143.732530
2	New	East Barwon River @ Dewings Bridge Road	-38.434878	143.747933
3	CO_WES010	West Barwon River @ 7 bridges Road	-38.474669	143.689396
4	New	Barwon River immediately u/s of Boundary Ck conf.	-38.418236	143.742025
5	CO_BOU009	Boundary Creek, Colac-Forrest Road	-38.421122	143.710475
6	New	Barwon River immediately d/s of Boundary conf.	-38.416717	143.742383
7	New	Barwon River u/s CO_BAR016	-38.402291	143.757554
8	CO_BAR016	Barwon River @ Colac-Lorne Road	-38.388771	143.768956
9	CO_BAR020	Barwon River @ Birregurra	-38.339105	143.790971

10	CO_BAR030	Barwon River @ Conns Lane	-38.325134	143.832385
11	CO_BAR040	Barwon River @ Winchelsea Deans Marsh Road	-38.278018	143.978382
12	CO_BAR060	Barwon River @ Princes Hwy bridge, Winchelsea	-38.240445	143.989326

- **Table 1: Site locations and descriptions**

2.2. Sampling methodology

Macroinvertebrates and *in situ* water quality, vegetation, site descriptions and photos were collected with specific sampling methods detailed below.

2.2.1. In-situ water quality

In-situ water quality parameters were measured at each site including dissolved oxygen (mg/L), temperature (ºC), specific conductivity (µS/cm) and pH using a YSI ProPlus water quality meter. Turbidity (NTU) and alkalinity (mg/L) were measured using HACH meters and test kits respectively.

2.2.2. Metals in water

Water samples were collected for metals analysis and kept refrigerated prior to delivery to the NATA accredited Eurofins Laboratory (Spring 2019- Spring 2020) and ALS Laboratory (Autumn 2021).

2.2.3. Macroinvertebrates

The benefit of monitoring the biological community is that it is affected by numerous types of toxicants and disturbances and the impacts can be evident over months or years unlike chemical testing which may not capture an episodic event.

Macroinvertebrates were collected at each site and photos and site assessment sheets were completed as per Victorian EPA guidelines (EPA, 2021). In the absence of riffle habitats, two edge samples were collected (EPA, 2021) using a 250µm mesh dip net to sample ten metres of representative habitat at two locations at each site on the 17th and 18th May, 2021. The contents of the net are placed into a white tray to be picked through for 30 minutes with the aim of picking over 100 animals into 70% ethanol for later identification to family level following the Rapid Bioassessment Methodology for Rivers and Streams (EPA, 2021). Macroinvertebrates were identified in the laboratory in accordance with the guidelines; to class for Oligochaeta and Mites, chironomids to sub-family and all other taxa to family except those that are not included in EPA Victoria biotic calculations (EPA, 2021).

2.2.4. Site descriptions

EPA Victoria field sampling and habitat assessment sheets were filled out at each site and site photos taken (EPA, 2021). This information has been summarised in Appendix 1. The reported habitat parameter score is not expected to change over the short term unless works have been undertaken at the site such as riparian revegetation or fencing or large woody debris introduction or the site is experiencing changes in flow such as drought conditions.

3. Results

3.1. Survey Trips and Stream Flow States

Table 2 lists all survey trips undertaken since the start of this project along with discharge data for both Boundary Creek (at Yeodene) and the Barwon River at Ricketts Creek.

- **Table 2. Flow conditions of Boundary Creek and Barwon River at Ricketts Creek during each survey (Department of Environment, Land, Water and Planning, 2021). Zero flow is below gauge height.**

Survey Dates	Season	Boundary Ck at Yeodene (ML/d) Gauge #233228			Barwon Rv at Ricketts Creek (ML/d) Gauge #233224		
		Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
2-4/10/19	Spring 19	6.04	5.49	5.30	155	124	96.1
31/3–2/04/20	Autumn 20	0	0	0	8.77	8.74	9.50
7-8/12/20	Spring 20	5.87	5.44		17.9	20.0	
20/01/21	Summer 21	6.11			19.2	22.0	
17-18/05/21	Autumn 21	7.05	6.46		77.1	82.4	

The Summer 2021 survey was necessary after metal samples collected in the Spring 2020 survey were incorrectly analysed for total rather than filterable metals. For further discussion see the Upper Barwon Investigation Spring 2020 data report (Austral Research and Consulting, 2021).

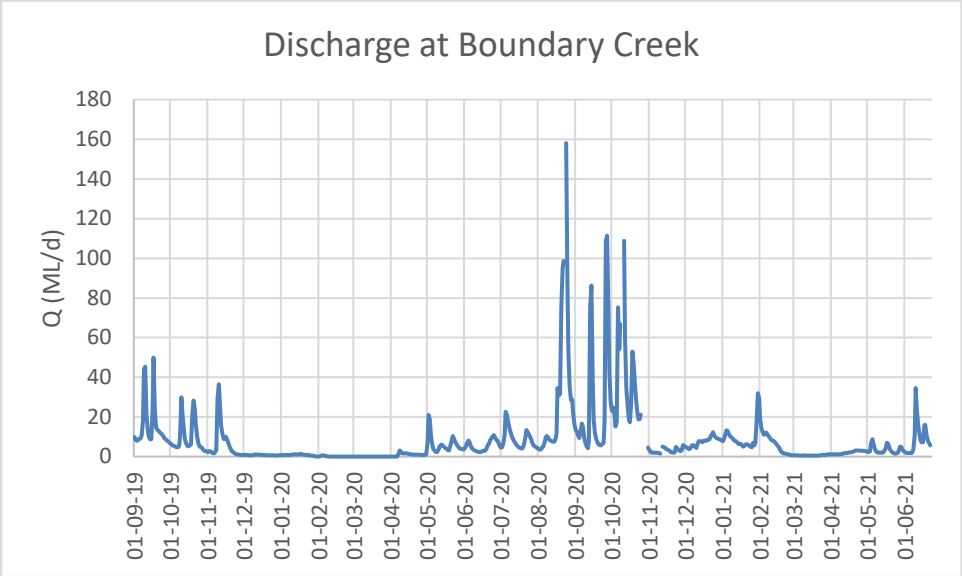
Flow statistics for both stream gauging stations over the survey period are given in Table 3.

- **Table 3. Flow statistics for Boundary Creek at Colac-Forrest Rd and the Barwon River at Ricketts Creek during the Survey period (Dept. Env., Land, Water and Planning, 2021)**

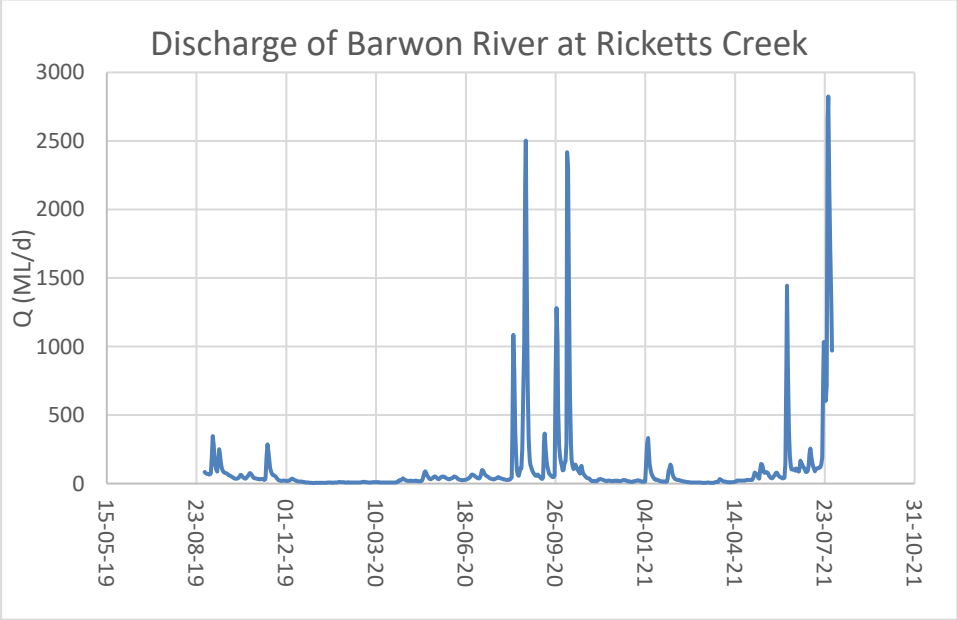
Quantity	Boundary Creek (#233228)	Barwon River (#233224)
Flow Period	1 Sep 2019 – 22 June 2021	1 Sep 2019 – 31 July 2021
Mean Q (ML/d)	8.25	106
Median Q (ML/d)	4.00	33.1
Maximum Q (ML/d)	158	2825
Minimum Q (ML/d)	0	4.35

During each survey discharge was only slightly less than the mean flow of Boundary Creek and substantially less than its maximum discharge. Flow conditions in the Barwon River at Ricketts Creek were highest (and above mean flow) during the Spring 2019 survey. Second highest flow conditions were during the Autumn 2021 survey (but below mean discharge). The Barwon flow conditions for the other 3 surveys were all below median flow at the Ricketts Creek gauge.

Daily discharge data for Boundary Creek at Colac Forrest Rd (Figure 2) and the Barwon River at Ricketts Creek (Figure 3) both peaked during winter and spring. Smaller “freshes” occurred in both streams during November 2019 and January 2021 but did not coincide with surveys.



- **Figure 2. Daily discharge in Boundary Creek, Colac-Forrest Rd, Yeodene between 1 September 2019 and 22 June 2021 (Dept. Env., Land, Water and Planning, 2021)**



- **Figure 3. Daily discharge of the Barwon River at Ricketts Creek from 1 September 2019 to 31 July 2021 (Dept. Env., Land, Water and Planning, 2021)**

3.2. Water Quality

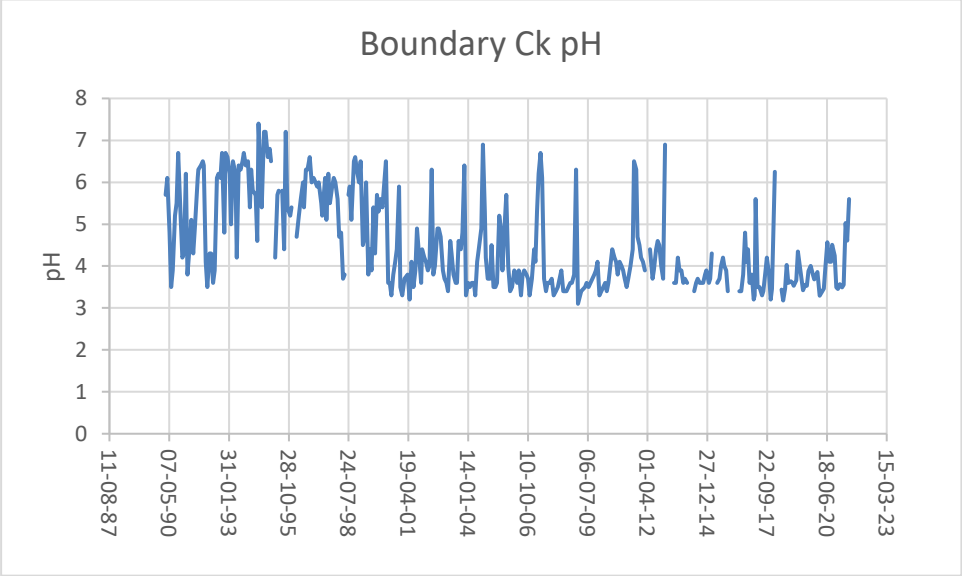
Water samples were collected and *in situ* readings taken at all 12 sites at the same time as macroinvertebrates were collected giving an indication of conditions at the time of sampling.

- **Table 4: In-situ water quality data- Spring 2019, Autumn 2020, Spring 2020, Autumn 2021**

Site Waterway	Season	Temp. (°C)	pH	Conductivity (µS/cm)	Specific Conductivity (µS/cm@25°C)	Dissolved oxygen (mg/L)	DO %	Alkalinity (mg/L)	Turbidity (NTU)
Site 1 East Barwon Rv	Spring 19	13.2	6.2	186.7	240	13.07	123	5	9.09
	Autumn 20	14	8.67	161.8	210.2	4.42	47.5	5	2.6
	Spring 20	16.0	7.2	123.7	149.5	7.79	80.1	35	2.88
	Autumn 21	10.6	6.5	93.5	129.0	7.59	68.7	25	8.5
Site 2 East Barwon Rv	Spring 19	15.5	6.3	544	664	6.8	66.8	10	9.97
	Autumn 20	16	7.71	180.7	218.2	5.85	59.9	10	9.49
	Spring 20	16.2	7.6	272.0	327.3	10.86	110.4	55	9.35
	Autumn 21	11.2	6.58	228.9	311.8	8.21	74.2	35	8.2
Site 3 West Barwon Rv	Spring 19	14.7	5.26	473.4	590.6	7.3	73.5	10	16.3
	Autumn 20	14.4	8.23	179.6	224.0	4.45	42.9	10	3.28
	Spring 20	12.7	7.1	195.2	255.0	6.12	56.3	50	5.58
	Autumn 21	9.7	7.3	165.1	233.4	7.21	63.9	30	3.65
Site 4 Barwon Rv	Spring 19	17.9	7.4	575	664	9.15	96.4	10	8.01
	Autumn 20	17	6.60	211.2	248.4	6.08	64.3	10	41.5
	Spring 20	14.1	7.4	248.1	326.1	8.25	80.5	55	17.7
	Autumn 21	10.8	7.03	224.3	308.7	7.96	71.2	35	14.2
Site 5 Boundary Ck	Spring 19	12.1	3.94	777	1030	7.43	67.6	0	2.92
	Autumn 20	10.4	4.05	680	944	2.05	18.5	0	260
	Spring 20	12.9	3.1	614	798	5.31	50.6	0	6.82
	Autumn 21	10.0	4.0	286.6	401.2	8.76	76.8	20	35.7
Site 6 Barwon Rv	Spring 19	14.4	7.34	608	756	7.3	71.3	10	9.43
	Autumn 20	15.8	6.88	207.7	250.6	6.58	66.1	10	31.7
	Spring 20	13.9	7.0	298.5	378.8	6.88	67.1	50	12.2
	Autumn 21	10.9	7.19	254.2	347.7	7.03	62.9	25	20.6
Site 7 Barwon Rv	Spring 19	13.4	7.9	599	770	7.2	71.7	5	10
	Autumn 20	15.4	6.46	207.9	256.2	7.46	75.6	5	21.8
	Spring 20	14.2	7.1	276.6	348.2	7.15	69.7	45	13.6
	Autumn 21	10.7	7.14	258.1	354.6	6.78	61.9	20	17.9
Site 8 Barwon Rv	Spring 19	16.2	7.8	660	795	8.8	87.9	10	13.5
	Autumn 20	15.9	6.79	234.8	284.8	3.22	32	10	5.13
	Spring 20	16.3	7.3	286.3	344.2	6.55	67.7	50	5.61
	Autumn 21	11.0	6.74	255.8	350.3	7.10	64.2	25	12.4
Site 9 Barwon Rv	Spring 19	15.4	7.8	1049	1288	9.7	98	15	16.6
	Autumn 20	16.2	6.79	494.4	600.6	6.65	69.8	15	11.1
	Spring 20	16.7	7.5	477.8	568.1	8.28	86.6	25	16.2
	Autumn 21	10.0	7.62	372.9	523.3	7.69	67.0	40	23.7
Site 10 Barwon Rv	Spring 19	14.6	7.9	1252	1561	8.1	86.1	15	18
	Autumn 20	16.2	5.56	511	613	3.96	40.2	15	19.2
	Spring 20	15.6	7.5	756	920	7.45	76.2	75	22.2
	Autumn 21	11.1	7.14	372.5	506.5	9.03	81.5	40	33.7
Site 11	Spring 19	13	7.9	1707	2227	9.23	87	15	26.1

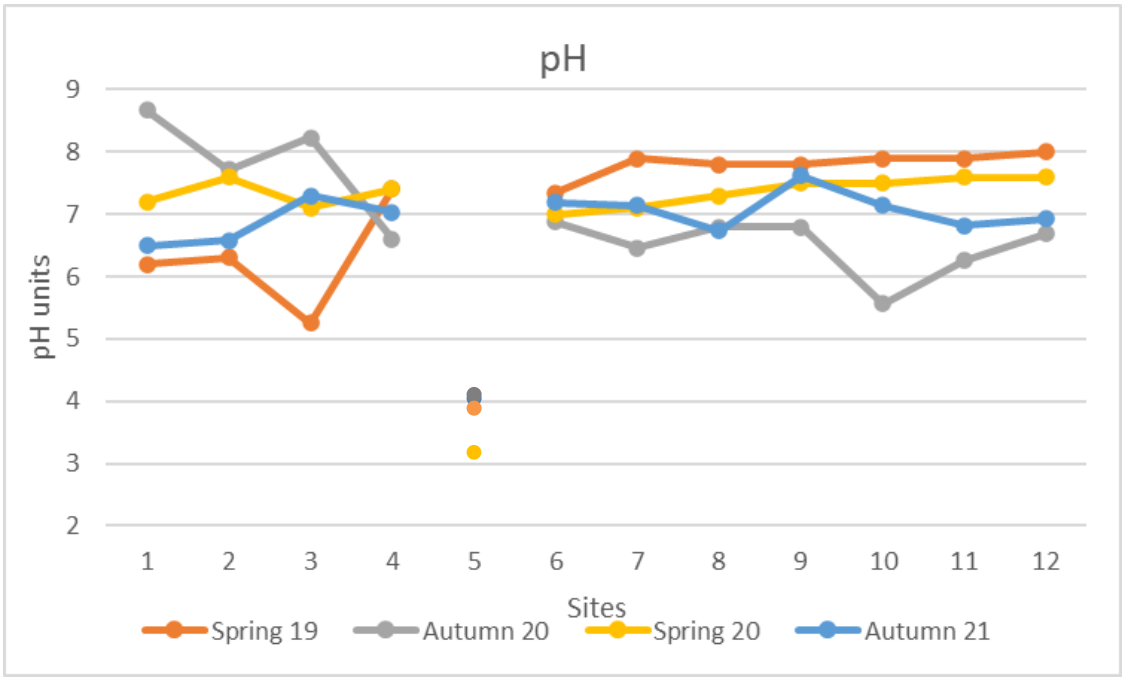
Site Waterway	Season	Temp. (°C)	pH	Conductivity (µS/cm)	Specific Conductivity (µS/cm@25°C)	Dissolved oxygen (mg/L)	DO %	Alkalinity (mg/L)	Turbidity (NTU)
Barwon Rv	Autumn 20	15.6	6.26	762	929	3.62	35.2	15	13.3
	Spring 20	15.5	7.6	863	1054	6.28	64.7	75	13.7
	Autumn 21	11.0	6.82	401.9	548.8	8.35	74.1	40	31.5
Site 12 Barwon Rv	Spring 19	12.4	8	1788	2364	8.4	82.1	15	19.9
	Autumn 20	15.9	6.69	924	1117	5.25	54.5	15	20.7
	Spring 20	15.0	7.6	1048	847	6.25	62.8	85	17.1
	Autumn 21	10.8	6.93	466.1	639.8	8.18	72.7	40	31.2

A property of major concern is the acidity of Boundary Creek. As Table 4 shows, the pH of the Creek (Site 5) was 4 or less on each field trip. This is a common state of the Creek after 2000 (Figure 4). The alkalinity of Site 5 is negligible as expected from the low pH (the Autumn 21 value is considered to be an error).



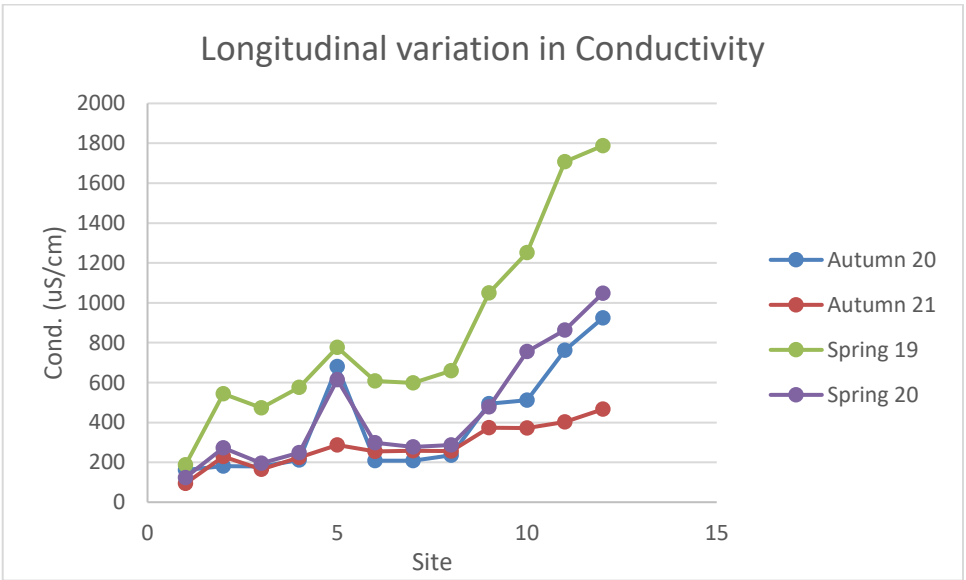
- **Figure 4. Variation of pH in Boundary Creek at Colac-Forrest Rd from January 1990 to June 2021 (Dept. Env., Land, Water and Planning, 2021)**

The pH value in the mainstem Barwon River appear unaffected by the low pH in Boundary Creek reflecting the Barwon's good buffering capacity (Figure 5) and the River's greater flow (typically 3 or more times that of Boundary Creek (Tables 2, 3). The Barwon River pH values lie predominantly within the ecologically optimum range of 6.5 to 8.5.

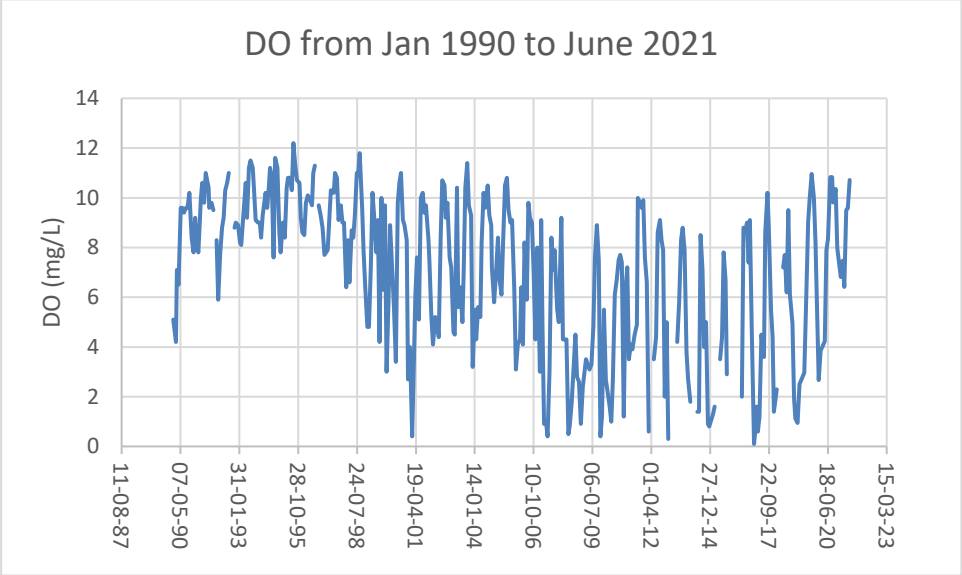


- **Figure 5. pH values during sampling events along upper Barwon River and Boundary Creek (Site 5) over two seasons and two years.**

The relatively high conductivity (a measure of dissolved salt concentration) of Boundary Creek also has little effect on the conductivity of the Barwon River immediately downstream because of efficient dilution by the river flow (Figure 6). Below Site 8, conductivity in the Barwon River rises significantly with distance downstream. This suggests a diffuse salt source, most likely regional groundwater although the larger increase under the higher flow encountered during the Spring 2019 survey suggests there may also be a catchment contribution.



- **Figure 6. Variation of electrical conductivity along the Barwon River during 4 surveys. Note that Site 5 is the tributary Boundary Creek.**



- **Figure 7. Measurements of dissolved oxygen (DO) in Boundary Creek at Colac- Forrest between January 1990 and June 2021 (Dept. Env., Land, Water and Planning, 2021).**

Dissolved Oxygen (DO) concentrations of 50% or less were measured at Site 5 during the Autumn 2020 and Spring 2020 surveys (Table 2). As the long term dataset of Figure 7 shows low DO concentrations (<5mg/L; < ~50% saturation) have become common since 2000. DO measurements were not continuous but were taken at intervals of weeks to a month. DO fluctuations have also become more extreme since 2000. The reasons for these changes are not clear. Many factors can affect DO – the balance between photosynthesis and respiration in the aquatic community, turbulence generated by wind or water flow and inflows of anoxic groundwaters can all cause DO to vary. Lower DO concentrations were observed along the length of the Barwon River in the Autumn 2020 survey, a time of very low flow. Lack of turbulence and/or groundwater inflows having greater influence may have been factors.

3.3. Heavy Metal Concentrations

Aluminium, iron and manganese are metals whose chemistry is strongly linked to geochemical and redox process associated with weathering of earth minerals including pyrite (FeS_2), manganese sulphide (MnS) and clays (aluminium silicates). Under anoxic conditions as found in sediments and anoxic waters manganese and iron exist in the +2 valence state as simple ions. Under oxidising conditions both metals are oxidised to insoluble oxides in higher valence states (+3 and +4 respectively). Aluminium does not take part in redox processes. Both iron and aluminium form hydrous oxyhydroxides in oxygenated waters at pH values above around 5 but remain as the simple cations under acidic conditions. The high (to very high) concentrations of aluminium and iron at Site 5 have been mobilised by the strongly acid waters of Boundary Creek. Manganese concentrations are not elevated however indicating low availability of this element in the Boundary Creek catchment. The oxyhydroxides of iron and aluminium can occur as colloids that can pass through 0.45µm filters and this may be the reason for slightly elevated concentrations found at other sites along the Barwon River in some of the surveys. At the neutral pH values observed along the Barwon the two metals are unlikely to be bioavailable.

During the Autumn 2021 survey an iron oxyhydroxide floc was observed on vegetation at Site 6 (see photos in Appendix 1). The filterable iron concentration at the site was also elevated (Table 3). Such flocs can be associated with respiration in iron bacteria which convert iron (II) to iron (III) - which is then converted to the red hydrous oxide ("rust"). Often this happens at an anoxic/oxic interface such as where groundwater enters a stream. As catchments "wet up" in autumn and water tables rise, inflows of groundwater into streams increase before appreciable surface runoff. At Site 6 it is likely iron (II) in the water is being oxidised by iron bacteria on the vegetation – taking advantage of the oxygen produced by the plants during photosynthesis. This leads to a build up of $Fe(OH)_3$ on the plants. This could be a regular seasonal effect at this site given its proximity to Boundary Creek. (Site 5). Higher flows will dislodge the iron floc.

With the exception of zinc, all other metal concentrations are at or below ANZECC guidelines (the value for lead at Site 3 in the Autumn 2020 survey is equal to the guideline value within the experimental uncertainty (±0.001)). This observation applies to Site 5 in Boundary Creek as well as to the Barwon River.

Zinc concentrations are elevated in Boundary Creek suggesting a source for this metal in the catchment and its mobilisation under acidic conditions. At other sites elevated concentrations were found mostly during the Spring 2019 and Autumn 2021 surveys. These were times of higher flow (Table 2) and suggest a diffuse source in the catchment may be contributing the metal in runoff.

- **Table 4: Metal results (0.45µm filtered) for water samples (mg/L) and ANZECC water quality guidelines (2000, Table 3.4.1) for trigger values applying to typical *slightly–moderately disturbed systems.***

mg/L	Season	Aluminium	Antimony	Arsenic	Cadmium	Chromium	Copper	Iron	Lead	Manganese	Mercury	Selenium	Silver	Zinc
Site 1	Spring 19	< 0.05*	< 0.005	< 0.001	< 0.0002	< 0.001	< 0.001	0.33	< 0.001	0.04	< 0.0001	< 0.001	< 0.005	0.032
	Autumn20	< 0.05	< 0.005	< 0.001	< 0.0002	< 0.001	< 0.001	0.21	< 0.001	0.007	< 0.0001	< 0.001	< 0.005	< 0.005
	Summer21	0.06	< 0.005	< 0.001	< 0.0002	< 0.001	< 0.001	0.68	< 0.001	< 0.005	< 0.0001	< 0.001	< 0.005	< 0.005
	Autumn21	0.04	<0.001	<0.001	<0.0002	<0.001	<0.001	0.35	<0.001	0.046	<0.0001	<0.001	<0.001	0.002
Site 2	Spring 19	< 0.05*	< 0.005	< 0.001	< 0.0002	< 0.001	< 0.001	0.4	< 0.001	0.15	< 0.0001	< 0.001	< 0.005	0.008
	Autumn20	< 0.05	< 0.005	< 0.001	< 0.0002	< 0.001	< 0.001	0.08	< 0.001	0.037	< 0.0001	0.001	< 0.005	< 0.005
	Summer21	< 0.05	< 0.005	< 0.001	< 0.0002	< 0.001	< 0.001	0.22	< 0.001	0.006	< 0.0001	< 0.001	< 0.005	< 0.005
	Autumn21	0.01	<0.001	<0.001	<0.0002	<0.001	<0.001	0.19	<0.001	0.057	<0.0001	<0.001	<0.001	0.005
Site 3	Spring 19	< 0.05*	< 0.005	< 0.001	< 0.0002	< 0.001	< 0.001	0.31	< 0.001	0.31	< 0.0001	< 0.001	< 0.005	0.051
	Autumn20	< 0.05	< 0.005	< 0.001	< 0.0002	< 0.001	< 0.001	< 0.05	0.004	0.015	< 0.0001	< 0.001	< 0.005	< 0.005
	Summer21	< 0.05	< 0.005	< 0.001	< 0.0002	< 0.001	< 0.001	0.09	< 0.001	0.12	< 0.0001	< 0.001	< 0.005	< 0.005
	Autumn21	0.01	<0.001	<0.001	<0.0002	<0.001	<0.001	0.21	<0.001	0.035	<0.0001	<0.001	<0.001	0.006
Site 4	Spring 19	< 0.05	< 0.005	< 0.001	< 0.0002	< 0.001	< 0.001	0.33	< 0.001	0.15	< 0.0001	< 0.001	< 0.005	0.017
	Autumn20	< 0.05	< 0.005	< 0.001	< 0.0002	< 0.001	< 0.001	0.14	< 0.001	0.35	< 0.0001	< 0.001	< 0.005	< 0.005
	Summer21	<0.05	< 0.005	< 0.001	<0.0002	< 0.001	< 0.001	0.33	< 0.001	0.019	< 0.0001	< 0.001	< 0.005	< 0.005
	Autumn21	0.01	<0.001	<0.001	<0.0002	<0.001	<0.001	0.19	<0.001	0.06	<0.0001	<0.001	<0.001	0.002
Site 5	Spring 19	10*	< 0.005	< 0.001	0.0002	< 0.001	< 0.001	5.4	< 0.001	0.06	< 0.0001	< 0.001	< 0.005	0.34
	Autumn20	< 0.05*	< 0.005	< 0.001	< 0.0002	< 0.001	< 0.001	17	< 0.001	0.18	< 0.0001	< 0.001	< 0.005	0.015
	Summer21	7.2*	< 0.005	0.001	<0.0002	0.001	< 0.001	40	< 0.001	0.061	< 0.0001	0.001	< 0.005	0.23
	Autumn21	1.6*	<0.001	0.004	<0.0002	<0.001	0.005	50	<0.001	0.024	<0.0001	0.003	<0.001	0.08
Site 6	Spring 19	0.09	< 0.005	< 0.001	< 0.0002	< 0.001	< 0.001	0.13	< 0.001	0.17	< 0.0001	< 0.001	< 0.005	0.057

mg/L	Season	Aluminium	Antimony	Arsenic	Cadmium	Chromium	Copper	Iron	Lead	Manganese	Mercury	Selenium	Silver	Zinc
	Autumn20	< 0.05	< 0.005	< 0.001	< 0.0002	< 0.001	< 0.001	0.17	< 0.001	0.16	< 0.0001	< 0.001	< 0.005	< 0.005
	Summer21	<0.05	< 0.005	< 0.001	<0.0002	< 0.001	< 0.001	0.69	< 0.001	0.29	< 0.0001	< 0.001	< 0.005	< 0.005
	Autumn21	0.07	<0.001	<0.001	<0.0002	<0.001	<0.001	2	<0.001	0.069	<0.0001	<0.001	<0.001	0.012
	Spring 19	0.07	< 0.005	< 0.001	< 0.0002	< 0.001	< 0.001	0.15	< 0.001	0.08	< 0.0001	< 0.001	< 0.005	0.013
Site 7	Autumn20	< 0.05*	< 0.005	< 0.001	< 0.0002	< 0.001	0.001	0.09	< 0.001	0.01	< 0.0001	< 0.001	< 0.005	0.006
	Summer21	0.06	< 0.005	< 0.001	<0.0002	< 0.001	< 0.001	0.37	< 0.001	0.023	< 0.0001	< 0.001	< 0.005	< 0.005
	Autumn21	0.07	<0.001	<0.001	<0.0002	<0.001	<0.001	0.61	<0.001	0.072	<0.0001	<0.001	<0.001	0.009
	Spring 19	0.1	< 0.005	< 0.001	< 0.0002	< 0.001	0.001	0.23	< 0.001	0.066	< 0.0001	< 0.001	< 0.005	0.015
Site 8	Autumn20	< 0.05	< 0.005	< 0.001	< 0.0002	< 0.001	< 0.001	< 0.05	< 0.001	< 0.005	< 0.0001	< 0.001	< 0.005	< 0.005
	Summer21	<0.05	< 0.005	< 0.001	<0.0002	< 0.001	<0.001	0.24	< 0.001	<0.005	< 0.0001	< 0.001	< 0.005	< 0.005
	Autumn21	0.04	<0.001	<0.001	<0.0002	<0.001	<0.001	0.33	<0.001	0.031	<0.0001	<0.001	<0.001	0.005
	Spring 19	< 0.05	< 0.005	< 0.001	< 0.0002	< 0.001	< 0.001	0.22	< 0.001	0.098	< 0.0001	< 0.001	< 0.005	0.01
Site 9	Autumn20	< 0.05	< 0.005	< 0.001	< 0.0002	< 0.001	< 0.001	< 0.05	< 0.001	0.016	< 0.0001	< 0.001	< 0.005	< 0.005
	Summer21	<0.05	< 0.005	< 0.001	<0.0002	< 0.001	< 0.001	0.37	< 0.001	0.037	< 0.0001	< 0.001	< 0.005	< 0.005
	Autumn21	0.02	<0.001	<0.001	<0.0002	<0.001	<0.001	0.24	<0.001	0.038	<0.0001	<0.001	<0.001	0.004
	Spring 19	< 0.05	< 0.005	< 0.001	< 0.0002	< 0.001	< 0.001	0.22	< 0.001	0.09	< 0.0001	< 0.001	< 0.005	< 0.005
Site 10	Autumn20	< 0.05*	< 0.005	< 0.001	< 0.0002	< 0.001	< 0.001	< 0.05	< 0.001	0.027	< 0.0001	< 0.001	< 0.005	0.008
	Summer21	0.09	< 0.005	< 0.001	<0.0002	< 0.001	<0.001	0.6	< 0.001	0.045	< 0.0001	< 0.001	< 0.005	< 0.005
	Autumn21	0.02	<0.001	<0.001	<0.0002	<0.001	<0.001	0.25	<0.001	0.025	<0.0001	<0.001	<0.001	0.005
	Spring 19	< 0.05	< 0.005	< 0.001	< 0.0002	< 0.001	< 0.001	0.42	< 0.001	0.1	< 0.0001	< 0.001	< 0.005	< 0.005
Site 11	Autumn20	< 0.05*	< 0.005	< 0.001	< 0.0002	< 0.001	< 0.001	< 0.05	< 0.001	0.082	< 0.0001	< 0.001	< 0.005	< 0.005
	Summer21	0.16	< 0.005	<0.001	<0.0002	< 0.001	<0.001	1.4	< 0.001	0.028	< 0.0001	< 0.001	< 0.005	< 0.005
	Autumn21	0.03	<0.001	<0.001	<0.0002	<0.001	<0.001	0.18	<0.001	0.032	<0.0001	<0.001	<0.001	0.003
	Spring 19	0.07	< 0.005	< 0.001	< 0.0002	< 0.001	< 0.001	0.56	< 0.001	0.1	< 0.0001	< 0.001	< 0.005	< 0.005
Site 12	Autumn20	< 0.05	< 0.005	< 0.001	< 0.0002	< 0.001	< 0.001	< 0.05	< 0.001	0.044	< 0.0001	< 0.001	< 0.005	0.015
	Summer21	0.27	< 0.005	0.001	<0.0002	< 0.001	0.001	1.7	< 0.001	0.049	< 0.0001	< 0.001	< 0.005	< 0.005
	Autumn21	0.02	<0.001	<0.001	<0.0002	<0.001	<0.001	0.19	<0.001	0.03	<0.0001	<0.001	<0.001	<0.001
ANZECC		0.05 (>6.5pH) *ID (<6.5pH)	ID	0.013	0.0002	0.001	0.0014	ID	0.0034	1.2	0.00006	0.005	0.00005	0.008

ID= insufficient data *Shaded exceeds trigger values* **aluminium results where pH is <6.5*

3.4. Macroinvertebrates

Biotic indices such as AusRivAS, SIGNAL2, EPT (Ephemoptera, Plecoptera, Trichoptera) and taxa richness (number of families) scores were calculated in accordance with EPA Victoria biological objectives (EPA Victoria, 2004). EPA Victoria released their updated guidelines for the rapid bioassessment of rivers in 2021 which ties in with the Environmental Reference Standards (ERS, formally SEPP-Waters). The main change in the objectives contained within the ERS is that they are split into Seasons so that concurrent seasons do not need to be sampled in order for objectives to be applied. Previous work in the Murray Darling Basin has found no significant difference between seasons (EPA, 2021) although each project should be assessed for the appropriateness of single or dual season sampling. Other changes include removing SIGNAL scores and instead using SIGNAL2 scores. Also, individual edge sample scores are calculated and the average reported rather than combining families and reporting as a single sample when a riffle is not present.

A multi dimensional scaling (MDS) plot was also produced and SIMPER analysis to give an indication of how similar the macroinvertebrate community compositions are to each other. A list of macroinvertebrate families found at each site in Autumn 2021 is in Appendix 2.

AusRivAS scores and bands are considered to give the most accurate assessment of the health of a site as the program compares the test site to a number of reference sites that have similar physical and chemical characteristics but are relatively free of environmental impacts. The score indicates how many macroinvertebrate families were found compared to those found at reference sites. The statewide model for edge habitat over two seasons was applied to these samples.

- **Table 5: AusRivAS Bands, Observed/Expected scores and descriptions for Autumn and Spring edge models (AusRivAS Macroinvertebrate Predictive Modelling Version 3.2.2)**

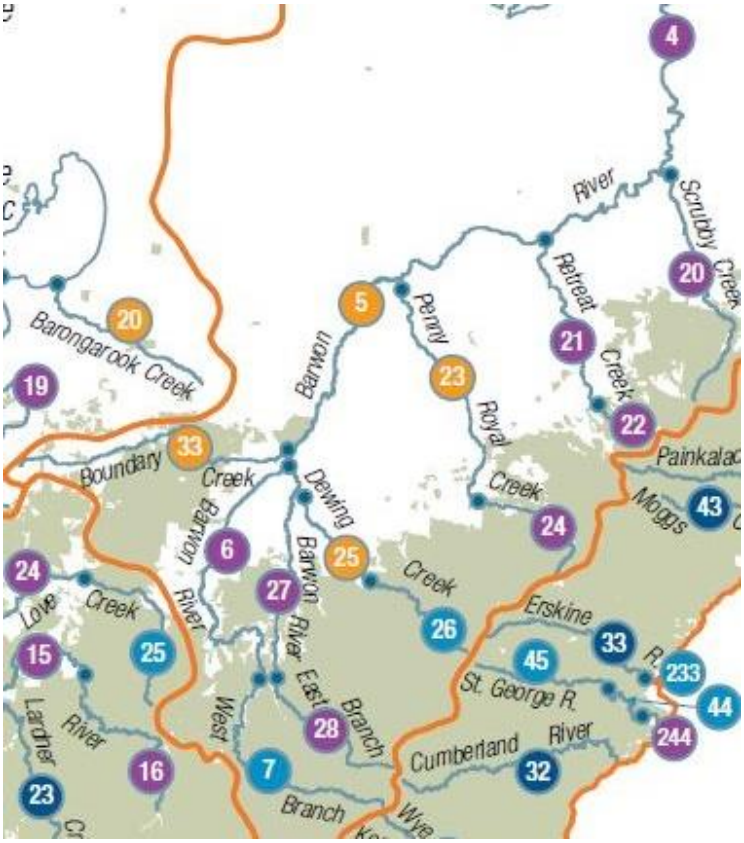
Band	OE 50 score		Description
	Autumn	Spring	
X	1.19+	1.19+	More biologically diverse than reference sites
A	0.81-1.18	0.81-1.18	Reference condition
B	0.42-0.80	0.43-0.80	Significantly impaired
C	0.03-0.41	0.05-0.42	Severely impaired
D	0-0.02	0-0.02	Extremely impaired

SIGNAL2 is a biotic index based on the tolerance or intolerance of biota (macroinvertebrates) to water pollution. Sites with high scores are likely to have low nutrient, salinity and turbidity levels and high oxygen levels but its accuracy in identifying toxicants is less certain (EPA, 2021).

The EPT score indicates the number of families that are sensitive to pollution that are present at the site with a low score usually indicating that there has been some type of disturbance. Together, these scores give a good picture of the health of the waterway at a site and potentially what is causing any disturbance.

Taxa richness, measured by the number of macroinvertebrate families collected, can give a good overview of the health of a waterway. High numbers are associated with diverse habitats present at the site but can also be influenced by mild nutrient enrichment which can increase the food supply. The score can be combined with SIGNAL2 scores as in Figure 2 to help interpret results.

Every six years the Department of Environment and Primary Industries conducts Index of Stream Condition (ISC) assessments using information collected from a range of sources to give a benchmark of river condition in Victoria. The five sub-indices measured are Hydrology, Physical Form, Streamside Zone, Water quality and Aquatic Life and are reported in reaches (10-30km river lengths with similar characteristics). Figure 2 has been taken from the latest Index of Stream Condition data and shows the extent of the reaches in the study area. Table 7 identifies which sites are in each reach. Aquatic Life sub-index is a score out of 10 and is calculated by adding three Aquatic Life indicator scores according to the formula found in the Aquatic Life sub-index fact sheet (DEPI, 2014). The Aquatic Life sub-index was included in this report as it was included as a target in the discussion paper by Baldwin (2018).



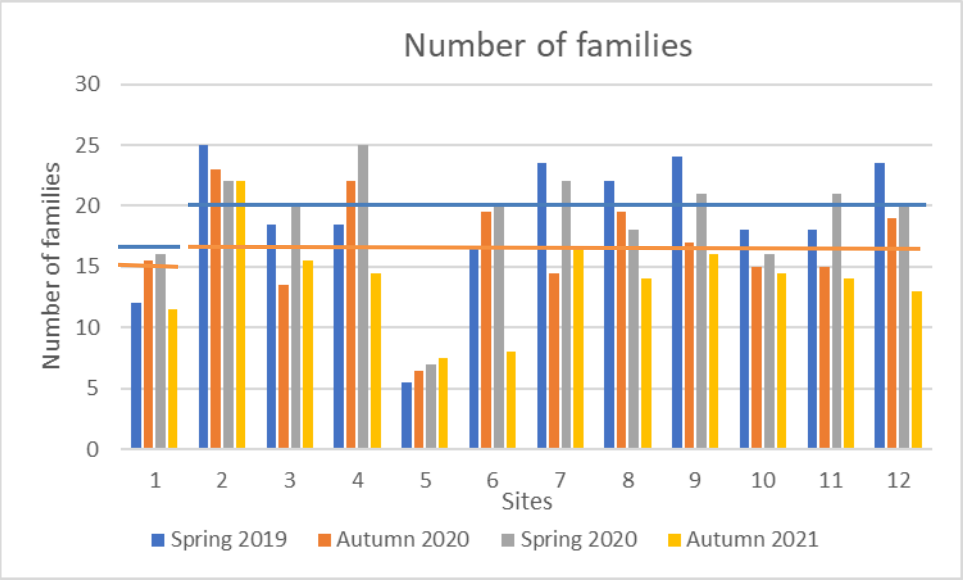
- **Figure 8: ISC reaches in the upper Barwon catchment. Taken from Index of Stream Condition ISC3 Corangamite Region. https://www.water.vic.gov.au/water-reporting/third-index-of-stream-condition-report**

Care should be taken when comparing Aquatic Life scores between reaches as whilst it is anticipated that reaches are similar along their length, sites can vary in their quality, influenced by riparian and instream vegetation condition, inputs by tributaries and stock access. The sites and the year sampled to form the ISC3 Aquatic Life score is also unknown. Aquatic Life scores for this study are in Table 7 below.

The study area crosses two biological regions. Site 1 (East Barwon River at Yaugher) is in Uplands B; characterised by upland reaches in the Otway Ranges where there is some clearing for forestry, grazing and some intensive agriculture. Sites 2 to 12 are in Central Foothills and Coastal Plains; incorporating the lower reaches of the Barwon River where the region has been substantially cleared for intensive agriculture (EPA, 2004).

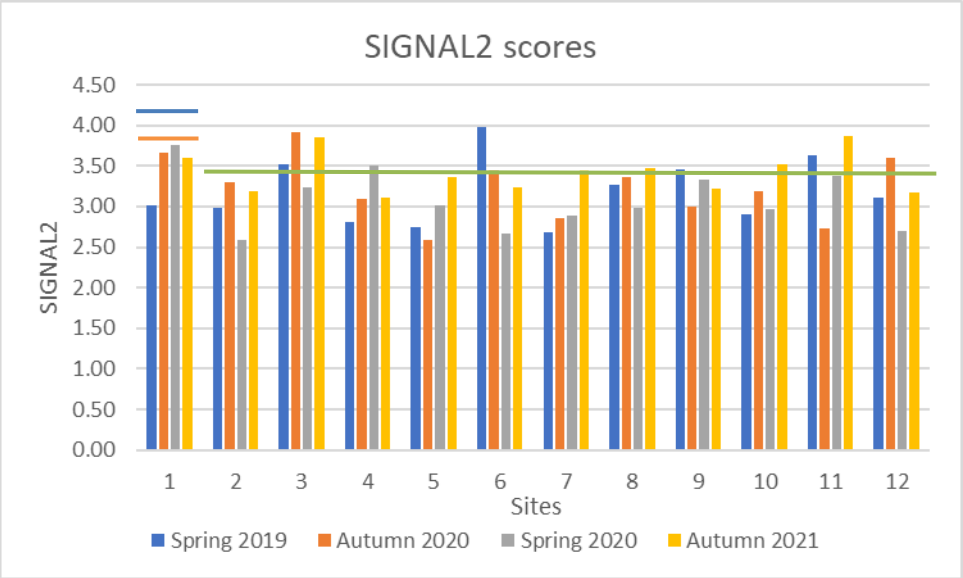
- **Table 6: Environmental Quality Objectives for Biological Indicators, edge habitat (EPA, 2021)**

Objective	Season	Number of Families	SIGNAL2 Index score	EPT Index score	AusRivAS Band
Uplands B	Autumn	15	3.8	N/A	A
	Spring	17	4.2	6	A
Central Foothills & Coastal Plains	Autumn	17	3.4	N/A	A
	Spring	20	3.4	N/A	A



- **Figure 9. Number of families found in edge habitats in the Barwon River and Boundary Creek. Blue line denotes Spring objective and orange line denotes Autumn objective.**

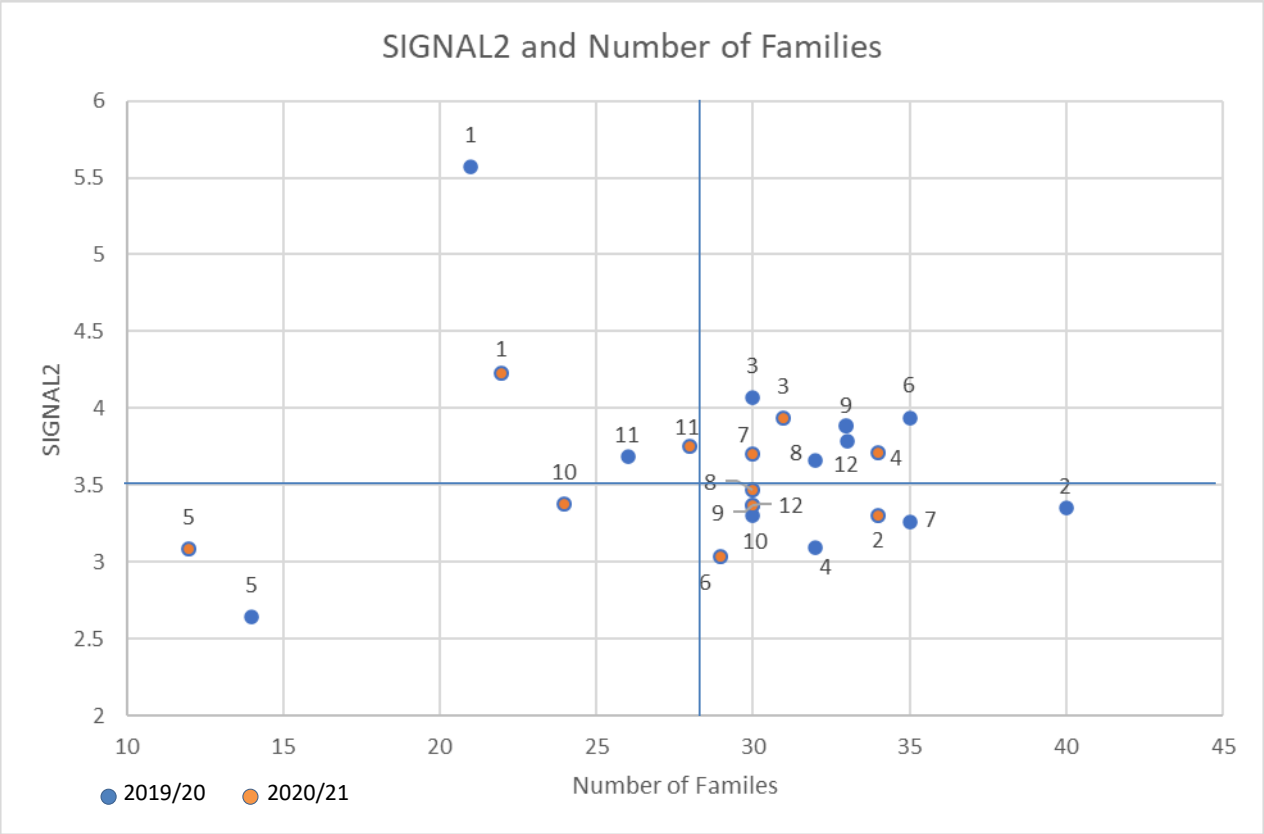
Figure 9 shows that the East Barwon River at Dewings Bridge Road (Site 2) is the only site to consistently meet the objectives for Number of Families. Site 6 immediately below the Boundary Creek confluence shows the greatest change in macroinvertebrate diversity in Autumn between years, most likely due to the impact on available habitat from the presence of iron floc on the aquatic vegetation and substrate (see site description in Appendix 1).



- **Figure 10. SIGNAL2 index scores from edge habitats in the Barwon River and Boundary Creek. Blue line denotes Spring objective and orange line denotes Autumn objective for Uplands B objectives and green line denotes both Spring and Autumn objective for Central Hills and Coastal Plains objective.**

Figure 10 shows that none of the sites consistently meet the objectives for SIGNAL2 but Site 3 on the West Barwon River at Seven Bridges Road has met objectives during three of the four sampling events. Five sites met objectives during the latest sampling event, at sites 3, 7, 8, 10 and 11.

Whilst SIGNAL2 scores give an indication of water quality in the river from which the sample was collected, combining the score with the richness score (how many different macroinvertebrate families are present), can provide an indication of the types of pollution and other physical and chemical factors that are affecting the macroinvertebrate community. This is shown in the plot in Figure 3 where quandrant boundaries are defined according to Chessman (2003) with the top right quadrant (Quadrant 1) containing the healthiest sites. As all sites are subject to human disturbance, those sites that met or were close to meeting EPA biological objectives for number of families and SIGNAL scores were included in Quadrant 1 and a cross check of which sites had the most EPT families (Table 5) confirmed the quadrant borders using 2019/20 data.

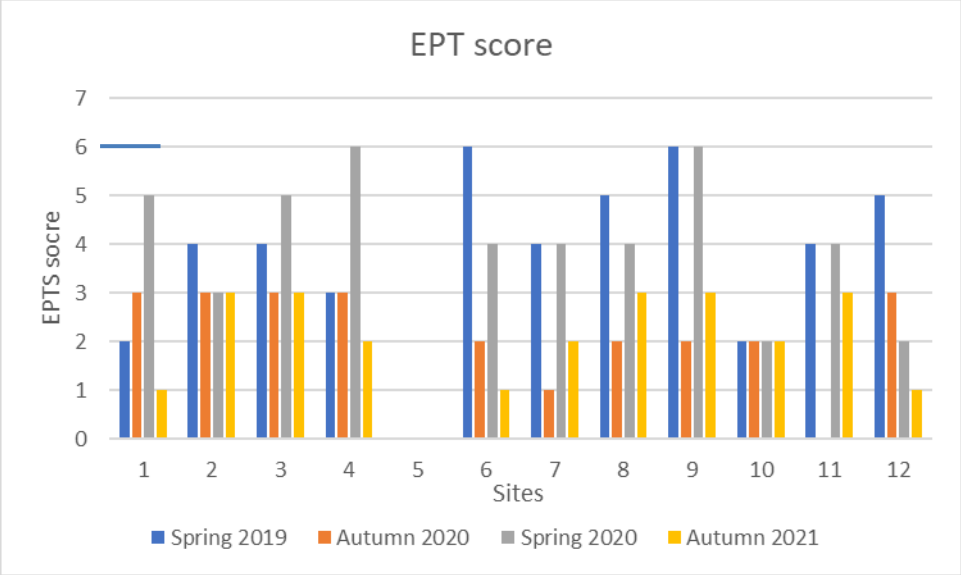


- **Figure 11. SIGNAL2 index plotted against number of families recorded for each site combined over season for two years.**

Figure 11 above shows that Site 3 (West Barwon Rv) has the highest number of families and SIGNAL2 scores for the two sampled years and Site 5 had the lowest number of families and SIGNAL2 scores over the two years. Site 4 and Site 11 recorded improved SIGNAL2 and number of families from 2019/20 to 2020/21. Sites 2, 6, 7, 9, and 12 recorded reduced number of families and SIGNAL2 scores between 2019/20 to 2020/21 with Site 6 (Barwon Rv immediately d/s of Boundary Ck) recording the largest decrease from the highest number of families and SIGNAL2 score in 2019/20 to one of the lowest SIGNAL2 scores in 2020/21.

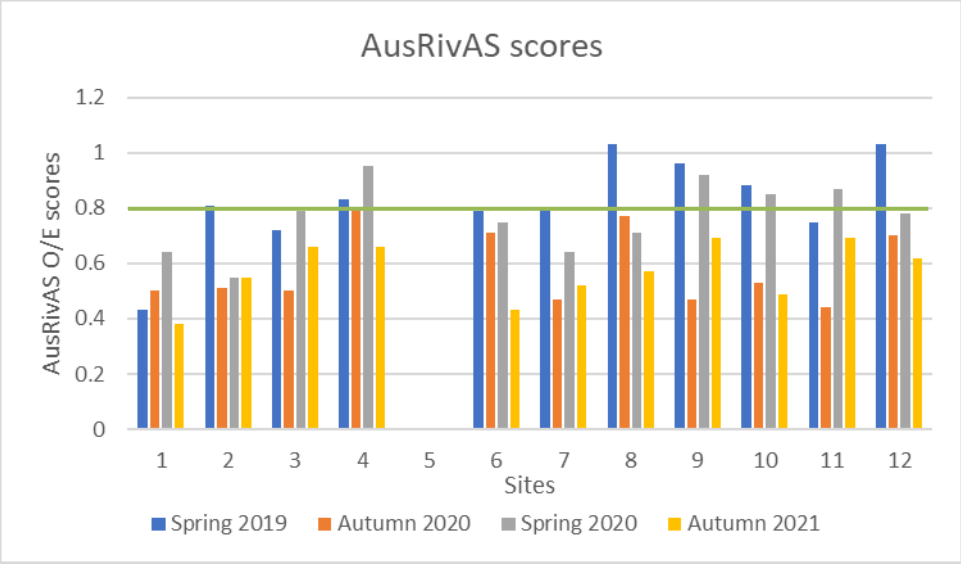
In 2020/2021, Site 3 (West Barwon Rv), Site 4 (upstream of Boundary Ck confluence) and Site 7 (Barwon Rv u/s Colac- Lorne Rd) have high SIGNAL2 and number of macroinvertebrate families suggesting the habitat and water quality are favorable and stress factors are low. Site 2 (East Barwon Rv), Site 6 (Barwon Rv immediately d/s of Boundary Ck), Site 8 (Colac-Lorne Road), Site 9 (Birregurra) and Site 12 (Winchelsea) in the bottom right quadrant have slightly lower SIGNAL2 scores, possibly due to water quality influences but the high number of families present suggest that any toxicants are not present in large amounts. Sites 11 (Barwon Rv above Winchelsea) and 1 (East Barwon Rv, Yaugher) in the top left quadrant have high SIGNAL2 scores but fewer number of families. These sites are possibly affected by pollution other than what SIGNAL scores are based on (organic, nutrient enrichment or salinity). Site 5 (Boundary Creek) and Site 10 have lower SIGNAL2 scores and low numbers of families suggesting that they are subject to a number of impacts such as low pH and high metal concentrations (Site 5) or other industrial type pollution.

The pollution sensitive Ephemeroptera, Plecoptera and Tricoptera (EPT) macroinvertebrate families are seldom found in waterways within the Cleared Hills and Coastal Plains region, therefore no objectives have been set but have been reported in Figure 12 below.



- **Figure 12. EPT (Ephemoptera, Plecoptera, Tricoptera families) index scores from edge habitats in the Barwon River and Boundary Creek. There is only a Spring objective for Uplands B objective (blue line).**

Whilst the number of pollution sensitive taxa present (EPT score) does not have an objective for many of the sites in this study, Figure 12 shows that sites such as Site 9 at Birregurra have good numbers of these taxa present in Spring.



- **Figure 13. AusRivAS observed vs expected (O/E) scores from edge habitats in the Barwon River and Boundary Creek. Green line denotes both Spring and Autumn objective (Band A).**

Site 4 immediately upstream of the Boundary Creek confluence meets AusRivAS objectives on three of the four sampling occasions suggesting that the macroinvertebrates present are not adversely impacted by water quality or habitat condition. Site 5 on Boundary Creek is always outside the experience of the model, presumably due to the unusually low alkalinity at this site. Sites usually

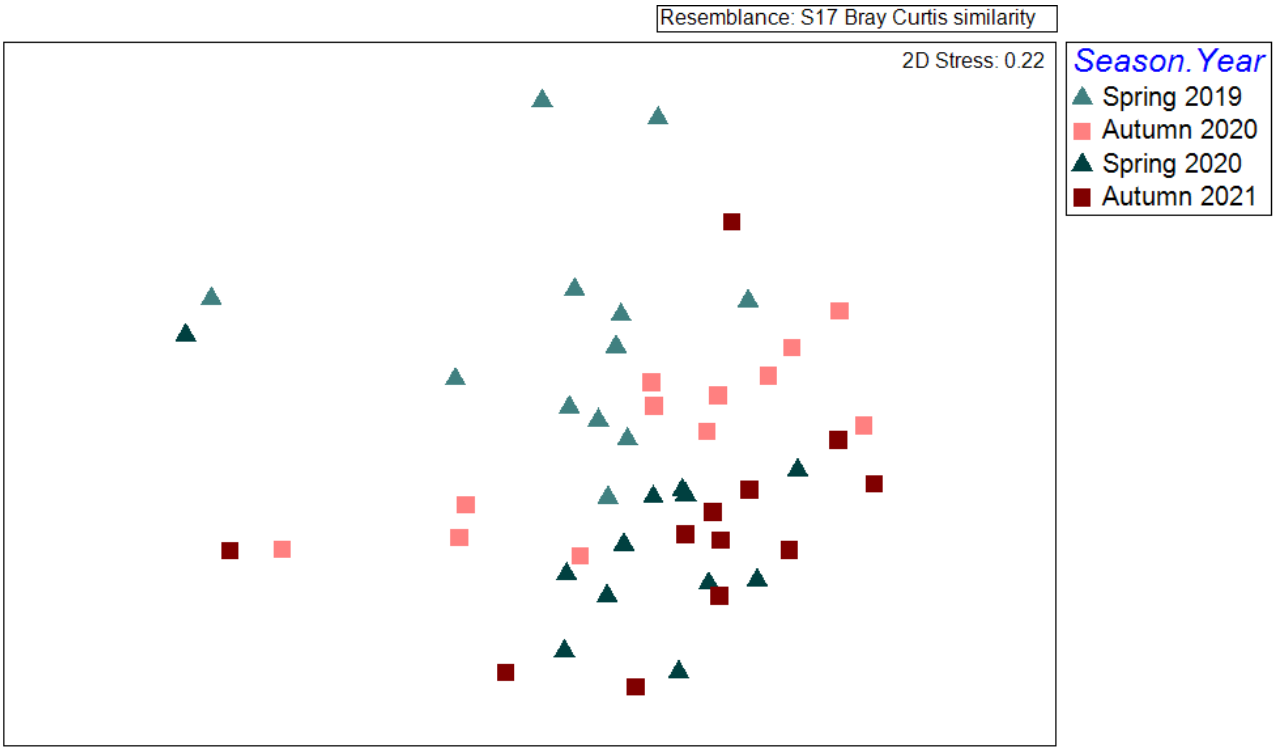
record better AusRivAS scores in Spring than in Autumn with sites 9 and 10 meeting objectives during the two Autumn sampling events.

- **Table 7. Aquatic Life scores comparing latest Index of Stream Condition (ISC) results (DEPI, 2019) to those collected during this study.**

ISC reach	Study sites	Aquatic Life subindex		
		ISC3 (2004-2010)	2019/2020	2020/2021
6	1, 3	9	6	6
27	2, 4	6	7	7
33	5	4	2	0
5	6, 7, 8, 9, 10	6	8	6.4
4	11, 12	8	7	7

The Aquatic Life score for each ISC reach was calculated by averaging the scores recorded at each site (Table 7). As this score combines a number of biotic indices over a number of sites along the reach, it is not considered to be particularly useful in detecting the smaller scale impacts of Boundary Creek on the Barwon River.

The MDS (Multidimensional Scaling) plot (Figure 14) shows how similar, or dissimilar, the macroinvertebrate community compositions at each site are to one another.



- **Figure 14: MDS plot of families over two seasons and two years at twelve sites.**

The Multi Dimensional Scaling plot in Figure 14 shows some seasonal effect on the macroinvertebrate community composition with Site 5 being the outlier to the left of the other sites. Sites in Spring 2020 and Autumn 2021 appear more similar to each other than with other sampling events, confirmed by SIMPER (Similarity Percentages) that calculated 37% similarity between those

sampling events. SIMPER calculated Spring 2019 and Autumn 2021 macroinvertebrate community compositions were the least similar to each other (27%).

3.5. Discussion

Boundary Creek water quality remains poor. Acidic waters are common under low flow conditions and can be associated with low dissolved oxygen concentrations. Both these parameters show a deterioration after the year 2000. In addition, high concentrations of aluminium and iron have the potential to form insoluble flocs which can smother vegetation and clog gills of aquatic organisms. Zinc also exceeds the ANZECC guidelines and is mobilised within the catchment probably in response to acidic conditions. Predictably, macroinvertebrate community composition continues to be poor at the only site on Boundary Creek (Site 5) with no families from the pollution sensitive Ephemoptera, Plecoptera and Trichoptera orders collected during the last four sampling events and consistently low number of families present.

The effects on Barwon River's water quality from Boundary Creek inflows are of very limited downstream effect. The buffering capacity of the river combined with its larger discharge mean pH and conductivity show little difference at sites above and below the confluence of the two streams (ie mixing and dilution are efficient). Overall, stream health as measured by macroinvertebrate community composition is good downstream of the confluence, with sites identified as reference condition (AusRivAS Band A) at most downstream sites at least once in the last two years.

During the Autumn 2021 survey, the presence of iron flocs at Site 6 immediately downstream of Boundary Creek show the potential for iron rich waters to enter the Barwon from Boundary Creek – either as groundwater or surface flows. This floc most likely contributed to the reduction in the quality and quantity of macroinvertebrates present at Site 6 but the impact was not evident at Site 7, 1.8km further downstream.

Elsewhere along the Barwon River conductivity rises abruptly downstream of Site 8 indicating a salt source in regional groundwater and/ or surface runoff. Lower pH and DO concentrations have been observed at different sites on various surveys. The reasons for them cannot be determined within the scope of this study. Whilst sites along the Barwon River do not consistently meet environmental quality objectives, overall indicator scores such as AusRivAS have been met over the last two years at sites downstream of the Boundary Creek confluence, reflecting the ability of the River to absorb catchment impacts on water quality or habitat condition. Comparison

3.6. Recommendations

Continued sampling of metals in the water along the Barwon River during the Boundary Creek remediation works should give an indication of whether they are being mobilised by the low pH water coming into the system. If not required by the remediation plan, those metals that have consistently been below detectable levels could be removed from the sampling program although this would be unlikely to have any cost benefit. Whilst best practice in the updated biological sampling guidelines and associated objectives is for only one season to be sampled, the variability seen in macroinvertebrate community composition between seasons suggests that annual macroinvertebrate sampling in Autumn and Spring during the Boundary Creek remediation works will give the most accurate assessment of the health of the Barwon River. The number of sites sampled could be reduced to Site 4 (above the confluence with Boundary Creek), Site 5 (Boundary Creek) and sites 6, 7, 8 and 9 (Birregurra) if required as any impact on the Barwon River from Boundary Creek past this point has not been observed during the past two years of surveys.

4. References

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Leon Metzling, EPA Victoria (12 August, 2019) pers. comm. Updated EPA sampling protocols.

Appendix 1:

4.1. Site 1- East Barwon River@ Kents Road


Site 1: upstream Spring 2019


Site 1: downstream Spring 2019


Site 1: upstream Autumn 2020


Site 1: downstream Autumn 2020


Site 1: upstream Spring 2020


Site 1: upstream Spring 2020

Site 1: upstream Autumn 2021

Site 1: downstream Autumn 2021

The East Barwon at Kents Road has diverse habitat with large deep pools and some riffle/ run areas. These runs had dried to trickles in Autumn 2020 but were flowing well in Spring and Autumn 2021. The average stream width is eight meters and bank full but had contracted to five meters in Autumn 2020. Willows dominate the riparian zone and are growing within the stream channel. The substrate is a mix of clay and silt with a number of aquatic macrophytes growing in the margins and shallow pool areas. The majority of the riparian zone is exotic vegetation, dominated by blackberries (possibly poisoned in Summer 20/21), willows and pasture grass. One larval fish was collected as bycatch during macroinvertebrate sampling. A concurrent snapshot study by EnviroDNA (2019) found evidence of platypus at this site. Overall analysis of the health of the waterway using EPA habitat parameters for Low Gradient Streams gives this site a score of 74 out of 140.

4.2. Site 2- East Barwon River@ Dewings Bridge Road


Site 2: upstream Spring 2019


Site 2: downstream Spring 2019


Site 2: upstream Autumn 2020


Site 2: downstream Autumn 2020


Site 2: upstream Spring 2020


Site 2: downstream Spring 2020

Site 2: upstream Autumn 2021

Site 2: downstream Autumn 2021

The East Barwon at Dewings Bridge Road consists of a slow flowing channel with extensive backwaters. There is very little riparian zone present but a number of submerged and emergent macrophytes provide good habitat for macroinvertebrates and fish. One larval fish was found in the sample net in Autumn 2020 and three pygmy perch in Spring 2020. The substrate is a mix of clay and silt with some sand. The average stream width at this site was seven meters and was bank full during both Spring and Autumn sampling. The majority of the riparian zone is pasture grass with stock access on both sides. Overall analysis of the health of the waterway using EPA habitat parameters for Low Gradient Streams gives this site a score of 59 out of 140.

4.3. Site 3- West Barwon River@ Seven Bridges Road


Site 3: upstream Spring 2019


Site 3: downstream Spring 2019


Site 3: upstream Autumn 2020


Site 3: downstream Autumn 2020


Site 3: upstream Spring 2020


Site 3: downstream Spring 2020

Site 3: upstream Autumn 2021

Site 3: downstream Autumn 2021

The West Barwon River at Seven Bridges Road has large deep pools with a number of large deep backwaters. The average stream width at this site is seven meters, narrow at the top of the surveyed reach and widening into a large pool near the bridge. The substrate is clay and silt mixed with 20% sand. There are some macrophytes present along with trailing bank vegetation, roots and instream large woody debris (primarily willow branches). Willows dominate the riparian zone a mix of shrubs and native and pasture grasses in the understory. Four larval fish were collected as bycatch during macroinvertebrate sampling in Spring 2019. One mountain galaxias, one common galaxias, one pygmy perch and a laval galaxias were collected in Spring 2020. A concurrent snapshot study by EnviroDNA (2019) found evidence of platypus at this site in Spring 2019. Overall analysis of the health of the waterway using EPA habitat parameters for Low Gradient Streams gives this site a score of 85 out of 140.

4.4. Site 4- Barwon River upstream of Boundary Creek confluence


Site 4: upstream Spring 2019


Site 4: downstream Spring 2019


Site 4: upstream Autumn 2020


Site 4: downstream Autumn 2020


Site 4: upstream Spring 2020


Site 4: downstream Spring 2020

Site 4: upstream Autumn 2021

Site 4: downstream Autumn 2021

The Barwon River immediately upstream of the Boundary Creek confluence is a large slow flowing channel with shallow side sections that support a number of macrophyte beds. The average stream width at this site is nine meters. The substrate is clay and black silt with some large woody debris and filamentous algae present in addition to the macrophytes. *Juncus, Typha, Triglochin* and *Polygonum* species are all present instream though riparian vegetation is limited to some isolated trees, a narrow native plantation and pasture grass with stock access. The introduced Gambusia (mosquito fish) were collected as bycatch during macroinvertebrate sampling in Spring 2019 and in Autumn 2021. Overall analysis of the health of the waterway using EPA habitat parameters for Low Gradient Streams gives this site a score of 79 out of 140.

4.5. Site 5- Boundary Creek @ Colac- Forrest Road

Site 5: upstream Spring 2019

Site 5: downstream Spring 2019

Site 5: upstream Autumn 2020

Site 5: downstream Autumn 2020

Site 5: upstream Spring 2020

Site 5: downstream Spring 2020

Site 5: upstream Autumn 2021

Site 5: downstream Autumn 2021

Boundary Creek at Colac- Forrest Road has a mix of large deep pools, a large shallow pool at the bridge and shallow runs. It has been bankfull with an average stream width of four meters, narrow at the top of the surveyed reach and widening into a large pool upstream of the bridge during each sampling event excepting during Autumn 2020 sampling when the creek had contracted to a pool approximately 4 meters long by 2.5 meters wide. During this time the pooled water was stagnant, with low oxygen concentrations and very high turbidity. The substrate is a mix of cobble, pebble, gravel, sand, clay and silt. There are no macrophytes but there was some filamentous algae in Spring 2019, (absent since) and trailing bank vegetation present. Foam was present at the top of the reach in Spring 2020 and Autumn 2021. The riparian zone is wide and a mix of native and exotic vegetation except the ground cover which is dominated by *Convolvulus* sp. and pasture grasses. Overall analysis of the health of the waterway using EPA habitat parameters for Low Gradient Streams gives this site a score of 81 out of 140.

4.6. Site 6- Barwon River downstream of Boundary Creek confluence


Site 6: upstream Spring 2019


Site 6 : downstream Spring 2019


Site 6: upstream Autumn 2020


Site 6: downstream Autumn 2020


Site 6: upstream Spring 2020


Site 6 : downstream Spring 2020

Site 6: upstream Autumn 2021

Site 6: downstream Autumn 2021

Floc on aquatic vegetation in Autumn 2021

The Barwon River immediately downstream of the Boundary Creek confluence is a narrow deep channel with wide shallow edges dominated by grasses and aquatic macrophytes. The average stream width at this site is five meters and was bank full in Spring 2019, Spring 2020 and Autumn 2021. There is a narrow channel at the top of the surveyed reach, narrowing to a confined channel downstream. The river had contracted to the main channel but remained flowing, leaving the fringes to dry out in Autumn 2020. The substrate consists of clay and silt usually with filamentous algae tangled through the macrophyte beds but in Autumn 2021 the macrophyte beds were covered in a smothering floc was easily disturbed, forming a thick plume (see photos above). Macrophyte species are varied with *Triglochin, Polygonum, Phragmites,* and *Juncus* species all present in addition to trailing grasses. Three different fish species were collected at this site as bycatch; southern pygmy perch, smelt and a galaxid in Autumn 2020, and gambusia and southern pygmy perch in Spring 2020. The riparian zone is limited to grasses and scattered native trees and shrubs with stock access to the site. Overall analysis of the health of the waterway using EPA habitat parameters for Low Gradient Streams gives this site a score of 70 out of 140.

4.7. Site 7- Barwon River upstream of CO_BAR16


Site 7: upstream Spring 2019


Site 7: downstream Spring 2019


Site 7: upstream Autumn 2020


Site 7: downstream Autumn 2020


Site 7: upstream Spring 2020


Site 7: downstream Spring 2020

	
Site 7: upstream Autumn 2021	Site 7: downstream Autumn 2021

The Barwon River upstream of CO_BAR16 adjacent to the northern boundary of the pine plantation has a large deep channel with any shallow areas dominated by beds of Phragmites. The average stream width at this site is seven meters. The substrate is clay and silt. In addition to the Phragmites beds there are beds of Triglochin, and scattered Polygonum, Juncus and other grasses. The riparian zone has a good mix of trees, shrubs and understory with a majority of native trees and shrubs. Overall analysis of the health of the waterway using EPA habitat parameters for Low Gradient Streams gives this site a score of 90 out of 140.

4.8. Site 8- Barwon River @ Colac- Lorne Road


Site 8: upstream Spring 2019


Site 8: downstream Spring 2019


Site 8: upstream Autumn 2020


Site 8: downstream Autumn 2020


Site 8: upstream Spring 2020


Site 8: downstream Spring 2020

Site 8: upstream Autumn 2021

Site 8: downstream Autumn 2021

The Barwon River at Colac- Lorne Road has large deep pools with a shallow areas at the sides and willow trees growing in the channel and some substrate exposed. The average stream width at this site is eight meters with a predominantly clay and silt substrate mixed with some sand. There are beds of *Triglochin* and *Phragmites* in addition to trailing grasses and large willows. The riparian zone consists of willow trees, pasture grasses and blackberries and allows stock access. Overall analysis of the health of the waterway using EPA habitat parameters for Low Gradient Streams gives this site a score of 69 out of 140.

4.9. Site 9- Barwon River @ Birregurra


Site 9: upstream Spring 2019


Site 9: downstream Spring 2019


Site 9: upstream Autumn 2020


Site 9: downstream Autumn 2020


Site 9: upstream Spring 2020


Site 9: downstream Spring 2020

Site 9: upstream Autumn 2021

Site 9: downstream Autumn 2021

The Barwon River at Birregurra consists of a large deep slow flowing pool. The average stream width at this site is five meters with steep clay banks. The substrate is clay and silt with willow roots, some snags and *Triglochin* beds scattered along the edges of the channel. There were recent willow removal works and replanting of the riparian zone in Spring 2019 in amongst the pasture grass and blackberry groundcover. The riparian zone is now growing well (Autumn 2021) although follow up ground cover weed management may be required. Rakali footprints were evident in the soft sediment edge in Spring 2019. Overall analysis of the health of the waterway using EPA habitat parameters for Low Gradient Streams gives this site a score of 67 out of 140.

4.10. Site 10- Barwon River @ Conns Lane

Site 10: upstream Spring 2019

Site 10: downstream Spring 2019

Site 10: upstream Autumn 2020

Site 10: downstream Autumn 2020

Site 10: upstream Spring 2020

Site 10: downstream Spring 2020

	
Site 10: upstream Autumn 2021	Site 10: downstream Autumn 2021

The Barwon River at Conns Lane has large deep pools with some small deep backwaters and a narrow deep run at the top of the reach. The average stream width at this site is six meters. The substrate is clay and silt mixed with some sand and gravel. Phragmites beds line the channel and there are isolated patches of *Triglochin* in addition to *Polygonum* and trailing grasses along the waters edge. The trailing and shallow vegetation contained filamentous algae in Autumn 2021. Larval fish and gambusia were collected in the macroinvertebrate net in Spring 2020 and gambusia were plentiful in Autumn 2021. The riparian zone consists of relatively new and older native revegetation with pasture grass understory. Overall analysis of the health of the waterway using EPA habitat parameters for Low Gradient Streams gives this site a score of 98 out of 140.

4.11. Site 11- Barwon River@ Winchelsea- Deans Marsh Road

Site 11: upstream Spring 2019

Site 11: downstream Spring 2019

Site 11: upstream Autumn 2020

Site 11: downstream Autumn 2020

Site 11: upstream Spring 2020

Site 11: downstream Spring 2020

	
Site 11: upstream Autumn 2021	Site 11: downstream Autumn 2021

The Barwon River at Winchelsea- Deans Marsh Road has large deep pools with a shallow run at the top of the reach. The average stream width at this site is five meters and the substrate is clay and silt mixed with some sand and gravel. *Triglochin* is growing in the shallow areas of the channel and there are roots, large woody debris and trailing grasses. The riparian zone is predominately native trees and understory with a mix of grasses as groundcover. Rakali footprints were spotted at the waters edge in Spring 2019. Overall analysis of the health of the waterway using EPA habitat parameters for Low Gradient Streams gives this site a score of 90 out of 140.

4.12. Site 12- Barwon River @ Winchelsea

Site 12: upstream Spring 2019

Site 12: downstream Spring 2019

Site 12: upstream Autumn 2020

Site 12: downstream Autumn 2020

Site 12: upstream Spring 2020

Site 12: downstream Spring 2020

Site 12: upstream Autumn 2021

Site 12: downstream Autumn 2021

The Barwon River at Winchelsea has large deep pools with a large shallow pool at the top of the reach. The average stream width at this site is twelve meters. The substrate is clay and silt mixed with sand and some gravel. In addition to the Phragmites beds at the top of the reach and along some edges there are also patches of *Triglochin*. Large woody debris, trailing grasses and emergent vegetation such as *Polygonum* are also present. Riparian vegetation is predominantly native with many established eucalypts and groundcover is pasture grass. A concurrent snapshot study by EnviroDNA (2019) in Spring found evidence of platypus at this site. Overall analysis of the health of the waterway using EPA habitat parameters for Low Gradient Streams gives this site a score of 88 out of 140.

Appendix 2:

Macroinvertebrate families collected in Autumn 2021

	1	2	3	4	5	6	7	8	9	10	11	12
Aeshnidae		2						1				
Atyidae	5	9		12		12	32		27	24	15	10
Baetidae		4		2				9			27	
Caenidae		1										
Ceinidae	7	13		15			14	2	15	57	2	23
Ceratopogonidae					1							
Chironominae	75	13	11	26	34	8	8	19	33		3	3
Coenagrionidae		5					3	1	6	1		
Conoesucidae			8									
Corduliidae								1				
Corixidae	1	51	4	28	1	14	30	50	45	18	25	38
Crambidae		1										
Culicidae			1		34	3						
Dixidae	1	3	42	3			1			1		1
Dugesiidae		1	1				8		6	21		
Dytiscidae	3	9	4	1	4				1			2
Glossiphoniidae		3	1	1								
Gripopterygidae									1		13	
Gyrinidae		1		1			5	2		2	6	1
Hydrobiidae	15											
Hydrobiosidae								1				
Hydrophilidae		3		1					1			
Hydroptilidae	1		19				2	1	19	8		
Janiridae							1			2		
Leptoceridae	1	37	42	61		2	28	24	17	1	8	50
Leptophlebiidae			6				1		1	2		
Lymnaeidae				1		4	5	1	3	1		
Mites	2	1	22	1		3	2		1	26	35	37
Naucoridae		1		2					1			1
Notonectidae		2	1	5			10	4	10		3	7
Notonemouridae			1									
Oligochaeta	4	12	2		2			3	4	2		1
Orthocladiinae	7	24	3	17		17	2	7	3	8	6	1
Paramelitidae		3	1				6	1		3	4	1
Perthiidae		2					3					
Physidae	24	14	23	3			18	34	19	10	16	17
Planorbidae			4	2		1						
Scirtidae			1		26							
Simuliidae	5							17			15	
Sphaeriidae		1										
Stratiomyidae								1	1		1	1

	1	2	3	4	5	6	7	8	9	10	11	12
Tanypodinae	1	2	3									2
Telephlebiidae			1									
Tipulidae					3		1					
Veliidae			16	1	5		3	1	5	1	2	1