

1 **Community structure and ecological responses to hydrological changes**
2 **in benthic algal assemblages in a regulated river: Application of algal**
3 **metrics and multivariate techniques in river management**

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22

23 **Abstract**

24 The flow regime of the Wimmera River was substantially modified due to the
25 construction of a water supply reservoir. Samples of diatoms, soft algae and
26 measurements of water quality were analysed at ten sampling sites for three years
27 (between February 2012 and November 2014) along the MacKenzie River, a tributary of
28 the Wimmera River, in different seasons and under different flow regimes, to understand
29 the spatial and temporal variation in the relationship between algal communities, water
30 quality and stream condition. Baseline information on algal communities and water
31 quality was collected during base flow conditions, while experiments on the effect of
32 water releases on algal communities were based on flow regime variations (manipulated
33 flow regimes), specifically on the algae community structure, water quality and
34 ecosystem function. Algal species composition changed along the river under different
35 flow regimes and different seasons. Under base flow, Bacillariophyta (diatoms) were
36 more abundant upstream and filamentous green algae were more abundant downstream.
37 The results showed that the algal composition shifted downstream after water release
38 events. Chlorophyta (Green algae), Cyanophyta (Blue-green algae) and Chrysophyta
39 gradually increased from upstream to downstream under base flow conditions, and
40 before water releases, whereas diatoms were greater upstream and increased
41 downstream after water releases. The results suggest that by tailoring the discharge and
42 duration of the river flows, through the amalgamation of consumptive and
43 environmental flows would improve the condition of the stream, and supplementing the
44 positive effects of the flows dedicated to improving environmental outcomes.

45

46 **Keywords:** River management; Ecological assessment; Algae; Flow regime; Aquatic
47 ecology

48 **Introduction**

49 The main challenge for river scientists and water managers is to keep rivers healthy
50 whilst sustaining productive industries and communities. To achieve this, the in-stream
51 flow recommendations used by water management agencies, and their acceptance by
52 regional communities, is related to an understanding of the interdependencies of
53 management actions and ecosystem health (Ryder et al. 2010, Bunn 2016, Atazadeh
54 2017, Atazadeh et al. 2020). Many terms such as environmental water allocations
55 (EWAs), environmental flows (E-flows), ecological and environmental water
56 requirements (EEWRs), ecological water demands, in-stream flow and environmental
57 water consumption are widely used by aquatic ecologists to refer to the flows that
58 maintain and preserve ecological and biophysical characteristics of rivers (Acreman and
59 Dunbar 2004, Arthington 2012, Atazadeh et al. 2012, Atazadeh et al. 2014a, Zeiringer
60 et al. 2018). The concept of the environmental flow regime has been accompanied by an
61 expectation, and prediction, that ecologists can provide environmental flow
62 prescriptions that sustain and improve the condition of riverine ecosystems (Arthington
63 et al. 2006b, Arthington 2012). The environmental flow regime relates not only to the
64 volume of flow through a river system, but also the pattern of those flows (e.g. water
65 frequency, water speed and depth of water) (Arthington 2012, Atazadeh 2017).

66 There is the potential for a range of environmental benefits to be obtained during
67 large and small pulsed flows. Pulsed flows are water releases from structures, such as
68 dams, reservoir and weirs, to affect water transfers between storages to meet certain
69 demands and water supply requirements (Watts et al. 2009a, Palmer et al. 2014, Palmer
70 and Ruhi 2019). The general form of consumptive water transfers is via a pulse flow
71 (Richter et al. 1996, Watts et al. 2009b, Powell et al. 2013, Nichols et al. 2017). Flow
72 regime has a significant effect on physical form, streamside zone, water quality and

73 aquatic biota of a river (Ladson et al. 1999, Bunn and Arthington 2002, Gordon et al.
74 2004, Poff et al. 2010). The regulation of flow from reservoirs and dams, coupled with
75 water abstraction, leads to severe stress on many river ecosystems. The deviation from
76 the natural flow regime must be considered when trying to understand water quality and
77 the allocation of water for environmental benefits (Poff et al. 1997, Norris and Thoms
78 1999, Arthington et al. 2006a, Poff et al. 2010). Initially, the use of environmental flows
79 was focused on the minimum required flow in a river and according to this concept, a
80 river's health could be evaluated by flow characteristics alone. It has since been
81 recognised that other factors for evaluating river health must be considered. This
82 includes (but is not limited to) water quality, water levels and inundation frequencies,
83 floods, human impacts, irrigation, public water supply and industry needs (Acreman and
84 Dunbar 2004, Arthington 2012, Arthington et al. 2018).

85 Understanding river health through the measurement and response of aquatic flora
86 and fauna is also useful because many of these organisms are sensitive to changes in the
87 broader aquatic ecosystem (Norris and Thoms 1999, Bond et al. 2012, Bunn 2016,
88 Wang et al. 2019, Atazadeh et al. 2020). Contemporary water quality monitoring
89 programmes regularly focus on both water chemistry and biological communities as
90 indicators of aquatic ecosystem health. Over the last few decades, biological monitoring
91 has risen to the forefront of environmental impact assessment and river biomonitoring
92 programs (Chessman et al. 1999, Atazadeh et al. 2007, Chessman et al. 2007, Mangadze
93 et al. 2019, Asadi Sharif et al. 2020). Biological indices can be useful tools for water
94 resource managers in the assessment of river health and decision making with regards to
95 water sharing amongst the consumptive users in order to improve environment benefits
96 and river health while considering potential impacts on consumptive users. The main
97 aim of this paper is to biomonitor the short-term and longer-term responses to water

98 release events using periphytic algal communities to understand ecosystem response to
99 hydrologic disturbances and their applications in river management. In the present
100 paper, we hypothesized that: a) water release events affect structure and habitat of algal
101 periphyton communities; b) species diversity and density would change under water
102 release events; c) adapted taxa would tolerate the water release events and any other
103 hydrological disturbances and anthropogenic stressors; d) DSIAR (Diatoms Species
104 Index for Australian Rivers) represents a suitable index for assessing water quality in
105 the river.

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107 **Study area**

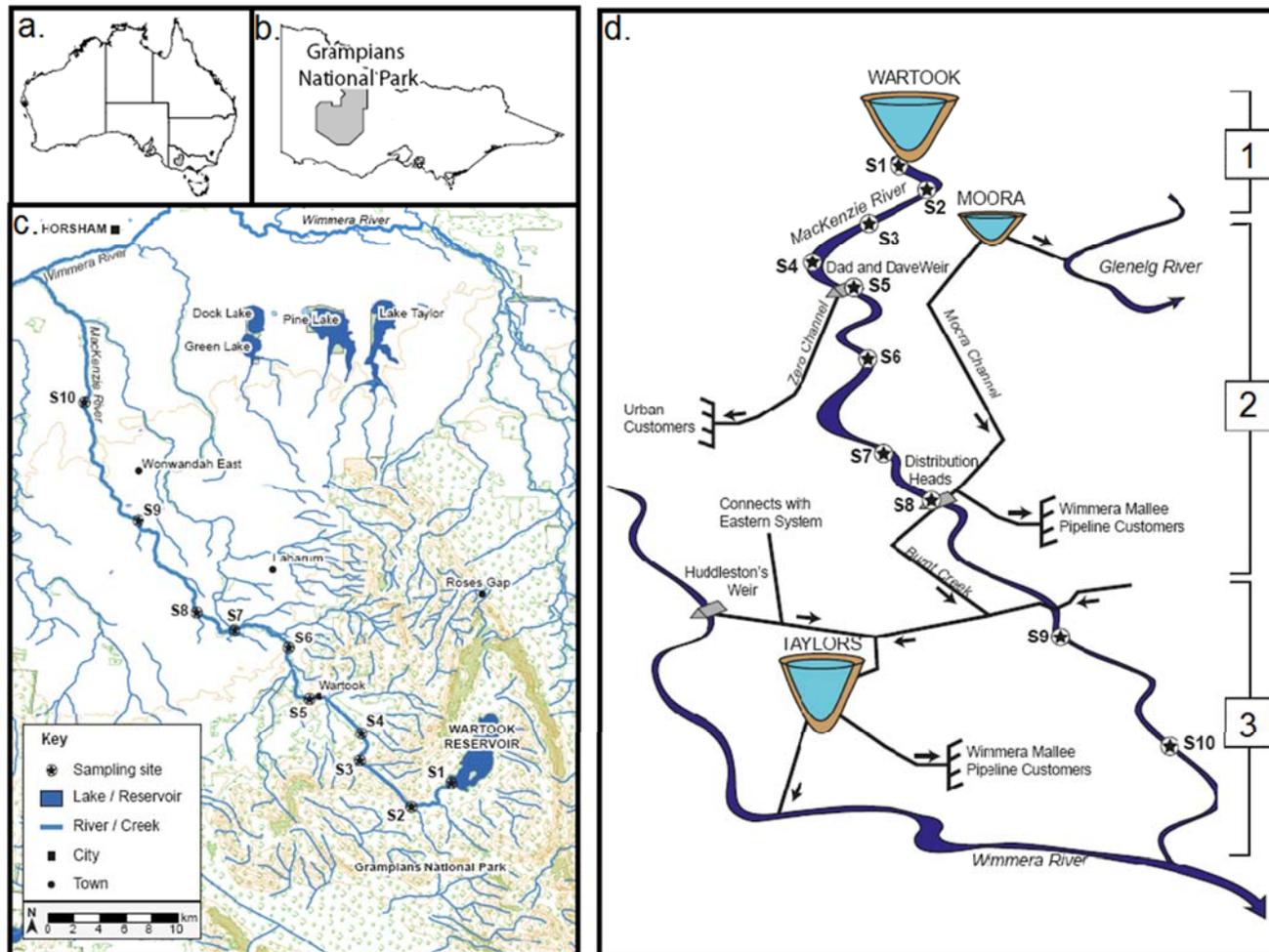
108 The MacKenzie River, which drains the northern slopes of the Grampians Ranges in
109 western Victoria, is one of the main tributaries of the Wimmera River (Figure 1a-c). The
110 headwaters feed into Lake Wartook in the Grampians National Park, which has a
111 maximum capacity of 29,360 ML. The river flows approximately 50 km from Wartook
112 Reservoir before its confluence with the Wimmera River. The catchment lies to the
113 south of the city of Horsham and covers an area of approximately 597 km² (WCMA
114 2004). The MacKenzie River is classified as a highly modified river as a consequence of
115 this anthropogenic modification, where consumptive flows will dominate the flow
116 regime in some years (Figure 1d).

117 Annually, a total of 10,000 ML of water is released from Lake Wartook into the
118 MacKenzie River. Of this volume, only about 4,000 ML (about one third) is released
119 explicitly for environmental purposes. The remaining 6,000 ML (about two thirds) is
120 released to meet consumptive demands and to transfer water to downstream reservoirs
121 (personal communication; GWMWater). Routine water releases from Wartook
122 Reservoir are up to 50 ML/day in summer and 15 ML/day in winter.

123 Ten sampling locations were established along the river, and the river was divided
124 into three reaches: Reach 1 included sampling stations S1, S2, S3 and S4 (Lake Wartook
125 to ‘Dad and Dave’ Weir); Reach 2 included sampling stations S5, S6, S7 and S8 (‘Dad
126 and Dave’ Weir to Distribution Heads) and Reach 3 included sampling stations S9 and
127 S10 (Distribution Heads to the Wimmera River) (Figure 1a-d). The upstream section
128 (Reach 1) tends to receive water most days of the year due to the water supply
129 requirements of the city of Horsham and is highly appreciated for its recreational and
130 conservation values. The middle and downstream sections (Reaches 2 and 3) receive a
131 more intermittent supply. The lower part of the river (Reach 3) has had the potential to
132 change greatly over time, evidenced by the persistent drought conditions between 1998
133 and 2009 (GWMWater pers. comm.).

134 There are a number of channels, pipelines and waterways in the system which
135 supplies and delivers water to various users including urban usage, irrigation, water
136 storages, environmental and recreational needs. In the Wimmera-Glenelg supply system
137 there are a number of water storages including Lake Wartook, Lake Lonsdale, Lake
138 Bellfield, Lake Taylor and Lake Fyans (Wimmera system), and Rocklands and Moora
139 Moora Reservoirs (Glenelg System). The engineered Wimmera-Glenelg system is
140 complex because of the water supply operation itself, the different sized water storages
141 and the varying demands from customers and the environment for water delivery
142 (VEWH 2015). The efficiency and flexibility of the system allows waterway managers
143 to transfer water between reservoirs. Furthermore, the facility of the system allows the
144 off-stream storages to harvest water from channels and storages (Figure 2).

145



146

147 **Figure 1 (a-c)** Location of the ten sampling stations along the MacKenzie River system in the Wimmera catchment and **(d)** Schematic diagram

148 showing the location of the three Reaches of the MacKenzie River within a complex water supply system (Adapted from GWMWater).



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150 **Figure 2** Schematic diagram of the complex water supply in the Wimmera-Glenelg

151 system (Courtesy of GWMWater)

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156 **Materials and methods**

157 Algal periphyton was scraped from an area of about 20-30 cm² of cobbles, pebbles or
158 rocks with a toothbrush and rinsing the algal suspension into a collection bottle.
159 Samples were initially taken during different seasons (28 February 2012, 17 July 2012,
160 9 November 2012 and 25 May 2013) to obtain baseline information of the spatial and
161 temporal variations of the algae communities, water quality, aquatic biota and stream
162 condition before the first water release event. After the baseline sampling, samples were
163 taken before, during and after three water release events (event 1: 18 October 2013, 21
164 October 2013, 25 October 2013; event 2: 16 December 2013, 19 December 2013, 23
165 December 2013, 3 January 2014, 16 April 2014; event 3: 29 October 2014, 1 November
166 2014, 8 November 2014 and 22 November 2014). The duration of water release events
167 (Fishes and high flows) was 3 days in the river. Data of the flow regimes from 2011-
168 2015 were provided by GWMWater.

169 *In situ* measurements of temperature, pH, electrical conductivity, turbidity, depth,
170 oxidation reduction potential, and dissolved oxygen were obtained using an Horiba
171 multimeter (Water checker U-52G). Then, samples collected for water quality analyses
172 were filtered using 0.45 µm syringe filters at each site for total suspended solids, total
173 dissolved solids, total oxidised nitrogen, total phosphorous, total nitrogen, ammonia,
174 silica, cations and anions were undertaken in the laboratory at Federation University
175 Australia using a spectrophotometer and Gallery Automated Photometric Analyser and
176 Hach DR 2800 following standard methods (APHA 2007, Atazadeh et al. 2009,
177 Victoria EPA 2009).

178 Algal samples were collected from cobbles, pebbles or rocks to a collection bottle
179 (200 mL) and then added Lugol's solution (2 mL) for preservation. Temporary slides
180 were prepared for soft algae to identify the species composition and to enumerate algal

181 groups. The relative abundance of the different algal groups (green algae,
182 Cyanobacteria, diatoms and other algae) was calculated by placing 1 mL of each sample
183 into a Sedgwick-Rafter counting chamber. Several replications of counts of one mL
184 were applied for accurate calculation of the relative abundance. Cells were counted
185 using a Nikon Eclipse 80i microscope at 100-400× magnification.

186 For diatom species identification and enumeration, the samples were prepared
187 following the method of Battarbee (1986). Samples were digested with 10% hydrogen
188 peroxide in a beaker at 90°C on a hotplate for 2 hours, after which two drops of 10%
189 hydrochloric acid were added. The beakers were filled with distilled water and left to
190 settle overnight after which the supernatant was discarded. This process was repeated
191 four times. Subsamples of 400 µl were air-dried on coverslips and mounted using
192 Naphrax (Battarbee 1986). At least 300 diatom valves were identified and counted per
193 slide using a Nikon Eclipse 80i microscope with differential interference contrast at
194 1000× magnification.

195 Separate samples were collected for dry mass, AFDM and chlorophyll-*a*
196 measurement. Algal periphyton was scraped from an area of about 20-30 cm² of
197 cobbles, pebbles, stone or rocks. Samples for the determination of dry mass (DM) were
198 oven-dried for 24 hours at 60°C and weighed. Samples were then combusted at 525°C
199 in a muffle furnace for four hours, and reweighed. Ash-free dry mass (AFDM) was
200 estimated as the difference in the mass before and after combustion and expressed as
201 mg.cm⁻² of the original substratum (Steinman et al. 1996, Biggs and Kilroy 2000,
202 Lavoie et al. 2004).

203 For chlorophyll-*a* analysis, the samples were transferred into tubes containing 10
204 ml of 95% ethanol (Nusch 1980, Snow et al. 2000). The samples were stored overnight
205 in a freezer and then allowed to return to room temperature. The absorbance of the

206 supernatant at 665 nm was determined before and after adding two drops of 0.1N HCl
207 using a Shimadzu UV 1800 spectrophotometer. Chlorophyll-*a* concentration was
208 determined using Hilmer's equation (Hilmer 1990) that had been derived from Nusch's
209 equation (Nusch 1980).

210 Ecological condition of the MacKenzie River was evaluated using the Diatom
211 Species Index for Australian Rivers (DSIAR) as a local diatom index (Chessman et al.
212 2007). Sensitivity values (SV) in this index evaluate the tolerance of each species to
213 anthropogenic stress (e.g. industry, agriculture, urban and any other manipulation in the
214 catchment). The SV of all species are used to calculate numerical scores for each
215 sample in the dataset, weighted by the proportional abundance of each species. High
216 DSIAR scores signify a flora 'less impacted' by anthropogenic modification on aquatic
217 ecosystems. In contrast low scores are interpreted as indicating a greater anthropogenic
218 stress. Using the algae-based indices, each site can be categorised as being in bad, poor,
219 moderate, good or excellent condition. The sensitivity values of species to
220 anthropogenic stressors in the MacKenzie River were used to calculate algae-based
221 index scores for each sample in the datasets.

222

223 **Data analysis and interpretation**

224 The species and water quality data were transformed to reduce skewness and to, as far
225 as possible, ensure normal distribution of the data sets. Canonical Correspondence
226 Analysis (CCA) was used to determine the direct relationship between diatom and soft
227 algae communities and the environmental variables. CCA is a constrained ordination
228 which uses *a priori* hypotheses (in contrast with unconstrained tests which do not use *a*
229 *priori* hypotheses). The CCA analyses were applied to determine the most important
230 variables influencing the diatom and soft algae communities under different seasons and
231 under different flows along the MacKenzie River. Furthermore, the CCA analyses were

232 performed to examine how algal species respond to a range of variables under different
233 flows.

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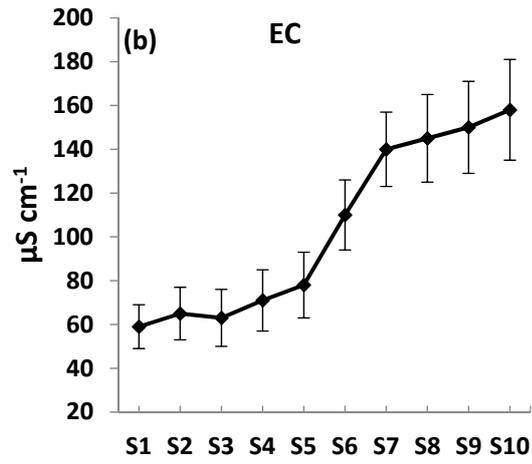
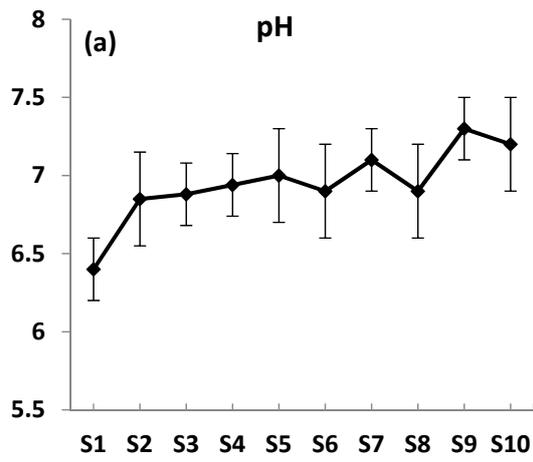
235 **Results**

236 **Average annual water chemistry in the MacKenzie River**

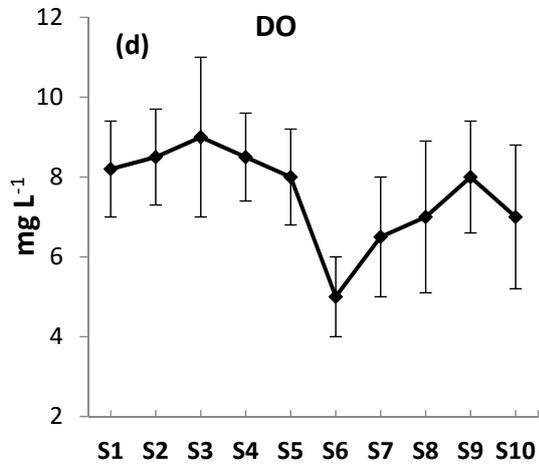
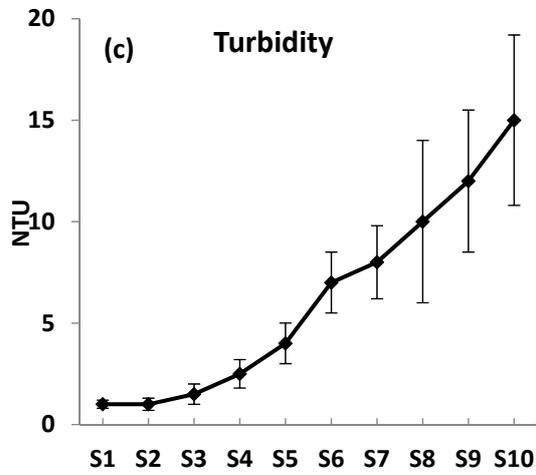
237 There were downstream trends in most of the physical and chemical water parameters
238 measured during the different flow regimes. Typically, the pH gradually increased
239 (became more alkaline) downstream. The upstream sites (S1-S4) also had lower
240 conductivity compared to those further downstream. Turbidity increased greatly
241 downstream and the concentration of dissolved oxygen (DO) changed along the river
242 where low concentrations were observed mid-stream due to standing water in the
243 middle of the river. Total suspended solid (TSS) values also increased in the lower parts
244 of the river particularly during water release events. The concentrations of nutrients (TN
245 and TP) also increased downstream (Table 1). The concentrations of cations (Mg^{2+} ,
246 Ca^{2+}) and anions (SO_4^{2-} , Cl^-) also increased downstream consistent with an increase in
247 total salinity and the concentration of Oxidation Reduction Potential (ORP), Total
248 Dissolved Solid (TDS) and Total Oxidative Nitrogen (TON). In contrast, the
249 concentration of ammonia decreased from upstream to downstream (Figure 3; Table 1).

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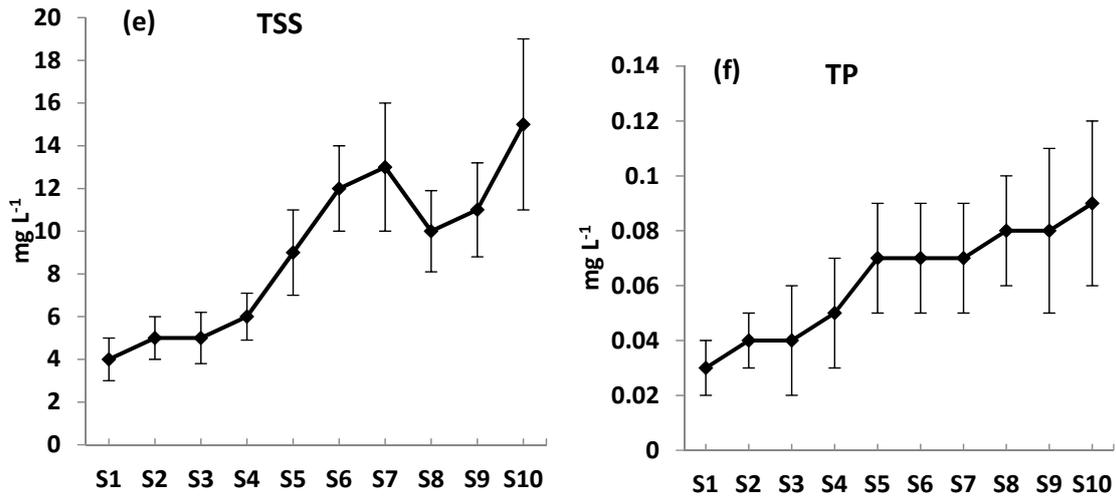
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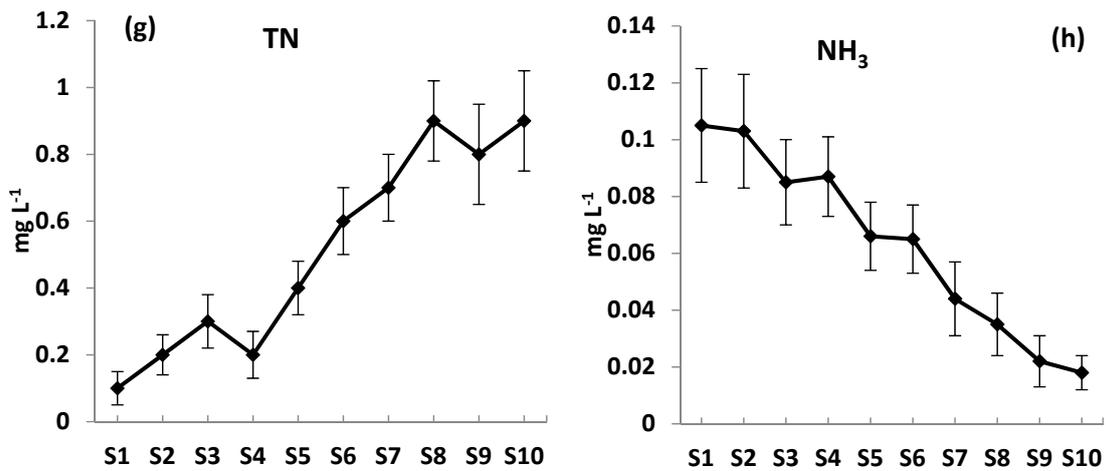
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274 **Figure 3** Physical and chemical characteristics of water at the sampling stations (S1-
275 S10) along the MacKenzie River: **(a)** pH; **(b)** Electrical Conductivity; **(c)** Turbidity; **(d)**
276 Dissolved Oxygen; **(e)** Total Suspended Solids; **(f)** Total Phosphorus; **(g)** Total
277 Nitrogen; **(h)** Ammonia. Data indicate means \pm SD

278 **Table 1** Average annual physical and chemical water quality characteristics at the sampling stations on the MacKenzie River from February
 279 2012 to Nov 2014 (Temp = temperature, ORP= Oxidation Reduction potential, TDS= Total Dissolved Solids, TON= Total Oxidative Nitrogen).
 280 Data indicate means \pm SD.

281

282

	Unit	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
Temp	°C	14 \pm 7	16 \pm 6	16 \pm 7	16 \pm 8	17 \pm 6	18 \pm 4	19 \pm 8	19 \pm 6	19 \pm 7	19 \pm 6
Depth	m	0.6 \pm 0.2	0.6 \pm 0.2	0.6 \pm 0.3	0.7 \pm 0.4	0.6 \pm 0.3	1 \pm 0.5	0.8 \pm 0.4	1 \pm 0.3	0.5 \pm 0.2	0.8 \pm 0.5
ORP	mV	212 \pm 22	222 \pm 32	223 \pm 35	258 \pm 41	245 \pm 42	255 \pm 64	255 \pm 58	244 \pm 51	299 \pm 75	298 \pm 74
TDS	mg L ⁻¹	22 \pm 11	35 \pm 15	41 \pm 22	68 \pm 31	71 \pm 23	86 \pm 32	91 \pm 33	98 \pm 38	106 \pm 41	111 \pm 35
Mg²⁺	mg L ⁻¹	1.1 \pm 0.5	1.8 \pm 0.8	2.2 \pm 0.9	2 \pm 0.8	3 \pm 0.5	3 \pm 0.5	3 \pm 1	4 \pm 1.2	4 \pm 1.2	4.1 \pm 1.3
SO₄⁻²	mg L ⁻¹	0.55 \pm 0.08	0.51 \pm 0.1	0.52 \pm 0.1	0.55 \pm 0.1	0.61 \pm 0.1	0.44 \pm 0.09	0.64 \pm 0.1	0.63 \pm 0.08	0.55 \pm 0.09	0.45 \pm 0.1
Ca⁺²	mg L ⁻¹	1.5 \pm 0.5	2.1 \pm 0.6	2.5 \pm 0.5	3 \pm 0.6	3.2 \pm 0.5	3.2 \pm 0.6	3.6 \pm 0.2	3.4 \pm 0.5	3.5 \pm 0.5	3.9 \pm 0.6
SiO₂	mg L ⁻¹	0.22 \pm 0.05	0.28 \pm 0.06	0.36 \pm 0.09	0.38 \pm 0.06	0.49 \pm 0.08	0.65 \pm 0.05	0.75 \pm 0.04	0.79 \pm 0.09	0.78 \pm 0.08	0.88 \pm 0.09
TON	mg L ⁻¹	0.07 \pm 0.01	0.08 \pm 0.02	0.07 \pm 0.01	0.06 \pm 0.02	0.08 \pm 0.02	0.07 \pm 0.03	0.07 \pm 0.01	0.08 \pm 0.04	0.1 \pm 0.05	0.1 \pm 0.05
Cl⁻¹	mg L ⁻¹	19 \pm 8	22 \pm 8	23 \pm 4	28 \pm 8	29 \pm 7	30 \pm 10	30 \pm 6	29 \pm 8	33 \pm 8	40 \pm 10

283 **Water chemistry during water release events**

284 The changes in water quality observed during water release events were such that the water
 285 quality downstream was relatively similar to that upstream. Nevertheless, the results show this
 286 phenomenon (similarity of the water quality between upstream and downstream reaches) is
 287 only temporary in nature and antecedent conditions return within a few days of water release.
 288 Whilst the water quality does vary during different seasons, the main changes in water quality
 289 are observed during the water release events (Table 2).

290
 291 **Table 2** Physical and chemical water quality characteristics during water release events at the
 292 sampling stations on the MacKenzie River.

293

	Unit	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
pH	-	6.7	6.7	6.7	6.8	6.8	6.8	6.7	6.5	6.6	6.7
Temp.	°C	16.2	16.5	16.3	16.2	17.4	17.2	17.5	17.2	18.1	17.9
Cond.	µS cm ⁻¹	75	76	79	76	79	82	82	85	88	86
Turb.	NTU	8.5	9.3	8.2	10	8.5	12	15.2	16	14.2	14.5
Depth	m	0.8	0.8	0.7	1.2	1.3	1.5	1.6	1.7	1.2	1.3
ORP	mV	250	251	256	264	261	258	269	274	261	271
DO	mg L ⁻¹	8.5	9.2	9.8	8.4	9.2	7.2	7.8	8.5	7.8	8.2
TDS	mg L ⁻¹	45	46	42	55	65	64	71	75	71	85
TSS	mg L ⁻¹	6	6.2	7.4	6.6	7	8.2	7.3	6.8	8.2	7.1
TN	mg L ⁻¹	0.2	0.2	0.3	0.2	0.3	0.3	0.4	0.5	0.7	0.8
TP	mg L ⁻¹	0.05	0.05	0.06	0.07	0.07	0.07	0.08	0.08	0.08	0.08
Mg²⁺	mg L ⁻¹	1.2	1.7	2	2.2	2.1	2.5	2.6	3.3	3.3	3.3
NH₃	mg L ⁻¹	0.1	0.1	0.09	0.09	0.08	0.09	0.07	0.08	0.07	0.09
SO₄²⁻	mg L ⁻¹	0.45	0.52	0.46	0.48	0.55	0.54	0.62	0.55	0.42	0.56
Ca⁺²	mg L ⁻¹	1.6	1.8	2.2	3.3	3.2	3.3	3.1	3.2	3.4	3.6
SiO₂	mg L ⁻¹	0.024	0.25	0.23	0.25	0.39	0.51	0.55	0.66	0.62	0.77
TON	mg L ⁻¹	0.06	0.08	0.07	0.08	0.05	0.07	0.06	0.08	0.08	0.08
Cl⁻¹	mg L ⁻¹	22	25	23	25	31	32	33	28	29	28

294 **Algal response under base flow**

295 This investigation showed that during base flow the algal flora of the MacKenzie River was
296 composed of typical acidic taxa, especially in the upper reaches, while more alkaline taxa
297 were recorded in lower reaches. In this study, 126 diatom species (43 genera), 44 green algae
298 species (23 genera), 24 blue-green algae species (10 genera), and 9 other algae (6 genera)
299 were recorded from samples collected during base flow conditions. The diatom community
300 (species composition) was the most abundant and dominated the river samples, displaying
301 high diversity in the upstream sites while the relative proportions of green algae and
302 Cyanobacteria increased in the mid and downstream reaches.

303 The most common algal species in the upstream reaches (S1-S5) were diatoms (based
304 on relative abundance) including: diatoms - *Brachysira brebissonii* R.Ross, *Eunotia*
305 *bilunaris* (Ehrenberg) Schaarschmidt, *Frustulia rhomboides* (Ehrenberg) De Toni,
306 *Gomphonema affine* Kützing, *Melosira arentii* (Kolbe) Nagumo & Kobayashi, *Navicula*
307 *heimansioides* Lange-Bertalo, *Tabellaria flocculosa* (Roth) Kützing; green algae -
308 *Stigeoclonium flagelliferum* Kützing, *Ulothrix flacca* (Dillwyn) Thuret; and Cyanobacteria -
309 *Tolypothrix tenuis* Kützing ex Bornet & Flahault. In the downstream reaches (S6-S10), the
310 most common algal species were: diatoms - *Eunotia serpentine* (Pantocsek) Hustedt,
311 *Nitzschia capitellata* Hustedt, *Planothidium frequentissimum* (Lange-Bertalot) Lange-
312 Bertalot, *Surirella angusta* Kützing; green algae - *Oedogonium undulatum* A.Braun ex Hirn;
313 and Charophytes – *Chara* sp., *Nitella cristata* A.Braun and Cyanobacteria - *Schizothrix*
314 *arenaria* Gomont (Table 3).

315

316 **Table 3** Taxonomic composition of algae by genus and number of taxa present in each genus,
 317 in the MacKenzie River

Bacillariophyta	Taxa No.	Chlorophyta	Taxa No.	Cyanophyta	Taxa No.	Other groups	Taxa No.
<i>Achnanthes</i>	2	<i>Ankistrodesmus</i>	1	<i>Anabaena</i>	2	<i>Cryptomonas</i>	1
<i>Achnantheidium</i>	1	<i>Bambusina</i>	1	<i>Chroococcus</i>	1	<i>Ceratium</i>	2
<i>Asterionella</i>	1	<i>Bulbochaete</i>	1	<i>Lyngbya</i>	3	<i>Dinobryon</i>	2
<i>Aulacosira</i>	2	<i>Chara</i>	2	<i>Merismopedia</i>	1	<i>Euglena</i>	1
<i>Brachysira</i>	6	<i>Chlorella</i>	1	<i>Nodularia</i>	1	<i>Gymnodinium</i>	1
<i>Brevisira</i>	1	<i>Cladophora</i>	1	<i>Nostoc</i>	1	<i>Peridinium</i>	2
<i>Caloneis</i>	2	<i>Closterium</i>	3	<i>Oscillatoria</i>	5		
<i>Cocconeis</i>	1	<i>Cosmarium</i>	4	<i>Phormidium</i>	2		
<i>Craticula</i>	1	<i>Euastrum</i>	3	<i>Schizothrix</i>	1		
<i>Cyclostephanos</i>	1	<i>Gonium</i>	1	<i>Tolypothrix</i>	1		
<i>Cyclotella</i>	2	<i>Micrasterias</i>	1	<i>Nostoc</i>	1		
<i>Cymbella</i>	3	<i>Monoraphidium</i>	1	<i>Oscillatoria</i>	5		
<i>Cymbopleura</i>	4	<i>Nitella</i>	2				
<i>Diatoma</i>	3	<i>Oedogonium</i>	2				
<i>Diplonies</i>	1	<i>Oocystis</i>	2				
<i>Discostella</i>	1	<i>Pediastrum</i>	2				
<i>Encyonema</i>	2	<i>Rhizoclonium</i>	1				
<i>Epithemia</i>	1	<i>Scenedesmus</i>	6				
<i>Eunotia</i>	16	<i>Spirogyra</i>	1				
<i>Envekadea</i>	1	<i>Staurodesmus</i>	1				
<i>Fragilaria</i>	4	<i>Staurastrum</i>	5				
<i>Frustulia</i>	5	<i>Stigeoclonium</i>	1				
<i>Gomphonema</i>	7	<i>Ulothrix</i>	1				
<i>Gyrosigma</i>	2						
<i>Hantzschia</i>	1						
<i>Luticola</i>	1						
<i>Melosira</i>	1						
<i>Navicula</i>	8						
<i>Neidium</i>	4						
<i>Nitzschia</i>	5						
<i>Pinnularia</i>	9						
<i>Planothidium</i>	1						
<i>Psammothidium</i>	2						
<i>Pseudostaurosira</i>	1						
<i>Rhopalodia</i>	1						
<i>Sellaphora</i>	1						
<i>Stauroforma</i>	1						
<i>Stauroneis</i>	7						
<i>Staurosira</i>	1						
<i>Stenopterobia</i>	3						
<i>Surirella</i>	3						
<i>Synedra</i>	3						
<i>Tabellaria</i>	3						
Total	126		44		24		9

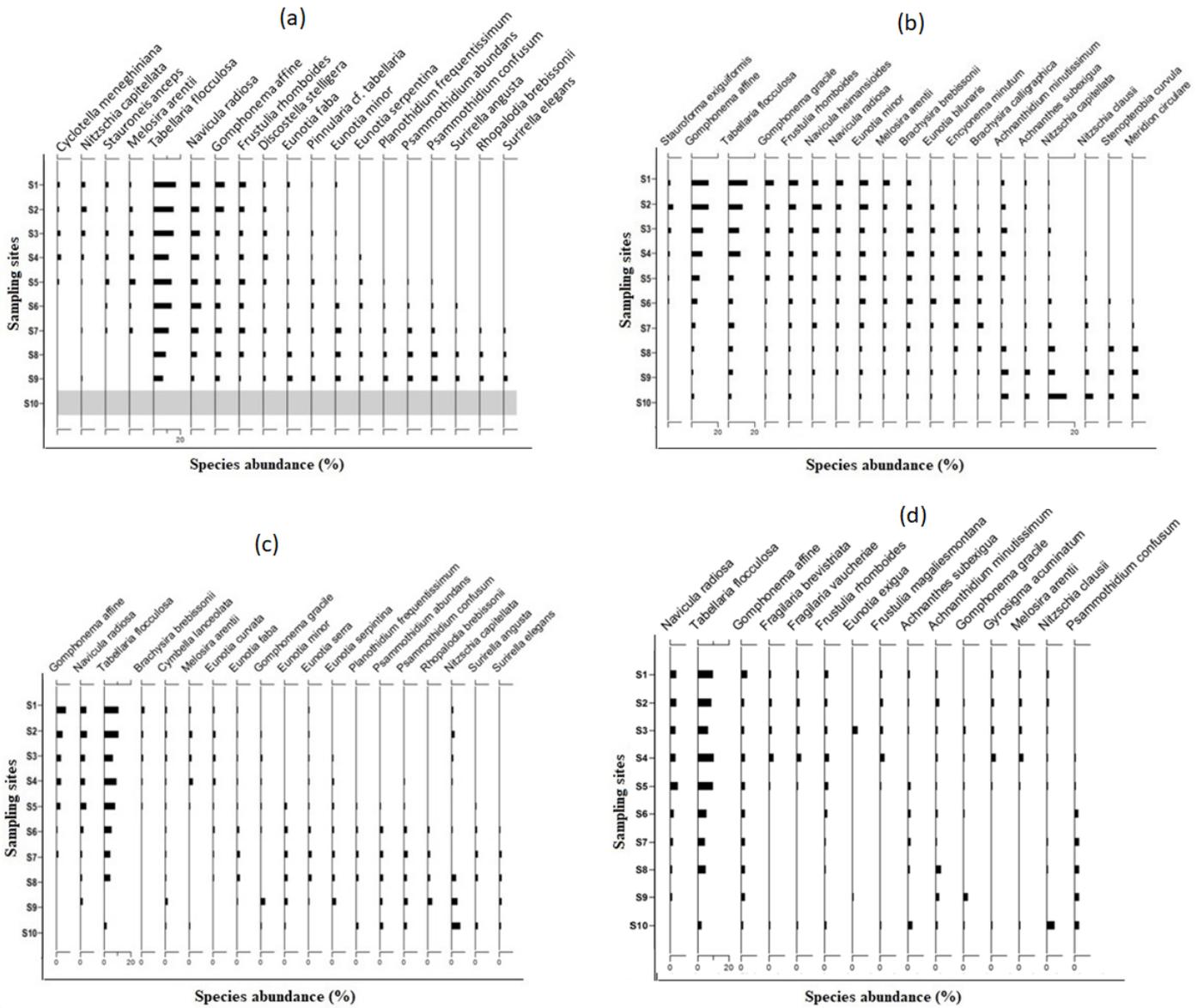
318 **Diatom variations under base flow**

319 Diatom species composition varied from upstream to downstream and between seasons. The
320 summer (February 2012) samples presented in Figure 4a showed, *Frustulia rhomboides*
321 *Gomphonema affine*, *Navicula radiosa* Kützing, *Tabellaria flocculosa* to be common in the
322 upstream reaches, however, the relative abundance of those diatoms decreased downstream.
323 In contrast, those most strongly associated with downstream reaches were *Eunotia*
324 *serpentina*, *Psammothidium abundans* (Manguin) Bukhtiyarova & Round, *Rhopalodia*
325 *brebissonii* Krammer and *Surirella elegans* Ehrenberg. In contrast, the winter (July 2012)
326 samples showed the upstream sites supported *Frustulia rhomboides*, *Gomphonema affine*,
327 *Gomphonema gracile* Ehrenberg, *Navicula heimansioides* Lange-Bertalot, *Navicula. radiosa*
328 and *Tabellaria flocculosa* while the downstream sites supported assemblages including
329 *Nitzschia capitellata*, *Nitzschia clausii* Hantzsch, *Meridion circulare* (Greville) C.Agardh and
330 *Stenopterobia curvula* (W.Smith) Krammer (Figure 4b). The results of the spring survey
331 (November 2012) showed the algal community to be similar to those in summer. The most
332 common diatom taxa collected in the upstream (S1-S5) samples were *T. flocculosa*, *G. affine*,
333 *N. radiosa* and *M. arentii* while in the downstream reaches the most common species were
334 *Eunotia serpentina*, *P. abundans*, *Psammothidium confusum* (Manguin) van de Vijver, *N.*
335 *capitellata* and *S. angusta* (Figure 4c). The results of the diatom community structure in the
336 following winter, in June 2013, showed the most common diatom species in upstream sites
337 (S1-S5) are *T. flocculosa*, *G. affine*, *N. radiosa*, while in the downstream section (S6-S10) the
338 most common species were *Achnantheidium minutissimum* (Kützing) Czarnecki, *N. clausii*
339 and *P. confusum* (Figure 4d).

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 344 **Figure 4** Results of diatom analysis along the MacKenzie River sampling stations; **(a)**
 345 February 2012, Station 10 was dry at the time of sampling; **(b)** July 2012; **(c)** November
 346 2012; **(d)** June 2013. Data indicate >4% in any one sample, sorted by weighted-averaging
 347 [ascending].

348

349 **Soft algae variations under base flow**

350 The soft algal species composition differed along the MacKenzie River and between seasons.
351 The soft algae were classified into three different groups comprising Chlorophyta (green
352 algae), Cyanobacteria (blue-green algae) and other algae (Chrysophyta, Charophyta and
353 Euglenophyta). The results showed that the algal assemblages differed between sites, and
354 from upstream to downstream. The unicellular species were mostly found in the upstream
355 sites while the downstream sites (S6-S10) supported both unicellular and filamentous algae.

356 Soft algae species composition was different from upstream to downstream and
357 between the seasons. The summer results (February 2012) showed *Chlorella vulgaris*
358 Beyerinck [Beijerinck], *Closterium kuetzingii* Brébisson, *Dinobryon sertularia* Ehrenberg,
359 *Oocytis parva* West & G.S.West, *Pediastrum angulosum* Ehrenberg ex Meneghini and
360 *Peridinium lomnickii* Woloszyńska, were the most common of the soft algae in the upstream
361 sites whilst *C. vulgaris*, *Cosmarium sportella* Brébisson ex Kützing, *O. parva*, *P. angulosum*
362 and *Stigeoclonium flagelliferum* Kützing were common in the midstream sections. In
363 contrast, filamentous algae such as *Lyngbya* Agardh Ex Gomont and *Schizothrix arenaria*
364 were more often present downstream.

365 The winter results (July 2012) showed upstream sites of the river had more unicellular
366 soft algae including *Ceratium cornutum* (Ehrenberg) Claparède & J.Lachmann, *C. vulgaris*,
367 *Closterium* sp., *D. sertularia*, *Peridinium lomnickii*, *P. angulosum* and *O. parva* while
368 filamentous soft algae species that were found to be more abundant in the downstream sites
369 included *Cladophora glomerata* (Linnaeus) Kützing, *Lyngbya* sp., *Oedogonium undulatum*,
370 *Oedogonium* sp. and *S. arenaria*.

371 **Relative abundance of the algal groups under base flow**

372 Base flow sampling results showed that relative abundances of the algal groups varied by
373 stream reach and also by season. Relative abundance of diatoms was highest in the upstream
374 sites while filamentous green algae were more abundant downstream. The taxonomic

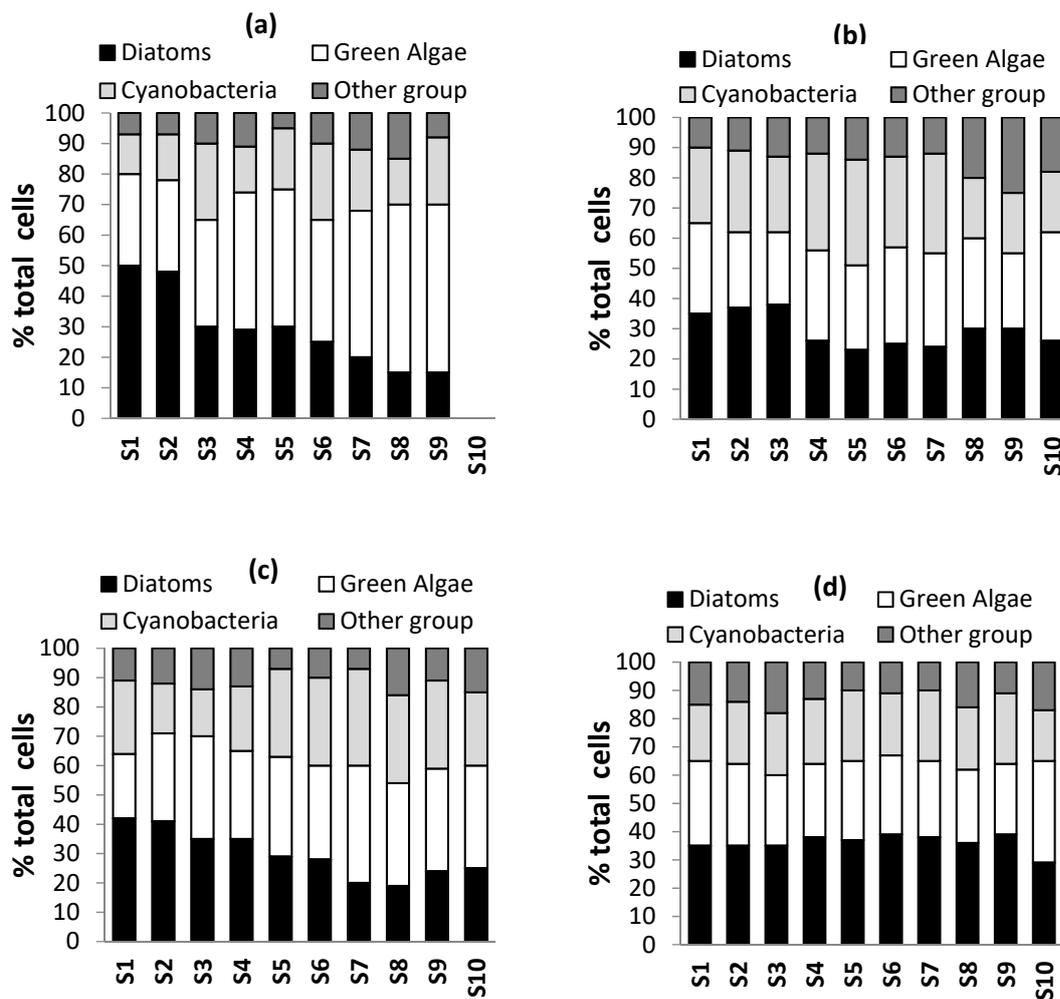
375 composition of the algae varied among reaches along the river. In February 2012, diatoms
376 represented the highest proportion of algal cells (c. 48%) upstream (S1 and S2) but decreased
377 gradually from the midstream (c. 30%) to the lower reaches (c. 20%). In contrast, the relative
378 abundance of green algae increased from approximately 20% in upstream sites to 40% in
379 downstream sites, while the relative abundance of Cyanobacteria increased from 15%
380 upstream to 20% downstream. The other algae (Chrysophyta, Charophyta and Euglenophyta)
381 varied slightly between sites (Figure 5a).

382 The algal group assemblages in the July 2012 samples (Figure 5b) showed that the
383 relative abundance of diatoms, green algae, and Cyanobacteria were approximately 35%,
384 30% and 25% respectively in the upstream sites (S1-S3). The relative abundances of diatoms
385 and green algae were slightly lower in the midstream sites with both approximating 25%,
386 whereas Cyanobacteria and other algae such as Chrysophyta increased to 35% and 15%
387 respectively. In the downstream sites the relative abundance of the diatoms slightly increased
388 (c. 30%) while that of the Cyanobacteria decreased (c. 20%). The other algal groups also
389 increased in relative abundances downstream, but were not dominant in the MacKenzie
390 system.

391 The relative abundances of the algal groups again changed downstream seasonally, and
392 in November 2012 (spring) (Figure 5c) diatoms were more abundant in the upstream sites
393 while green algae and Cyanobacteria were more abundant in downstream sites. The relative
394 abundance of the cells of diatom, green algae, and Cyanobacteria and other algae were 42%,
395 23%, 22% and 13% respectively in the upstream sites, changing to 25%, 30%, 25% and 20%
396 respectively downstream. Together, the soft algal groups (especially filamentous and colonial
397 algae) increased markedly in mid and downstream reaches in spring and summer whilst
398 diatoms had a higher relative abundance in upstream sites in the same seasons. The results of
399 the June 2013 sampling (Figure 5d) showed diatoms and Cyanobacteria to be the most

400 abundant algal groups in the upstream reaches of the MacKenzie River, with diatom
 401 abundance decreasing downstream. The relative abundance of diatoms, green algae,
 402 Cyanobacteria and other algae groups was approximately 35%, 24%, 26% and 15%
 403 respectively in the upstream sites whilst downstream their relative abundances were 30%,
 404 35%, 18% and 17% respectively.

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409 **Figure 5** Percent of total cells of the algal communities in the MacKenzie River: (a) February
 410 2012; (b) July 2012; (c) November 2012; (d) June 2013. (Station 10 was dry in February
 411 2012).

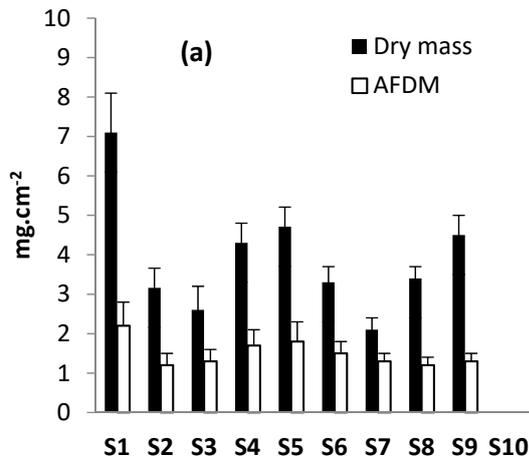
412 **Biological properties of algae under base flow**

413 The results of the analyses of biomass vary significantly between sampling sites and events.
414 The dry mass (DM) and ash-free dry mass (AFDM) showed values varied downstream in
415 February 2012. The DM value in the upstream, midstream and downstream reaches in the
416 February 2012 samples was $7\text{mg}\cdot\text{cm}^{-2}$, $5\text{mg}\cdot\text{cm}^{-2}$, and $4\text{mg}\cdot\text{cm}^{-2}$ respectively. The river was
417 dry in February 2012 at the most downstream site (S10) and so DM samples could not be
418 taken for S10 in February 2012 (Figure 6a).

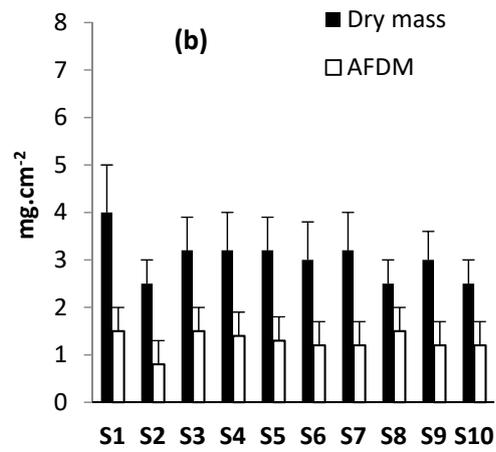
419 In July 2012, the accumulation of DM and AFDM were also the highest in the
420 uppermost site (S1) but sharply decreased by S2. At S3 the DM and ADFM results were
421 higher than S2, but gradually decreased further towards the mid and lower parts of the river
422 (Figure 6b). In contrast, there was a trend of DM and ADFM results decreasing with distance
423 downstream in November 2012 (Figure 6c). There was no clear trend for the DM and AFDM
424 results in June 2013 (Figure 6d).

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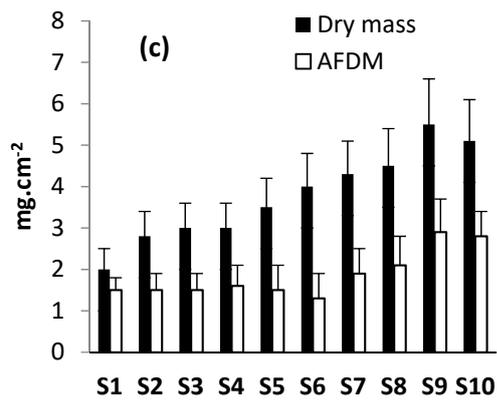


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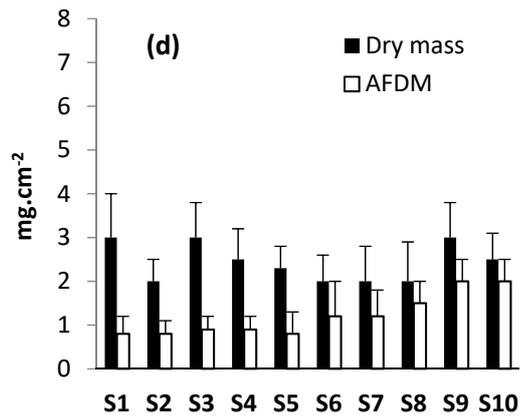


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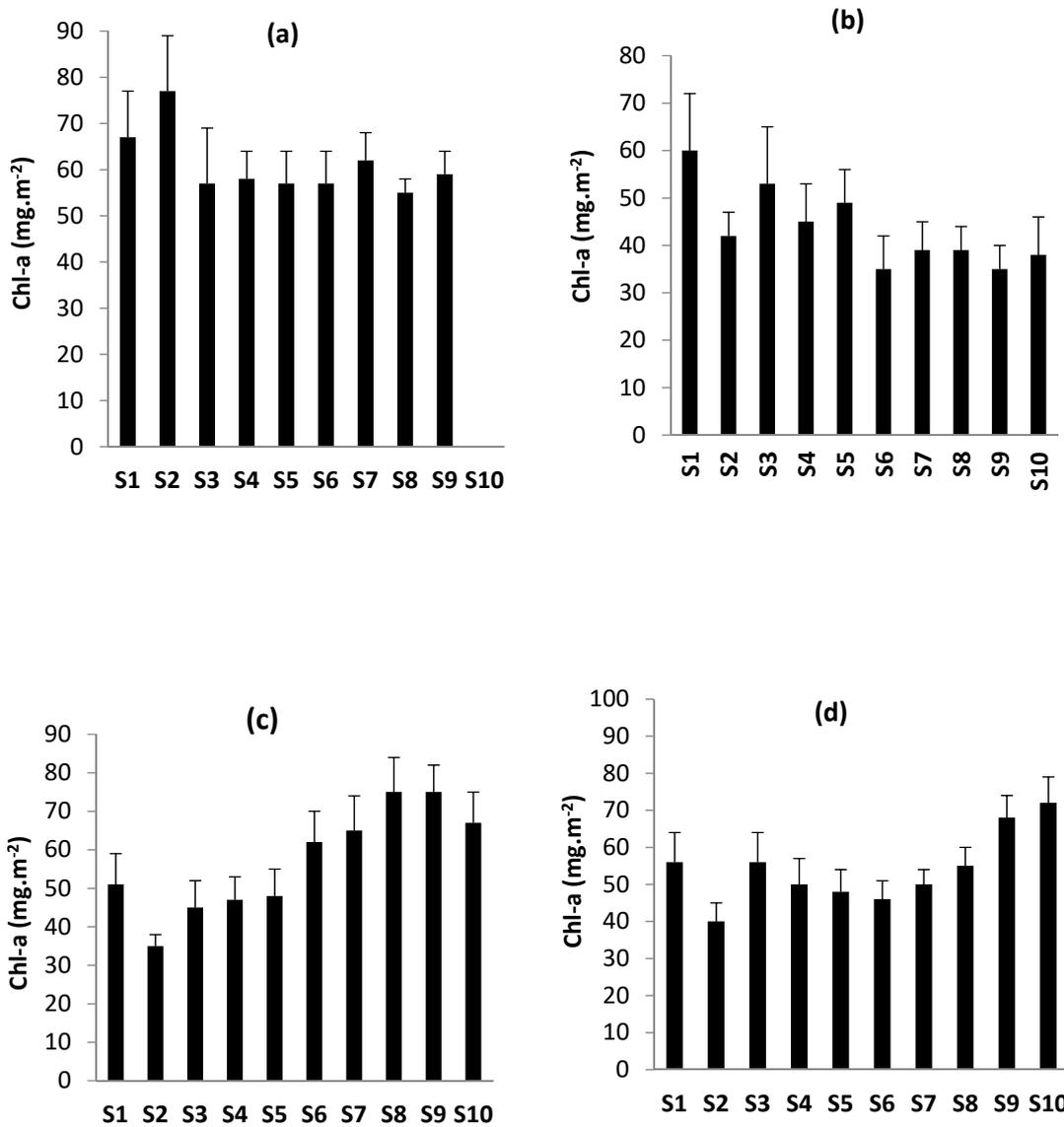
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431 **Figure 6** The accumulation of dry mass and ash-free dry mass at each of the sampling
432 stations along the MacKenzie: (a) February 2012; (b) July 2012; (c) November 2012; (d)
433 June 2013. Data indicate means \pm SD. (Station 10 was dry in February 2012).

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435 The upstream and downstream values for chlorophyll-*a* concentrations in February
436 2012 ranged from approximately 90 mg.m⁻² (highest value in S2) and 60 mg.m⁻² (mid and
437 lower reaches of the river) with no real pattern evident (Figure 7a). The results from July
438 2012 showed that chlorophyll-*a* concentration gradually decreased downstream. The highest
439 chlorophyll-*a* concentration was greatest upstream with approximately 60 mg.m⁻² recorded at
440 S1, with the concentration gradually decreasing through mid and downstream reaches and
441 correlating negatively with turbidity (Figure 7b). In contrast, the concentration of
442 chlorophyll-*a* increased downstream in November 2012 (Figure 7c) ranging from
443 approximately 35 mg.m⁻² in the upstream sites increasing to 80 mg.m⁻². Overall, it seems the
444 algal productivity is greater in spring, especially in the lower parts of the river. The
445 chlorophyll-*a* concentrations for June 2013 (Figure 7d) showed an unusual pattern of gradual
446 increases (S3 to S5; S6 to S10), with substantial declines at sites (S1 to S2; S5 to S6).
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Figure 7 The concentration of chlorophyll-a at each of the sampling stations along the MacKenzie River; **(a)** February 2012; **(b)** July 2012; **(c)** November 2012; **(d)** June 2013. Data indicate means \pm SD. (Station 10 was dry in February 2012).

462 **Algal response under manipulated flow regimes (freshes and high flow)**

463 Algae and water quality were monitored under different water release regimes to determine
464 their response to river flow and so its effect on river health. Algal community structure was
465 documented in terms of the major algal groups and then biological properties of the
466 periphytic algal communities were measured before, during and after water release events (1
467 week before water release, 3 days during water release and three weeks after water release (3
468 times).

469 **Soft algae and diatoms community structure before, during and after freshes**

470 Algal species composition varied between sites under freshes (35-40 ML/day). The algal
471 composition shifted downstream after water release events. Diatoms were the most abundant
472 group (50% of cells) upstream (Site 1 and 2 in Reach 1) before the water release whilst green
473 algae were most abundant downstream (55% of cells at Site 10 in Reach 3). The proportion
474 of green algae and Cyanobacteria tended to be greater downstream before the water release,
475 whereas diatoms had lower relative abundance downstream (to 10% at site S10). However,
476 Cyanobacteria and other algae were relatively more abundant (25% and 15% respectively) in
477 some mid-stream sites (Reach 2) (Figure 8a).

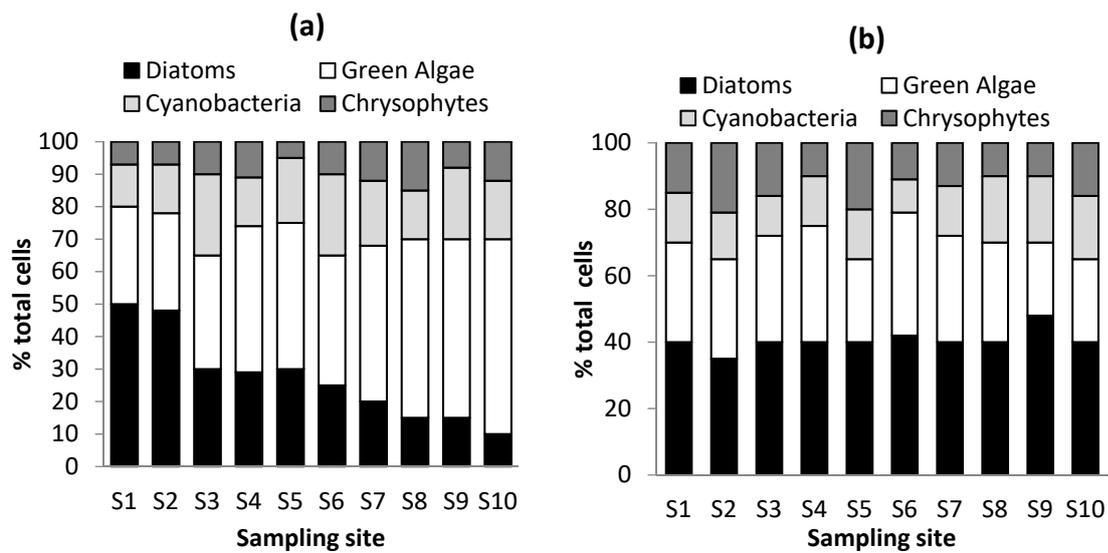
478 There were substantial changes in algal communities during and after water release
479 events. The relative abundance of diatom cells (40%) increased during and after freshes,
480 whilst green algae decreased downstream (23%); the proportion of green algae during and
481 after freshes was lower in the downstream sites. Therefore, the algal taxonomic composition
482 became more uniform across the reaches following a release event. For example, blooms of
483 green algae, typical of base flow conditions in downstream reaches, were reduced by the
484 freshes (Figure 8a-c).

485 The diatom species composition after freshes at upstream sites was different to those at
486 downstream sites. The most common diatom taxa found at the upstream sites before freshes

487 were *Melosira arentii*, *Frustulia. rhomboides*, *Gomphonema affine*, *Navicula radiosa*,
 488 *Neidium iridis* (Ehrenberg) Cleve, *T. flocculosa* whilst at downstream sites the common
 489 diatoms found were *Encyonema minutum*, *Eunotia bigibba*, *E. serpentina*, *Nitaschia*
 490 *capitellata* and *Stenopterobia delicatissima* (F.W.Lewis) Brébisson ex Van Heurck.

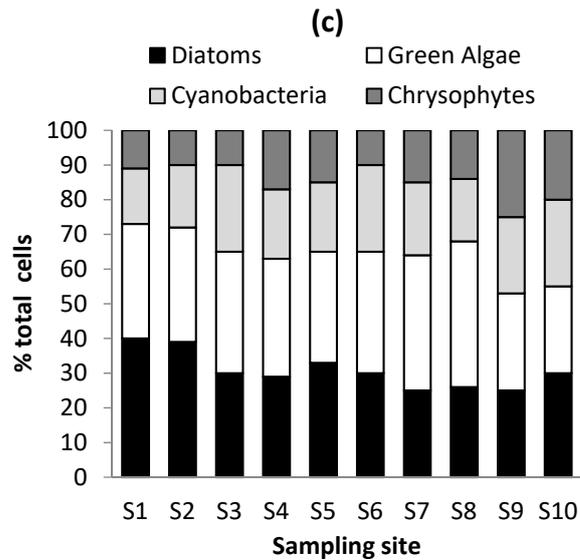
491 The community structure changed during and after freshes. The most abundant diatoms
 492 in the upstream reach during and after the water release were *Brachysira brebissonii*, *M.*
 493 *arentii*, *E. minor*, *G. affine*, *N. heimansioides*, *Brevisira* Karammer, *S. exiguiiformis* and *T.*
 494 *flocculosa* whilst those sampled from the downstream reach were *E. serpentina*, *N.*
 495 *capitellata*, *N. clausii*, and *S. curvula*

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500 **Figure 8** Relative abundance of total cells of algae in sampling sites along the MacKenzie
 501 River; (a) before freshes (15 ML/day); (b) during freshes (35-40 ML/day); (c) after freshes
 502 (15ML/day).

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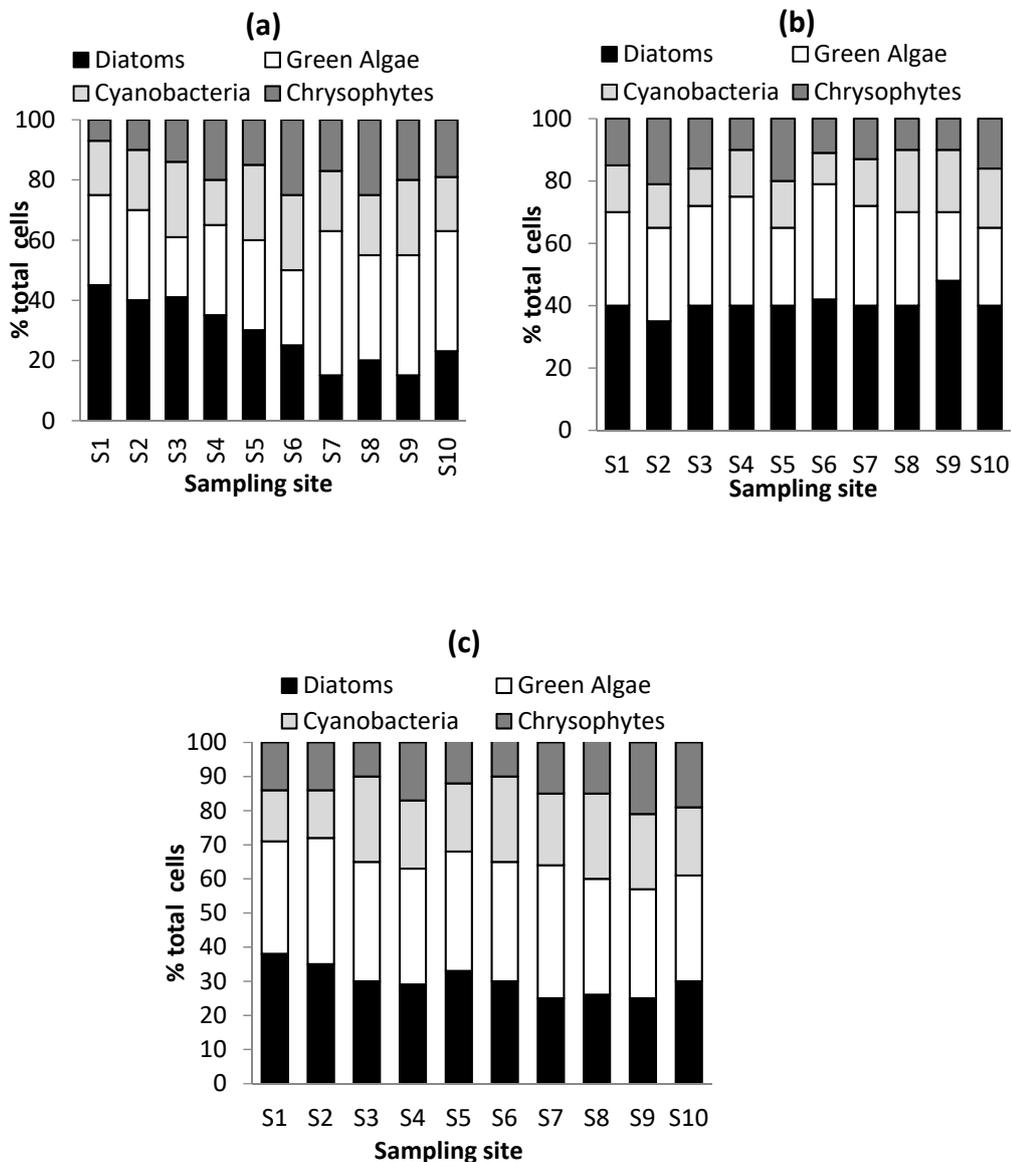
504 **Algal community structure before, during and after high flow**

505 Diatoms were common in upstream sites (45%), while green algae (33%), Cyanobacteria
 506 (12%) and Chrysophyta (10%) were less abundant before high flow events (Figure 9a).
 507 However, this pattern in the algae community structure changed gradually downstream. In the
 508 midstream (Reach 2), the percentage of diatoms, green algae and Cyanobacteria were similar.
 509 In contrast to the upstream sites green algae were more abundant from sites 7 to 10.

510 Diatoms had higher relative abundances downstream (sites 5 to 10) during high flow (c.
 511 40%) and after the high flow event (c. 35%). In contrast, green algae and Cyanobacteria
 512 relative abundances decreased downstream (c. 30% and c. 20% respectively). The results
 513 showed that high flows had a major influence on algae communities. The abundance of
 514 Cyanobacteria and Chrysophyta increased from upstream to downstream during base flow
 515 and before high flow, whilst their composition was relatively uniform spatially during and

516 after high flow. Overall the algal taxonomic composition became more uniform along the
 517 MacKenzie River during and after the high flow events (Figure 9b-c).

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522 **Figure 9** Relative abundance of cells of algae from sampling sites along the MacKenzie
 523 River; (a) before high flow (15 ML/day); (b) during high flow (55 ML/day); (c) after high
 524 flow (15ML/day)

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528 **Algal biomass under high flows**

529 Before the high flows (55ML/day) were released, dry mass generally increased from the
530 upstream to the downstream sites with the highest value of dry mass (6.5 mg.cm⁻²) found at
531 site 9; under the same conditions however, there was more fluctuation evident at midstream
532 and downstream sites in the values of the dry mass. Dry mass decreased dramatically during
533 high flows and partially recovered after the high flows (Figure 10a). Overall the accumulation
534 of dry mass decreased during high flow conditions within the system.

535 The accumulation of AFDM increased in upstream and midstream and subsequent
536 decreased in downstream sites before high flows while during high flows the AFDM
537 decreased at site 1, remained unchanged at sites 2 and 3 and increased at the other sites. After
538 the high flows, AFDM values were almost the same as during high flows (Figure 10b).

539 The chlorophyll-*a* concentration fluctuated downstream (before, during and after high
540 flows); chlorophyll-*a* concentration increased before water release (high flows) but decreased
541 substantially during high flows and then partially increased again afterwards but still did not
542 reach the same concentrations as before the releases (Figure 10c).

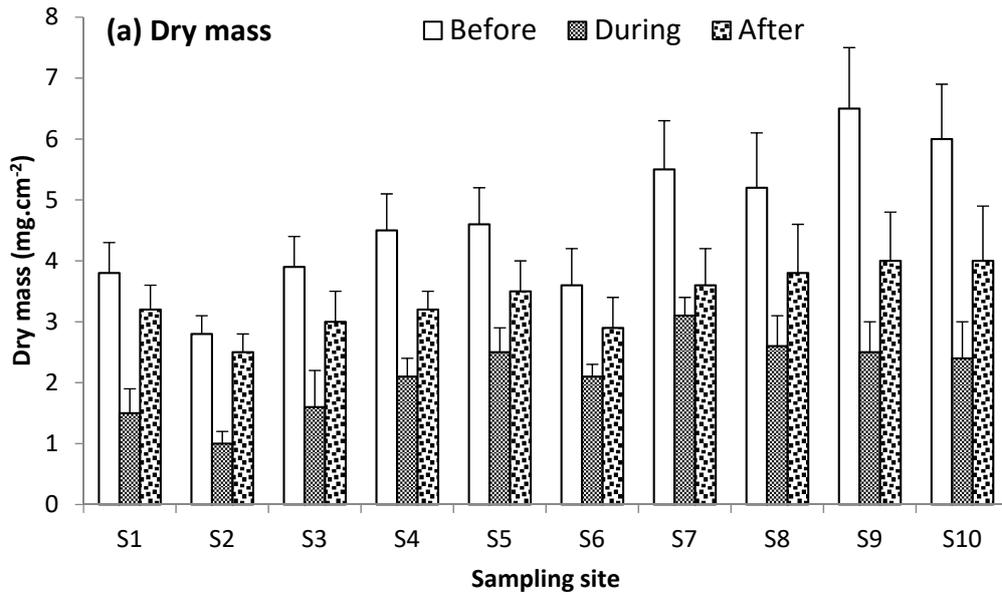
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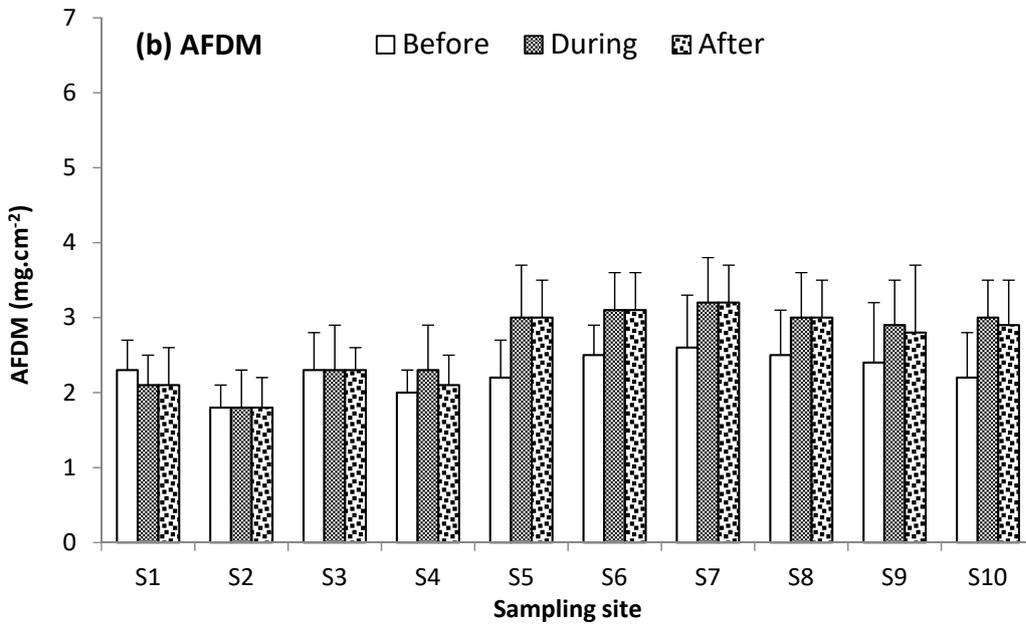
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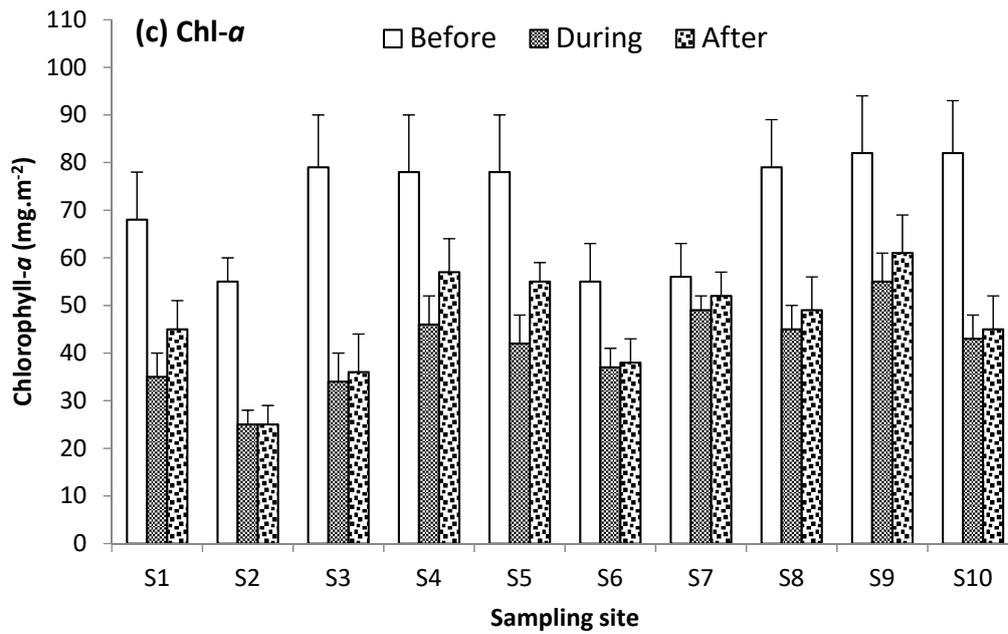


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555 **Figure 10** Accumulation of algal biomass before high flows (15 ML/day), during high flows

556 (55ML/day) and after high flows (15ML/day) at each of the sampling stations along the

557 MacKenzie River; (a) Dry mass; (b) AFDM; (c) Chlorophyll-*a*. Data indicate means \pm SD.

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562 **Diatom Species Index for Australian Rivers (DSIAR)**

563 The DSIAR was calculated to classify the condition of the waterway. The results showed
564 sites S1 to S4 to be in ‘good’ condition with a DSIAR score above 60 under all flow regimes.
565 During baseflow conditions, sites S5 to S8 were in ‘moderate’ condition with DSIAR scores
566 between 41-60 and downstream sites ranged from ‘moderate’ to ‘poor’ condition (Table 4).
567 The DSIAR scores varied more in Reaches 2 and 3 compared to those upstream (Reach 1).
568 Overall the upstream sites had the highest scores (least impacted), with DSIAR scores
569 decreasing downstream. The site furthest downstream (S10) typically returned the lowest, or
570 near lowest, DSIAR score. However, during and after water release events (freshes and high
571 flows) the DSIAR score increased downstream to scores reflective of ‘good’ and ‘moderate’
572 conditions, albeit temporarily (Table 4).

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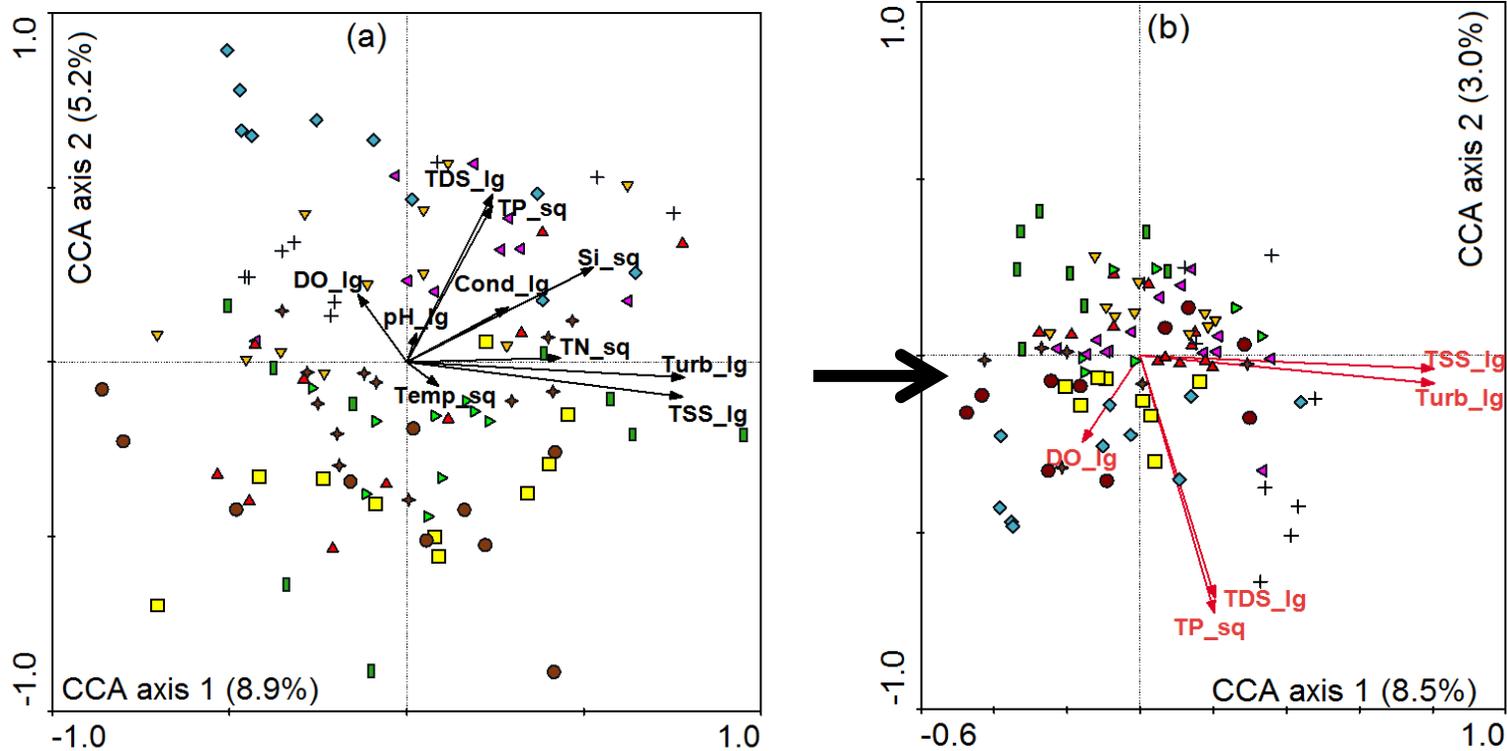
577 **Table 4** The Results of modified Diatom Species Index for Australian Rivers (DSIAR) at
 578 sampling sites along the MacKenzie River in different flow regimes. The scores were used to
 579 classify the waterway as **bad** (0-20), **poor** (21-40), **moderate** (41-60), **good** (61-80) and **high**
 580 (81-100).
 581

	Reach	1				2				3	
	Site	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
	Date										
Base flow (10 ML/day)	28/02/12	78	75	71	68	56	49	49	48	35	-
Base flow (15 ML/day)	17/07/12	77	72	72	66	58	48	42	48	42	38
Base flow (15 ML/day)	9/11/12	78	65	76	68	59	48	43	47	49	41
Base flow (15 ML/day)	25/05/13	75	72	75	67	55	49	45	49	47	48
During freshes (35 ML/d)	21/10/13	82	78	78	66	65	65	61	62	65	62
After freshes (15 ML/d)	25/10/13	81	75	79	69	66	65	61	63	66	65
Before freshes (15 ML/d)	16/12/13	76	74	72	71	55	42	46	48	37	38
During freshes (40 ML/d)	19/12/13	86	75	78	75	64	66	69	63	66	62
After freshes (15 ML/d)	23/12/13	78	75	72	72	57	56	55	55	59	52
After freshes (15 ML/d)	3/01/14	79	75	78	71	59	57	55	45	41	39
After freshes (15 ML/d)	16/04/14	74	71	68	62	61	49	45	41	41	39
Before high flow (15 ML/d)	29/10/14	71	72	73	74	55	42	46	48	37	38
During high flow (55 ML/d)	1/11/14	82	75	78	75	71	68	69	65	66	67
After high flow (15 ML/d)	08/11/14	78	75	72	72	67	66	65	61	59	52
After high flow (15 ML/d)	22/11/14	79	75	68	68	59	57	55	65	45	37

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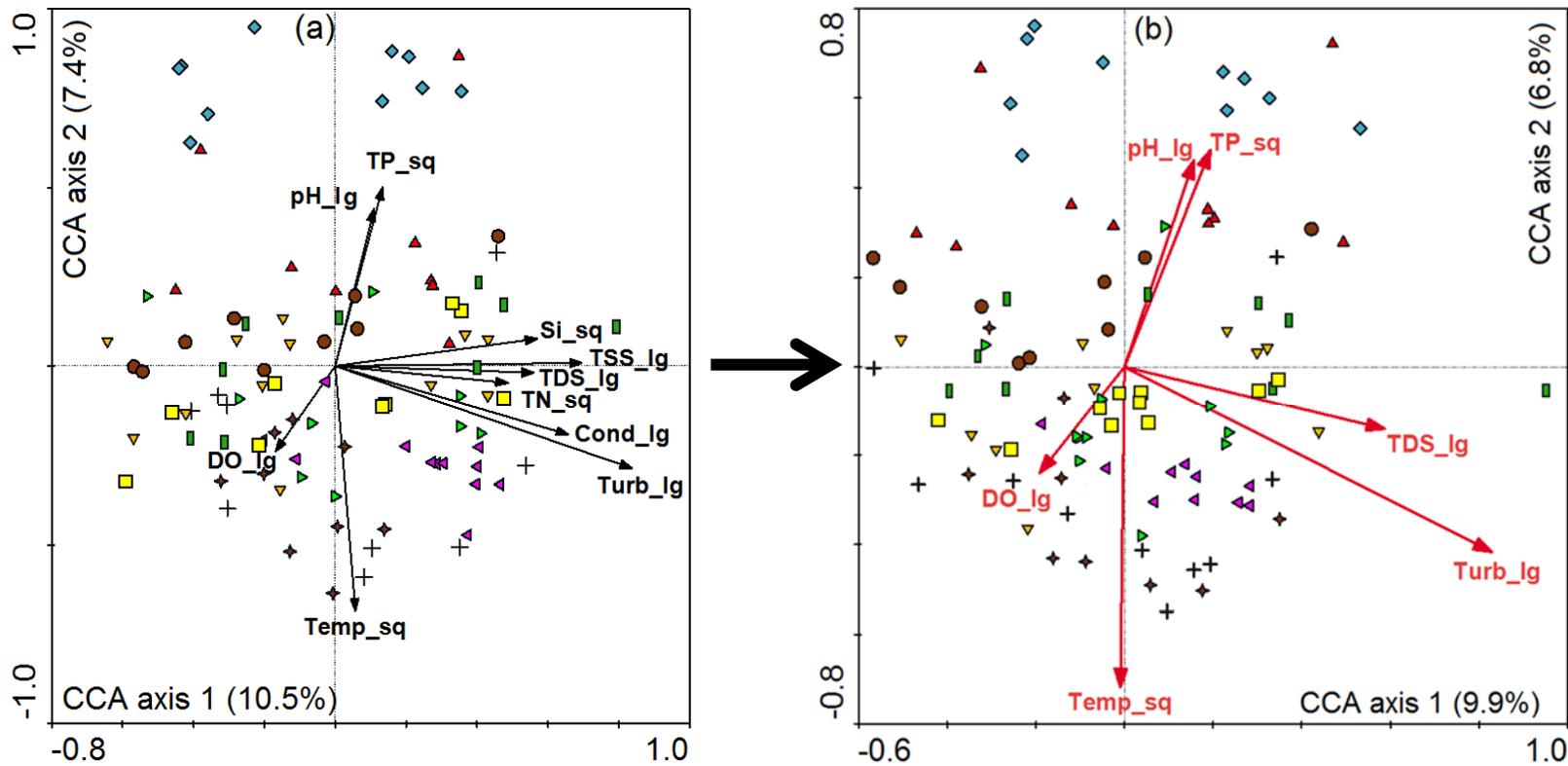
583 **Canonical Correspondence Analysis (CCA)**

584 The CCA was applied using forward-selection to determine the most influential variables,
585 environmental drivers of flow water chemistry and biological properties for all flows. The
586 soft algae were also evaluated under all flow conditions and their responses to environmental
587 variables analysed. This accounted for 8.5% of the species-environment interactions on axis 1
588 and 3.0% on axis 2 (Figure 11). The results highlight that nutrient availability is the major
589 driver in soft algal assemblages in the MacKenzie River. The results showed that algal
590 community assemblages were different during high flow events compared to low flow events.
591 The results showed that downstream species were more closely associated with turbidity,
592 TSS and conductivity. The CCA results indicated that turbidity is a significant factor
593 affecting both soft algae and diatoms. The statistical results of soft algae showed that that
594 upstream species such as *Chlorella vulgaris*, *Dinobryon sertularia*, *Peridinium lomnickii*,
595 *Scenedesmus acuminatus*, *Euastrum* sp. and *Scenedesmus armatus* were associated with high
596 DO, and low TP and TDS, whilst downstream species *Anabaena flos-aquae*, *Monoraphidium*
597 *subclavatum*, *Oocystis pusila* and *O. parva* were associated with high TSS, turbidity, pH, and
598 conductivity. The CCA results showed that the diatom assemblage pattern was different
599 during high flow compared with all the other flow scenarios. Water chemistry had a
600 significant influence on the diatom communities along the MacKenzie River under these
601 circumstances. Diatom assemblages were associated with TDS and turbidity, parallel with
602 axis 1 (9.9%), and pH and TP, temperature and DO parallel with axis 2 (6.8%) (Figure 12).
603 The statistical results of diatom showed that *G. affine*, *F. rhomboides*, *N. heimansioides*, *N.*
604 *radiosa* and *T. flocculosa* were associated with high DO, low pH, and low TP while
605 downstream species such as *E. serpentina* and *S. delicatissima* were associated with higher
606 Si, TDS, turbidity and temperature.



607

608 **Figure 11** Cononical Correspondence Analysis (CCA) of soft algae altogether in different seasons under baseline flows and under different
 609 treatment flow regimes along the MacKenzie River: (a) with all environment variables; (b) significant ($p < 0.05$) variables after forward
 610 selection. February 2012 (brown circle), July 2012 (blue diamond), November 2012 (green box), and June 2013 (red up-triangle), before freshes
 611 (yellow square), during freshes (pink left-triangle), after freshes (right-triangle), before high flow (yellow down-triangle), during high flow
 612 (cross) and after high flow (star)



613

614 **Figure 12** Cononical Correspondence Analysis (CCA) of diatoms altogether in different seasons under baseline flows and under different
 615 treatment flow regimes along the MacKenzie River: **(a)** with all environment variables; **(b)** significant ($p < 0.05$) variables after forward
 616 selection. February 2012 (brown circle), July 2012 (blue diamond), November 2012 (green box), and June 2013 (red up-triangle), before freshes
 617 (yellow square), during freshes (pink left-triangle), after freshes (right-triangle), before high flow (yellow down-triangle), during high flow
 618 (cross) and after high flow (star)

619 **Discussion**

620 Biological structure, ecological processes, ecosystem function and metabolism in riverine
621 ecosystems can change due to flow patterns, water quality and climatic variability. The results
622 presented here for the MacKenzie River show some similarities and dissimilarities to the
623 conclusions of Boulton et al. (2014) and the River Continuum Concept (Vannote et al. 1980,
624 Tornwall et al. 2015, Atazadeh 2017, Atazadeh et al. 2020). For example, the DO dramatically
625 decreases in the middle reach of the river and increases slightly downstream. The water
626 velocity is stagnant in the middle of the river whilst the River Continuum Concept suggests that
627 average velocity increases in downstream sections of rivers. These changes can appear
628 naturally or as a consequence of anthropogenic modification of lotic systems due to water
629 abstractions, diversions as well as through evaporation. Turbidity, total suspended solid (TSS),
630 cations and anions also increased with distance downstream along the MacKenzie River,
631 particularly downstream of points of sediment input and water abstraction in the lower reaches.
632 The range of turbidity, TSS, cations and anions were less in the MacKenzie River (Figure 3).
633 in comparison with the Wimmera and Glenelg Rivers (Anderson and Morison 1989, Chee et al.
634 2009, Alluvium 2013, VEWH 2015, WCMA 2015) likely as the MacKenzie River sits higher
635 in the catchment. The concentration of nutrients increases with distance downstream due to
636 agricultural activities and land use which contribute to increases in the concentration of
637 nitrogen and phosphorous in the MacKenzie River (Figure 3, Table 1). This study reveals the
638 water quality and stream condition of the MacKenzie River are influenced by flow
639 modifications (e.g. construction of Wartook Reservoir in 1887, human settlement in Zumsteins
640 and lower reaches of the river, water diversion to the Mt Zero Channel, water storages and
641 water plant treatment). In general, water quality varies in riverine ecosystems (spatially and
642 temporally) and these changes can provide good evidence to assist in understanding the effects
643 of human impacts and modifications (Heathwaite 2010).

644 The results under water release events (freshes and high flows) showed that the pH of the
645 upstream and downstream reaches became similar (Table 3). Indeed, water release events likely
646 bring acidic water from Wartook Reservoir to the lower parts of the MacKenzie River,
647 reducing the alkalinity of that reach. The pH can determine solubility and availability of
648 nutrients (e.g. N and P) in rivers and streams. The concentration of dissolved oxygen (DO)
649 changes substantially along the river, particularly in the midstream where DO increased greatly
650 in response to water release events. Nutrients (P, N, Si) are a main source of chemical energy
651 for both autotrophic (e.g. cyanobacteria) and heterotrophic (bacteria) microbes in rivers and
652 streams (Allan and Castillo 2007). Flow alteration and human activities (e.g. agriculture)
653 profoundly influenced nutrient dynamics along the MacKenzie River. The nutrients enter the
654 MacKenzie River as dissolved materials from the atmosphere, lithosphere and hydrosphere.
655 The nutrients also enter the MacKenzie River in organic form via biological assimilation
656 (nitrogen assimilation by cyanobacteria). The contribution of the dissolved inorganic and
657 organic nutrients can be more than that of organic materials under water release events, due to
658 greater sediment input into the water. The statistical analyses showed that total nitrogen (TN)
659 and total phosphorus (TP) were high under water release events (Figs 11-12).

660 Algal colonisation and structure are known to be highly responsive to shifts in water
661 quality and flow variation (Ryder et al. 2006, Robson et al. 2008, Atazadeh 2011, Atazadeh and
662 Sharifi 2012, Atazadeh et al. 2014b, Chester and Robson 2014, Stevenson 2014). The results of
663 base flow in the MacKenzie River revealed that upstream has high algal biodiversity whilst
664 middle and downstream reaches have low algal diversity despite a high incidence of algal
665 blooms. Watts et al. (2009b) found similar evidence in the Mitta Mitta River for increasing
666 incidence of algal blooms downstream under constant base flows.

667 Under freshes and high flows, algal monitoring surveys revealed the key indicator taxa
668 increased. Indeed, the relative abundance of diatoms increased during and after freshes and
669 high flows, especially epiphytic diatoms such as *Tabellaria flocculosa*, *Gomphonema affine*,

670 *Navicula radiosa*, and *Encyonema minutum* (epiphytic diatoms) Biggs and Hickey (1994) and
671 Ryder et al. (2006) found diatoms to increase while soft algae decreased under water release
672 events. In contrast, Davie and Mitrovic (2014) found diatom abundance to decline while
673 filamentous green algae and cyanobacteria increased downstream with high water releases in
674 the Severn River (NSW). The CCA results indicated that turbidity is a significant factor
675 affecting both soft algae and diatoms (Figs 11-12). Biggs and Hickey (1994) found
676 physiognomy of algal periphyton changed under different hydraulic gradients in the Ohau
677 River, South Island, New Zealand.

678 The allocation of water for environmental purposes usually requires considerable lead-
679 time, including that required to develop stakeholder support, a strategy for the delivery of
680 environmental water, and a monitoring program to measure benefit. Therefore, most studies
681 have focused on the environmental flow needs of rivers and the water volumes required for
682 regulated systems. The necessity of environmental flows has increased significantly because of
683 the incidence of drought, salinisation and the increased scarcity of water, especially in Australia
684 (Bond et al. 2008). However, there is little focus in water supply systems globally on
685 managing consumptive flows to provide ecological benefits. In fact, there are number of
686 constraints (e.g. existing water amount limitations, physicochemical and biological conditions,
687 social and economic restrictions and political issues) that affect the allocation of water among
688 the consumptive users (Richter and Thomas 2007). The allocation of water between the
689 consumptive users and the environment is dependent on the availability of water and,
690 ultimately, the decision to release. Hence, equitable and effective sharing of the water resource
691 between consumptive users and the environment is critical.

692 This paper, utilising flow releases within the MacKenzie River, provides an opportunity
693 to measure and assess responses to inform manipulations of the nature of releases, allowing for
694 fine-tuning, over short periods, toward an adaptive approach. These results showed that water
695 quality, algal community structure and biological properties respond to different flow regimes.

696 Depending on water availability, environmental watering plans will often seek to release water
697 along the MacKenzie River in order to improve water quality, stream condition and river
698 health, especially for the downstream reaches. These water release events (freshes) are
699 designed to keep the river healthy, but this research shows that the intended consequence of
700 pulsed releases is often short-lived, with no long-term benefit to the health of the river's
701 ecosystem. It seems the ecological impacts of the water release events are not constant in other
702 waterways as well (e.g. Allan et al. 2009). The algal responses to different flow regimes reveal
703 that the downstream sections of the MacKenzie River remain stressed and so require further
704 water release events to enhance its health. The statistical results show the algal community
705 patterns and ecosystem functions are different under different flow regimes where the water
706 release events (freshes) play major role to improve the ecosystem processes in the MacKenzie
707 River. The results show that the water release events (freshes) are important opportunities to
708 improve the ecological conditions of the MacKenzie River with limited water availability,
709 particularly in the middle and lower parts of the river. Clearly, additional work to develop the
710 model for configuring flow regimes for ecological/environmental benefits and setting
711 operational rules of water management with respect to social and economic objectives are
712 needed.

713

714 **Conclusions**

715 The algal communities responded under different flow regimes and showed the role of flows in
716 improving stream condition. These flows can come from consumptive water transfers which is
717 the new challenge for the river scientists and water engineers. This research provides the means
718 by which stream condition may be enhanced from consumptive flows to complement the
719 benefit derived from environmental flows. In addition, this research shows that benefits can
720 accrue when management moves from a contest between volumes for allocations, to a
721 coordinated approach to bring environments benefits without compromising consumptive

722 needs. The results reveal that the lower parts of the river remain under stress due to flow
723 alteration despite the allocation of environmental flows. However, good ecological condition
724 can be achieved if consumptive flows are also released in a manner that benefits the ecology of
725 the River. There are clear benefits that would accrue from integrating environmental flows and
726 consumptive flow operations. This would be achieved by respective operators exchanging flow
727 release plans and for this to be coordinated between water agencies. The agencies have this as a
728 planning goal and so the way is paved for more effective use of all water releases. The
729 integrated ecological response based on algal response to hydrological changes and water
730 policy initiatives will help to provide for a healthy and resilient working river which supports
731 the environmental values.

732

733 **Acknowledgments**

734 Thanks to Grampians Wimmera Mallee Water (GWMWater, Australia), Federation University
735 (Australia), Wimmera Catchment Management Authority (WCMA, Australia) and University
736 of Tabriz (Iran). The authors would also like to thank the anonymous reviewers and the editor
737 for their valuable comments and corrections that much improved the final version of the
738 manuscript.

739

740 **Ethical Approval:** Not Applicable

741 **Consent to Participate:** All authors have consented to participate in this paper.

742 **Consent to Publish:** All authors have consented to publish this paper.

743 **Authors Contributions:** EA carried out main tasks including the experiment design, data
744 writing and analysing, editing the paper. PG carried out in the experiment design and editing
745 the MS. KM carried out the data analysing and editing the MS. AB carried out the experiment
746 design. PN carried out editing the MS.

747 **Funding:** This project was funded by Grampians Wimmera Mallee Water (GMMWater,
748 Australia), Project number: 30096716

749 **Competing Interests:** The authors declare that they have no known competing financial
750 interests or personal relationships that could have appeared to influence the work reported in
751 this paper.

752 **Availability of data and materials:** All data generated or analysed during this study are
753 available upon the request.

754

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