1   **Decadal effects of climate, nitrogen removal from wastewater, and lotic**

2   **characteristics on phytoplankton in small phosphorus-rich prairie streams**

3

4   Nathanael T. Bergbusch[1,2], Nicole M. Hayes[1,2,3], Gavin L. Simpson[2], Vanessa J.

5   Swarbrick[1,2,4], Zoraida J. Quiñones-Rivera[2], and Peter R. Leavitt[1,2,5,*]

6

7   [1] Limnology Laboratory, Department of Biology, University of Regina, Regina,

8   Saskatchewan S4S 0A2 Canada

9   [2] Institute of Environmental Change and Society, University of Regina, Regina,

10   Saskatchewan, S4S 0A2, Canada

11   [3] Present address: Biology Department, University of Wisconsin Stout, Menomonie,

12   Wisconsin, 54751, USA

13   [4] Present address: Alberta Environment and Parks, Edmonton, Alberta, T6B 2X3, Canada

14   [5] Global Institute for Food Security, Queen's University Belfast, Belfast, Antrim, BT9

15   5DL, United Kingdom

16   [*]Author for correspondence P.Leavitt@QUB.ac.uk

17   **Contacts**:
18   NTB nbergbusch@gmail.com, ORCID 0000-0002-8062-6876
19   NMH hayesn@uwstout.edu; ORCID 0000-0002-5664-9939
20   GL Gavin.Simpson@uregina.ca, ORCID 0000-0002-9084-8413
21   VJS vanessa.swarbrick@gmail.com, ORCID 0000-0002-9323-6172
22   ZQR Zoraida.Quinones-Rivera@uregina.ca, ORCID 0000-0001-8418-8255
23   PRL Peter.Leavitt@uregina.ca, P.Leavitt@QUB.ac.uk, ORCID 0000-0001-9805-9307

24   **Running Head: Effluent nitrogen effects on streams**

Abstract

1. Reduction of urban nitrogen (N) pollution through biological nutrient removal (BNR) technologies has been proposed as an effective strategy to reduce eutrophication. However, little is known about the effects of selective N removal on streams.

2. Changes in phytoplankton abundance and community composition in connected first- and higher-order prairie streams were quantified over six years to determine how ecosystem health varied following a shift in wastewater treatment from tertiary (mainly phosphorus, P, removal) to BNR (NH_4^+ and P removal) technologies.

3. Time series of water-column biomarker pigment concentrations (carotenoids, chlorophylls) at nine stations sampled biweekly (May to September) were analyzed using generalized additive models (GAMs) to quantify spatio-temporal variation in phytoplankton assemblages and effects of wastewater treatment before (2010-2012) and after (2017-2019) installation of BNR processes.

4. GAMs explained 66-80 % of deviance in phytoplankton abundance and showed that BNR reduced effluent N concentrations (NO_3^- 67%, NH_4^+ 97%) and shifted community composition from chlorophytes and cyanobacteria towards siliceous algae (diatoms).

5. BNR upgrade reduced phytoplankton abundance ~50-60 % in effluent-impacted reaches relative to upstream values, although effects of BNR upgrade on total phytoplankton abundance were obscured by high inter-annual variation in discharge and baseline Chl a concentration.

6. Overall, BNR improved lotic ecosystems by reducing levels NH_4^+ to below toxicity guidelines, and favouring nutritious diatoms over colonial and potentially-toxic taxa.

1 Introduction

Excess influx of nitrogen (N) and phosphorous (P) from human activities has led to cultural eutrophication, freshwater degradation, and blooms of harmful algae and cyanobacteria (Carpenter et al., 1998). Such nutrient pollution is expected to increase during the 21st century, yet debate remains over the most effective management strategies to diminish its damage (Wurtsbaugh, Paerl, & Dodds, 2019). Freshwater management focuses on nutrient sources from municipal wastewater, stormwater, agricultural fertilizer, and livestock (Bennett, Carpenter, & Caraco, 2001; Bodirsky, Popp, Lotze-Campen, Dietrich, & Rolinski, 2014; Houlton, Boyer, Finzi, Galloway, & Leach, 2013), but given high processing costs, there is scientific uncertainty over the value of regulating one (P) or multiple (N, P) macronutrients (Paerl, Scott, McCarthy, Newell, & Gardiner, 2016; Schindler, 2006; Schindler, Carpenter, Chapra, Hecky, & Orihel, 2016). The historical paradigm for lakes is that P supply controls the productivity of lentic ecosystems (Schindler, 1977, 2006), whereas nutrient regulation of streams and rivers appears less singular (Dodds & Smith, 2017). In addition, recent evidence suggests that water quality in P-replete lakes, such as those in the northern Great Plains (NGPs; Bogard, Vogt, Hayes, & Leavitt, 2020; Donald, Bogard, Finlay, & Leavitt, 2011; Leavitt, Brock, Ebel, & Patoine, 2006) and other regions (Elser et al., 2007; Paerl et al., 2016), may be uniquely degraded by N pollution. However, to date, few studies have quantified the degree of recovery of P-rich freshwaters from reductions in N influx alone (Paterson, Schindler, Hecky, Findlay, & Rondeau, 2011; Schindler et al., 2016) or in conjunction with P (Shatwell & Köhler, 2019), particularly in lotic ecosystems (Dodds & Smith, 2017).

73   Urban wastewater treatment plant (WWTP) effluent can cause eutrophication,

74   anoxia, and degradation of freshwaters downstream of cities (Hamdhani, Eppehimer, &

75   Bogan, 2020; Holeton, Chambers, Grace, & Kidd, 2011; Leavitt et al., 2006). To reduce

76   these threats, some cities in developed countries have upgraded their facilities in the past

77   30 years to include biological nutrient removal (BNR), a process which uses microbial

78   nitrification and denitrification to substantially reduce or eliminate wastewater NH_4^+ and

79   NO_3^- (Carey & Migliaccio, 2009; Tchobanoglous, Burton, & Stensel, 2003) and reduce

80   eutrophication in downstream water bodies (Holeton et al., 2011). However, despite

81   expected environmental benefits, the high costs of upgrades (> \$100 million per WWTP)

82   (Cooper, Upton, Smith, & Churchley, 1995; Environmental Protection Agency, 2007),

83   combined with scientific debates over the incremental value of N removal (Paerl et al.,

84   2016; Schindler et al., 2016; Wurtsbaugh et al., 2019), has led to uneven adoption of

85   BNR protocols by cities (Organization for Economic Cooperation and Development

86   2020). Resolution of this impasse requires not only experiments demonstrating the

87   unique effects of N on P-replete systems (Bogard et al., 2020; Finlay, Patoine, Donald,

88   Bogard, & Leavitt, 2010; Harris et al., 2016;), but also evidence that N reductions benefit

89   downstream ecosystems (Schindler et al., 2016; Shatwell & Köhler, 2019). Further,

90   given that many urban centres and intensive livestock operations discharge into flowing

91   waters, documentation of the effects of BNR upgrades on streams and rivers is essential

92   to inform scientific, management, and policy decisions concerning nutrient pollution.

93   Despite impairment of many of the world's lotic ecosystems by nutrient pollution

94   and resulting algal and cyanobacterial blooms (Chambers et al., 2012; Paerl & Otten,

95   2013), little is known of how N regulates phytoplankton development in fluvial systems

4

96   (Dodds & Smith, 2017). Production of lotic phytoplankton in large rivers is controlled by

97   nutrients when light is not limiting (low turbidity, reduced canopy cover) (Hutchins et al.,

98   2010; Vannote, Minshall, Cummins, Sedell, & Cushing, 1980) and water has a long

99   residence time (Hardenbicker, Rolinski, Weitre, & Fischer, 2014; Leland, 2003;

100   Reynolds & Descy, 1996). Both P limitation (Chambers et al., 2012; Van Nieuwenhuyse

101   & Jones, 1996) and N-P co-limitation (Greene, Miller, Shiroyama, & Maloney, 1975) of

102   phytoplankton growth have been documented for large rivers, and fundamental effects of

103   nutrient pollution have been recognized in other lotic ecosystems in agricultural regions

104   or near urban centers (Paul & Meyer, 2001; Walsh et al., 2005; Walsh & Wepener, 2009).

105   In contrast, relatively little is known about the unique effects of N on suspended lotic

106   phototrophs in rivers (Dodds & Smith, 2017) or in small eutrophic streams (Breuer, Janz,

107   Farrelly, & Ebke, 2017; Wu, Schmalz, & Fohrer, 2011). Further research is needed to

108   improve management of flowing waters subject to nutrient pollution, particularly in

109   productive agricultural regions.

110   Experimental evidence suggests that eutrophication, and the chemical form of N

111   pollution (NH_4^+, NO_3^-, urea, organic N) in particular, can differentially affect component

112   populations within lotic phytoplankton (Dodds & Smith, 2017). While phytoplankton

113   were previously considered poor bioindicators of lotic trophic status (Wehr & Descy,

114   1998), diatoms rich in fatty acids (Opute, 1974; Taipale et al., 2013) are now assumed to

115   predominate in pristine or undisturbed lotic ecosystems (Lobo, Heinrich, Schuch, Wetzel,

116   & Ector, 2016; Mischke, Venohr, & Behrendt, 2011; Stevenson, Pan, & Van Dam, 2009),

117   whereas planktonic chlorophytes (Mischke et al. 2011) and potentially-toxic, colonial

118   cyanobacteria (Zurawell, Chen, Burke, & Prepas, 2005) are more common in eutrophied

5

119   waters (Affourtit, Zehr, & Paerl, 2001; Baker & Humpage, 1994; Kim et al., 2020).

120   Experiments in lentic systems (Bogard et al., 2020; Donald et al. 2011; Donald, Bogard,

121   Finlay, Bunting, & Leavitt, 2013; Swarbrick, Simpson, Glibert, & Leavitt, 2019;) and,

122   more recently, in streams and rivers (Breuer et al., 2017; Solomon, Jackson, & Glibert,

123   2019; Varol & Sen, 2018), suggest that cyanobacteria are favoured by intermediate levels

124   of NH_4^+ (1-4 mg/L), whereas chlorophytes benefit from extremely high influx of NO_3^- or

125   NH_4^- (> 8 mg/L). Diatoms prefer NO_3^-, while their growth is suppressed by exposure to

126   NH_4^+ in cool illuminated waters (Glibert et al., 2016; Swarbrick et al., 2019). However,

127   expected effects of N on phytoplankton community composition may be confounded by

128   intrinsic differences in characteristics of phototrophs (e.g., cell size, motility, generation

129   time, N metabolism, photo-sensitivity, etc.) that influence suitability of flowing-water

130   habitats (Reynolds, 1988; Reynolds, 1994). Further, while larger streams may favour

131   cyanobacteria (Chételat, Pick, & Hamilton, 2006; Stevenson & White, 1995), these

132   systems also often exhibit pronounced seasonal ontogeny from spring (and fall) diatom

133   peaks to mid- and late-summer blooms of chlorophytes and cyanobacteria (Breuer, Janz,

134   Farrelly, & Ebke, 2016; Moorhouse et al., 2018) which may obscure effects of N.

135   Together, these observations suggest that changes in N influx should interact with stream

136   size and seasonal community development to regulate lotic phytoplankton abundance and

137   composition.

138   In this paper, we studied the effect of improved wastewater treatment from

139   tertiary to BNR processes on phytoplankton assemblages in P-rich streams of the

140   northern Great Plains (NGPs). Prior to upgrade, effluent was characterized by extremely

141   high NH_4^+ (> 30 mg N-NH_4^+/L), but in 2016 the WWTP was upgraded from chemical P

142 removal (low P, NH_4^+-rich effluent) to BNR removal of N and P (low P- and NH_4^+,

143 moderate NO_3^-). Here we used generalized additive models (GAMs) to quantify

144 phytoplankton assemblages bi-weekly from May to September over six years, evenly

145 split between three years before and after the WWTP upgrade. We sought to quantify: 1)

146 effects of the WWTP on the spatio-temporal variation in phytoplankton abundance and

147 composition before and after the upgrade; 2) which physico-chemical parameters

148 associated with the WWTP regulated phytoplankton communities, and; 3) the relative

149 importance of the WWTP upgrade and natural controls arising from climate and regional

150 land-use. We hypothesized that: 1) phytoplankton biomass would increase in WWTP-

151 impacted reaches due to elimination of toxic NH_4^+ (Waiser, Tumber, & Holm, 2011); 2)

152 community composition would shift from chlorophytes and cyanobacteria to diatoms in

153 the impacted sites after NO_3^- replaced NH_4^+ as the main form of dissolved N (Glibert et

154 al., 2016; Swarbrick et al., 2019) and; 3) WWTP effects would overwhelm seasonal

155 patterns of phytoplankton ontogeny (Stevenson & White, 1995; del Giorgio, Vinocur,

156 Lombardo, Tell, 1991; Solomon et al., 2019).

157 **2 Methods**

158 **2.1 Study area**

159   The study area consisted of first-order Wascana Creek (WC) and the higher-order

160 (reach-dependent) Qu'Appelle River (QR) (Figure 1), within the 52,000 km^2 Qu'Appelle

161 River catchment in southern Saskatchewan, Canada (52.94° N, 106.45° W). These

162 streams drain mainly agricultural land (winter wheat, canola, mustard), urban centers

163 (Regina, Moose Jaw, Lumsden), livestock operations, and native prairie grassland (Hall,

164 Leavitt, Quinlan, Dixit, & Smol, 1999). Wascana Creek flows northwest through

7

165 Wascana Lake before receiving treated urban wastewater from the City of Regina and

166 joining the QR ~65 km downstream of the WWTP. The natural headwaters of the QR

167 arise near Eyebrow Lake, are supplemented by water from mesotrophic Lake

168 Diefenbaker, and flow eastward through eutrophic Buffalo Pound Lake into

169 hypereutrophic Pasqua Lake (Figure 1). Hypereutrophic Wascana and sub-saline, meso-

170 eutrophic Last Mountain lakes drain into the QR north of Regina (Hall et al., 1999; Haig

171 et al., 2020).

172 　　　　Streams were sampled at nine locations from WC headwaters to downstream QR

173 near Pasqua Lake during May-September 2010-2012 (pre-upgrade), and 2017-2019

174 (post-upgrade) (Figure 1). Two sites were located upstream of the WWTP outfall (before

175 and after Wascana Lake), three were located along WC between the WWTP outfall and

176 the WC-QR confluence, and three were located along QR after the confluence (one

177 before Last Mountain Creek inflow, two downstream). In addition, one QR station was

178 sampled upstream of the WC-QR confluence. In 2010, sites five and six were not

179 sampled, whereas during 2017, site four was not sampled (Figure 1).

180 **2.2 Field methods**

181 　　　　Stream stations were each sampled between 9:00 and 14:00 hrs on two successive

182 days every two weeks. Environmental parameters included air and water temperatures

183 (°C), cloud cover (% open sky), wind velocity (km/s), dissolved oxygen content (mg O_2/

184 L), specific conductivity (µS/cm), salinity (g total dissolved solids, TDS/L), and pH. *In*

185 *situ* parameters were recorded at surface, mid-column, and bottom-water depths using a

186 YSI Model 85 meter and a calibrated Oakten model 20 pH meter. Instantaneous water

187 velocity (m/s), maximum depth (m), and channel cross sectional area (m^2) were measured

188   for all wade-able locations (< 1.25-m depth) using a calibrated Swoffer Instruments

189   model 2100 current velocity meter, and discharge was calculated using Buchanan and

190   Somers' (1976) two-point and 6/10th-depth methods. Whole-water samples were

191   collected from each site at surface and mid-column depths in sterile containers,

192   combined, and screened through a 243-µm aperture mesh. All samples were processed in

193   the laboratory by 17:00 h on the day of collection.

194   **2.3 Laboratory methods**

195   Particulate matter from whole water was filtered onto GF/C 1.2-µm pore filters

196   for subsequent analysis of biomarker pigments by high pressure liquid chromatography

197   (HPLC) or elemental composition and stable isotopes via combustion. Water was also

198   filtered through 0.45-µm pore membrane filters and frozen for analysis of nutrient

199   chemistry and dissolved C and N isotope analyses. Filters and filtered water were frozen

200   at -20°C until analysis.

201   Phytoplankton abundance and community structure was quantified by analysis of

202   biomarker pigments (nmoles pigment/L) using standard trichromatic (Jeffrey &

203   Humphrey, 1975) and HPLC methods (Leavitt & Hodgson, 2001). Pigment analyses

204   were run on duplicate filters and under low-light conditions. HPLC used an Agilent 1100

205   HPLC system using in-line photodiode array and fluorescence detectors, as detailed in

206   Donald et al. (2013) and Bogard et al. (2020). Pigments were tentatively identified on the

207   basis of absorbance maxima and chromatographic position relative to an internal standard

208   (Sudan II dye), authentic pigment compounds from DHI Denmark, and laboratory

209   reference materials (Leavitt & Hodgson, 2001). Biomarker pigments included Chl a and

210   β carotene (total phytoplankton), fucoxanthin (siliceous algae, some dinoflagellates),

211 diadinoxanthin (dinoflagellates, some siliceous algae), peridinin (dinoflagellates),

212 diatoxanthin (mainly diatoms), alloxanthin (cryptophytes), chlorophyll b (chlorophytes),

213 lutein + zeaxanthin (chlorophytes and cyanobacteria), myxoxanthophyll (colonial

214 cyanobacteria), canthaxanthin (Nostocales cyanobacteria), aphanizophyll (N_2-fixing

215 cyanobacteria), and echinenone (total cyanobacteria) (Donald et al., 2011, 2013). We also

216 calculated the ratio of labile Chl a to its stable degradation product, pheophytin a, to

217 evaluate whether pigments represented live material or detritus. Observed ratios were

218 13.68 ± 0.57 in suspended particulate samples, therefore we assumed that pigments

219 mainly represented metabolically-active phototrophs (Bergbusch, 2020; Leavitt &

220 Hodgson, 2001). Consistent with this inference, preliminary microscopic examination

221 identified whole cells and colonies, and suspended communities grew rapidly in response

222 to nutrient amendments in bottle bioassay experiments (Bergbusch, 2020).

223   Nutrient chemistry was analysed using a Lachat QuikChem 8500 FIA automated

224 ion analyzer following standard procedures (APHA-AWWA/WEF, 1998) at the Institute

225 of Environmental Change and Society, University of Regina. Analytes included NH_3 +

226 NH_4^+ (hereafter ammonium), NO_2^- + NO_3^- (nitrite+nitrate, as nitrate), total dissolved

227 nitrogen (TDN), soluble reactive phosphorous (SRP), and total dissolved phosphorous

228 (TDP). In addition, monthly analyses of NH_4^+, NO_2^-, NO_3^- , TDN and TDP were

229 provided by City of Regina for all years and most sites. Managers of the municipal

230 WWTP (EPCOR, Alberta, Canada) supplied weekly nutrient concentration data for final

231 treated effluent.

232   Stable isotopes of carbon (C) and N from filtered water were measured for all

233 sampling sites using the protocols of Leavitt et al. (2006). Briefly, GF/C-filtered water

234   samples were freeze-dried (1 week, 0.01 Pa) to obtain residue for analysis. In general,

235   10-15 mg of solids from wastewater-impacted river sites and 20-25 mg was used for

236   isotope analysis of undisturbed sites, although masses were decreased for pre-upgrade,

237   effluent-impacted samples to allow for higher TDN content during 2010-2012. All solids

238   were folded into individual tin capsules and combusted using a Costech model ECS4010

239   elemental analyzer coupled to a Thermo Finnigan Delta V isotope ratio mass

240   spectrometer (IRMS) to quantify C and N content (% dry mass) and C and N isotope

241   values (‰) using standard notation ($\delta^{13}C$, $\delta^{15}N$) relative to calibrated laboratory standards.

242   **2.4 Hydrometic data**

243   Stream discharge (Q) was determined using a combination of three highly-

244   correlated methods: 1) direct measurement and calculation using Buchanan & Somers'

245   (1976) two-point and $6/10^{th}$-depth methods; 2) instrumental records from government

246   gauging stations, and; 3) values calculated using the drainage area ratio method following

247   Gianfagna, Johnson, Chandler, & Hofmann (2015) as modified by Swarbrick (2017). In

248   2018 and 2019, Q was calculated for all wade-able sites by summing the product of

249   discrete velocities and their respective cross-sectional areas, measured at 10-15 points

250   along a transect following the two-point (> 0.75 m; reading at 0.2 and 0.8 of depth) and

251   6/10th method (< 0.75 m; one reading at 0.6 x depth) (Buchanan & Somers, 1976). In

252   other years, we used Q recorded at provincial and federal hydrometric gauging stations

253   (sites 5 and 7 only), following Swarbrick (2017). For deeper sites, we applied the

254   drainage area ratio method of Gianfagna et al. (2015) to estimate prorated discharge

255   ($Q_{incremental}$) based on the incremental effective drainage area (EDA), distance to nearest

256   gauging station, and other inputs. $Q_{incremental}$ was calculated by multiplying Q at the

257　nearest gauging station (Q_{gauged}) by the ratio of the EDA at the sampling site to that at the

258　gauging station ($A_{incremental}$) and the EDA of the gauged site (A_{gauged}):

259　　　　　　　　　　　$Q_{incremental} = Q_{gauged} * (A_{incremental}/A_{gauged})$.

260　EDA was defined as the maximum area that could contribute runoff during average

261　hydrologic conditions (Mowchenko & Meid, 1983) and was estimated for each site using

262　the federal Agricultural and Agri-Food Canada Watersheds Project dataset (AAFC,

263　2008), or calculated from the AAFC EDA total boundary layer, topographic and

264　hydrologic data using ESRI ArcGIS 10.1 (Natural Resources Canada, 2016). These

265　estimates of Q were highly correlated ($R^2_{adj} = 0.86$, $p < 0.001$) with both gauging stations

266　and drainage-area ratio method values during 2018 and 2019 (Bergbusch, 2020).

267　**2.5 Numerical analyses**

268　　　　We employed generalized additive models (GAMs; Wood, Pya, & Säfken, 2016)

269　to estimate spatio-temporal trends of phytoplankton assemblages and their responses to

270　physico-chemical variables. We fitted five spatio-temporal models, one for each main

271　pigment: trichromatic Chl a (total phytoplankton), fucoxanthin (siliceous algae),

272　alloxanthin (cryptophytes), Chl b (chlorophytes), and echinenone (cyanobacteria). In

273　addition, we developed two models using physico-chemical parameters as predictors of

274　total phytoplankton abundance (as Chl a, µg/L) and community composition (other

275　biomarkers, nmoles/L). Other pigments were excluded from the main models either

276　because compounds were infrequent (e.g., peridinin) or exhibited significant taxonomic

277　overlap with selected biomarkers (e.g., cyanobacterial carotenoids). Additionally, due to

278　missing samples in 2017, we estimated trichromatic Chl a values in that year from the

279 overall relationship between HPLC- and trichromatic-derived Chl a (Supporting

280 Information Figure S1). To further evaluate the rigour of this substitution, we also

281 modeled spatio-temporal trends in HPLC Chl a in comparison to those developed solely

282 using trichromatic Chl a (Supporting Information Figure S2). In all GAMs, the QR site

283 immediately upstream of the WC-QR continuum was excluded from analysis as it

284 prevented us from explicitly modeling spatial patterns. Instead, this site was compared

285 separately to values in WC and the QR just above and below confluence, respectively, to

286 better identify the mechanisms contributing to downstream patterns in the QR.

287   Spatio-temporal GAMs included a random effect term for study year, a marginal-

288 smooth term for day of year (DOY) and distance along the stream flow path, and an

289 interaction term for DOY and distance modelled as a tensor-product smooth (Wood,

290 2017). Distance and DOY terms were included in tensor-product smooths with year to

291 allow for changes in the spatial and seasonal effects between years. These models

292 allowed us to quantify seasonal differences in phytoplankton along our lotic continuum.

293 We chose physico-chemical parameters based on biological relevance and included

294 distance, DOY, Q, pH, nutrients (NO_3^-, NH_4^+, SRP), the ratio of TDN to SRP

295 (TDN:SRP), specific conductivity, and water temperature. While 2-week lag effects

296 were also considered, they did not improve model fit and were discarded. To achieve

297 better dispersion, specific conductivity, NO_3^-, NH_4^+, and TDN:SRP were $\log_{10}$-

298 transformed, whereas SRP and discharge were square-root transformed. Correlations

299 between predictor parameters were not significant ($r < 0.35$, $p > 0.10$), suggesting

300 collinearity was low among variables. Both the range and null space of the smoothers'

301 penalty matrices were fully penalized to perform variable selection, improve model fit

302 and parsimony, and help deal with the potential concurvity of smooths (Marra & Wood,

303 2011).

304   Models were run in R (version 3.6.2; R Core Team, 2019) using *mgcv* (version

305 3.6.2) with residual marginal likelihood (REML) smoothness selection (Wood, 2011,

306 2017; Wood et al., 2017). We assumed the response variable to be conditionally gamma

307 distributed (positive, continuous responses). We used the log link function for GAMs of

308 Chl a biomass and fucoxanthin models because concentrations were always > 0 µg/L,

309 while a Tweedie distribution (non-negative, continuous responses) was used for other

310 pigments which occasionally exhibited concentrations of ~0 µg/L. Similarly, Tweedie

311 distributions were used for physico-chemical models that included all pigments as

312 responses. We did not fit models that accounted for data censoring because only ~5% of

313 values were 0 for the entire pigment dataset (< 20% for echinenone) and likely truly

314 absent (0.0 nmole pigment/L) and because preliminary analysis with Bayesian

315 Regression Models using *Stan* (BRMS, version 2.10.0) (Bürkner, 2018) greatly

316 overestimated observed pigment concentrations (i.e., exhibited a poor fit relative to *mgcv*

317 models).

318   The physico-chemical model used measured stream characteristics to estimate

319 global (all pigments) and pigment-specific smooth terms. Global terms were tensor-

320 product smoothers, whereas pigment-specific terms were factor smoothers (Pedersen,

321 Miller, Simpson, & Ross, 2019) because all parameters exhibited a marked decrease in

322 residuals and better homogeneity of residuals when taxa-specific responses were

323 included. We assessed basis size, dispersion of residuals, homogeneity of variance, and

324 the relationship between the observed and predicted response for all models to ensure

14

325 model assumptions were not violated. Smoothers were selected using REML (Wood,

326 2011). Physico-chemical model marginal smooths and spatio-temporal predictions were

327 visualized in R with plots produced with the *ggplot 2* package (version 3.2.1) (Wickham,

328 2016).

329 **3 Results**

330 **3.1 Stream conditions**

331 Discharge was generally higher in all stream reaches during 2010-2012 than in the

332 years after the BNR upgrade (Figure 2, Table 1). Prior to upgrade, mean ($\pm$ SE) flow in

333 headwaters (4.08 ± 1.28 m^3/s) was 10-fold greater than that during years following BNR

334 installation (0.41 ± 0.17 m^3/s), whereas flow in WC below the WWTP outfall was nearly

335 5-fold greater (5.14 ± 0.94 m^3/s vs. 1.17 ± 0.18 m^3/s). In general, elevated Q was

336 recorded mainly during 2010 (3.60 ± 0.88 m^3/s) and 2011 (9.37 ± 2.25 m^3/s), while mean

337 flow during 2012 (2.12 ± 0.51 m^3/s) was only modestly higher than that after the plant

338 upgrade ($0.85 - 1.49$ m^3/s). Because the volume of urban effluent was similar in all

339 years, the contribution of WWTP discharge to total flow in WC varied markedly among

340 seasons and years (Supporting Information Figure S3). For example, during pre-upgrade

341 years, the flow in WC was completely attributable to the WWTP for greater than half of

342 the growing season, whereas flow contribution was more variable among months (0-

343 100%) during 2010-2012 (Supporting Information Figure S3). In addition, downstream

344 QR experienced much faster mean flow during 2010-2012 (27.64 ± 2.73 m^3/s, max of

345 127 m^3/s) than that during 2017- 2019 (3.46 ± 0.22 m^3/s). Overall, discharge was

346 maximal during May in most reaches and years.

347   Temperature, pH, and specific conductivity were generally similar in years before

348   and after the WWTP BNR upgrade, although parameters varied markedly on both spatial

349   (pH, specific conductivity) and seasonal scales (temperature) (Table 1, Figure 2).

350   Specific conductivity was greatest in headwaters, decreased after Wascana Lake,

351   increased with urban effluent influx, and often remained elevated in downstream reaches.

352   Similarly, pH of WC decreased from 9 to 8 with effluent influx, before rebounding to 9

353   after WC confluence with the QR. Stream temperature was unchanged by urban

354   wastewater inputs, but ranged from 7 to 30°C within the study season (May-September)

355   and was greatest in all years during July.

356   In most years, influx of urban effluent increased concentrations of NO_3^- in WC

357   relative to levels in headwaters or downstream reaches of the QR (Table 1, Figure 2).

358   However, despite higher levels of NO_3^- in treated effluent prior to BNR upgrade

359   (Supporting Information Figure S3), mean ($\pm$ SE) in-stream concentrations of NO_3^- were

360   similar in effluent-impacted reaches before (1.98 $\pm$ 0.16 mg N/L) and after changes in the

361   WWTP (1.70 $\pm$ 0.16 mg N/L), with greatest NO_3^- values near the outfall after upgrade,

362   and highest concentration ~50 km downstream prior to BNR. Headwater levels of NH_4^+

363   were low in all years (< 0.5 mg N/L), before enriched effluent (Supporting Information

364   Figure S3) increased concentrations in urban-impacted reaches of WC prior to (6.64 $\pm$

365   0.96 mg N/L) but not after installation of BNR (0.33 $\pm$ 0.07 mg N/L) (Figure 3.2).

366   Despite higher dissolved N levels in WC during 2010-2012, NH_4^+ and NO_3^- levels were

367   comparatively low and similar (< 1.55 mg N/L) in all years in downstream QR.

368   Similarly, while influx of ^{15}N-enriched effluent ($\delta^{15}N$ 15-30 ‰ in all years) elevated $\delta^{15}N$

369   of dissolved N in stream water, those isotope values declined following confluence of

370   WC and QR in all years, reflecting influx of unenriched N from the upstream QR (Table

371   1). In general, mean SRP levels were elevated in pre-upgrade years in all reaches, then

372   remained the same or declined after effluent influx in pre-upgrade (0.36 ± 0.02 mg P/L)

373   and post-upgrade intervals (0.07 ± 0.01 mg P/L), respectively. Overall, TDN:SRP mass

374   ratios were much greater at all reaches during 2010-2012.

375   **3.2 Spatio-temporal distribution of phytoplankton**

376   Spatio-temporal GAMs of Chl a explained 71.4% of deviance in total

377   phytoplankton abundance, with significant effects of year, DOY, distance, and their

378   interaction, whether analyzed for trichromatic- (Figure 3) or HPLC-derived pigment

379   (Supporting Information Figure S2). Total phytoplankton abundance in WC generally

380   increased relative to headwater values following inputs of N-rich tertiary-treated effluent,

381   but declined after influx of BNR-treated wastewater, largely reflecting changes in

382   chlorophyte abundance (Chl b) (Table 1, Figure 3). Despite this pattern, mean Chl a in

383   effluent-impacted reaches of WC changed little as a result of the WWTP upgrade (~ 45

384   µg Chl a/L), largely reflecting high interannual variation in Q and phytoplankton

385   abundance in upstream reaches. For example, headwaters near Wascana Lake had higher

386   mean levels of phytoplankton Chl a during 2017-2019 (67.30 ± 5.60 µg/L) than in 2010-

387   2012 (28.52 ± 3.74 µg/L) (Figure 2, Supporting Information Figure S5), whereas values

388   in the QR showed little consistent pattern. Upstream of the WC-QR confluence in QR,

389   phytoplankton abundance was elevated during 2010-2012 (56.25 ± 8.08 Chl a/L) but not

390   in 2017-2019 (22.68 ± 3.71 µg Chl a/ L) (Table 1), while downstream of the confluence,

391   Chl a concentrations were elevated during 2012 (75.0 ± 8.6 µg/L) and 2017-2019 (65.72

392   ± 5.39 µg/L) relative to other years (< 45 µg/L). Taken together, these findings suggest

17

393   that BNR technologies improved water quality in a first-order lotic ecosystem with

394   eutrophic headwaters by reducing phytoplankton abundance compared to headwaters;

395   however, this effect was limited to a ~50-km reach between the WWTP and confluence

396   with a higher-order stream.

397   WWTP upgrade to BNR processes shifted the phytoplankton community to both

398   favour siliceous algae and diminish the historical importance of chlorophytes in effluent-

399   impacted reaches of WC (Figure 3). Overall, deviance explained for each biomarker

400   pigment was high, ranging from 65.7% for alloxanthin (cryptophytes) to 79.6 % for

401   echinenone (cyanobacteria), with intermediate values for fucoxanthin (siliceous algae;

402   74%) and Chl b (chlorophytes; 76.7%). In most models, effects of year, DOY, distance,

403   and their interaction were highly significant both before and after WWTP upgrade

404   (Figure 3). As with Chl a, concentrations of pigments from individual phytoplankton

405   groups increased modestly after influx of tertiary-treated effluent (2010-2012), but

406   declined sharply relative to upstream values following inputs of upgraded effluent,

407   particularly for cyanobacteria and cryptophytes (Figure 3b, Supporting Information

408   Figure S4a). Unlike those two taxa, abundance of siliceous algae and secondarily

409   chlorophytes recovered rapidly at downstream stations in WC prior to the QR confluence.

410   After the confluence, the abundance of taxa usually resembled that found in headwaters

411   in the respective years, although absolute pigment concentrations were often greater in

412   downstream QR in years following the BNR upgrade. Thus, while the phytoplankton

413   community of a first-order WWTP-influenced stream was comprised of diverse

414   assemblages of chlorophytes, siliceous algae, cryptophytes and cyanobacteria, pollution

415   with NH_4^+-rich tertiary wastewater favoured chlorophytes, whereas BNR-treated effluent

18

416   with NO_3^- resulted in selective increase in siliceous algae, with lesser responses of

417   chlorophytes and cryptophytes within urban-impacted reaches.

418   Seasonal patterns of lotic phytoplankton abundance and community composition were

419   more variable in years following the WWTP upgrade as compared to the earlier time

420   interval (Figure 4, Supporting Information Figure S4b). Prior to the BNR upgrade

421   (Figure 4a), phytoplankton seasonality was similar in all stream reaches, with a spring

422   Chl a peak derived from mainly diatoms (fucoxanthin), and a mid-summer maximum

423   related to chlorophytes (Chl b), with generally higher abundance of cyanobacteria

424   (echinenone) in late summer (Supporting Information Figure S4b), and less pronounced

425   seasonal changes in cryptophytes (alloxanthin; Figure 43a). In contrast, seasonal

426   progression of taxa varied among years and sites during 2017-2019 (Figure 4b). For

427   example, although spring blooms of diatoms generally gave way to summer maxima of

428   chlorophytes and cyanobacteria in the QR, spring peaks of siliceous algae were less

429   pronounced in headwater (-7 km) and WWTP-impacted reaches (36 km), except in 2018,

430   while timing of summer blooms of cyanobacteria varied greatly among years and did not

431   occur at reaches receiving upgraded wastewater (Figure 4b, Supporting Information Fig

432   S4b). As a result, timing of maximum phytoplankton abundance varied greatly among

433   years during the period following BNR installation (Figure 4b). Taken together,

434   community composition appears to vary both as a result of strong temporal changes in

435   stream conditions (Figure 2), as well as pronounced effects of different types of

436   wastewater treatment prior to the WC-QR confluence.

3.3 Response of phytoplankton to physico-chemical parameters

Analysis of trichromatic Chl a and biomarker pigments using GAMs suggested that phytoplankton assemblages were generally regulated by changes in similar physico-chemical processes in years before and after the BNR upgrade (Figure 5). Physico-chemical GAMs explained 39.8% of deviance in Chl a when including all years, 56.3% for pre-upgrade years, and 42.7% for post-upgrade years. Similarly, the physico-chemical GAM for all biomarkers and all years explained 68.8% of deviance, with even greater deviance explained when restricted to years before (78.2%) or after BNR upgrade (74.2%) (Figure 6). All Chl and aggregate biomarker models exhibited significant effects of year, distance downstream, and DOY.

In general, phytoplankton Chl a and overall community abundance increased with concentrations of NH_4^+ and secondarily water temperature, declined with discharge, specific conductivity, SRP, and NO_3^-, and had weak or inconsistent responses to pH and TDN:SRP ratios (Figures 5, 6). Similarly, responses recorded in models using all six years (e.g., Figure 5a) were most similar to those using only three years prior to WWTP upgrade (Figure 5b) rather than in years following BNR establishment (Figure 5c). In addition, phytoplankton taxa (siliceous algae, cryptophytes, chlorophytes, and cyanobacteria) often responded differentially to physico-chemical parameters (Figure 6) Modest flow had little effect on phytoplankton as Chl a (Figure 5a) or biomarker pigments (Figure 6a) until discharge exceeded ~9 m^3/s, after which abundance declined sharply until about 50 m^3/s. In general, community composition was insensitive to change in flow, other than more pronounced effects of chlorophytes and cyanobacteria

459   and diminished effects of siliceous algae, as Q increased slightly during post-upgrade

460   years (Figure 6c).

461   Change in stream pH had little consistent effect on phytoplankton abundance

462   (Figures 5a, 6a) despite variation of ~2 units among stations and time intervals (Figure 2)

463   and pronounced effects of the presence of siliceous algae and secondarily chlorophytes

464   under alkaline conditions (Figure 6a, c). Although phytoplankton abundance as Chl a

465   increased with pH during years prior to WWTP upgrade (Figure 5b), this effect was not

466   captured by aggregate analysis of biomarker pigments (Figure 6).

467   Effects of specific conductance on suspended phototrophs differed when analyzed as Chl

468   a (Figure 5) or all biomarker pigments (Figure 6). In general, Chl a decreased as

469   conductance increased in all modeled time intervals, whereas there were few changes

470   when all biomarkers were modelled at once until specific conductivity exceeded ~2500

471   µS/cm. Above that value, the abundance of phototrophs increased, largely reflecting

472   marked elevation of phytoplankton, particularly siliceous and green algae, at high

473   conductivity during 2017-2019, when flow was diminished.

474   Phytoplankton abundance increased with stream temperature, although the pattern

475   was more pronounced for GAMs modeling Chl a (Figure 5) than those modeling

476   biomarker pigments (Figure 6). The Chl a pattern generally followed the expected

477   seasonal phenology, with diminished concentrations during cool spring conditions

478   (~10°C; Figure 2) giving way to higher biomass in mid- to late-summer when water

479   temperatures exceed 25°C. The more muted, unimodal response relationship of

480   aggregate biomarkers in community model may reflect the differential contributions of

481   individual phytoplankton groups along the thermal gradient (Figure 6). In general,

21

482   cryptophyte prevalence decreased with temperature, siliceous algae exhibited an inverse

483   unimodal response, while chlorophytes increased linearly with rising temperature (Figure

484   6b, c). Overall, contributions of cyanobacteria to the community model were reduced at

485   high temperatures, even though their abundance tended to be elevated in warm summer

486   waters (Figure 4).

487   Phytoplankton abundance exhibited similar relationships to lotic nutrient levels whether

488   expressed as Chl a (Figure 5) or as various biomarker pigments (Figure 6). In both cases,

489   phytoplankton declined with increasing NO_3^- concentrations, particularly at values greater

490   than 0.2 mg N-NO_3/L. This effect was most pronounced after the WWTP upgrade

491   (Figures 5c, 6c) and reflected a greater effect of chlorophytes and secondarily siliceous

492   algae than other taxa at high NO_3^- levels. In contrast, both Chl a and biomarker pigments

493   increased with NH_4^+ levels both before and after BNR installation, although the precise

494   shape of the relationship varied slightly between years and type of pigment analyzed.

495   Similarly, while the relationship varied between a linear decline, a unimodal response,

496   and a descent to a plateau, phytoplankton abundance generally declined with increased

497   SRP concentrations and was usually lowest at values above ~0.35 mg P/L. The role of

498   chlorophytes was more pronounced under both elevated NH_4^+ and diminished SRP levels,

499   particularly prior to WWTP modification. In general, TDN:SRP ratios had little effect on

500   phytoplankton abundance (Figure 6c). Overall, these findings suggest that the chemical

501   form of N pollution was an important determinant of phytoplankton abundance and

502   community composition in small prairie streams receiving urban wastewater.

4 Discussion

Nutrient pollution from WWTP effluent is expected to impair stream ecosystems by increasing phytoplankton blooms of nuisance chlorophytes and cyanobacteria (Mischke et al., 2011; Solomon et al., 2019; Wager & Schumacher, 1970). In addition, nutrient pollution studies often record increased elevated benthic production by cyanobacteria (Peterson & Grimm, 1992), chlorophytes (Dodds, 1991), or eutrophic diatoms (Chambers et al., 2012; Hamdhani et al., 2020). In contrast, little is known about the response of phytoplankton in small streams to urban wastewater (Breuer et al., 2017; Wu et al., 2011) or of how changes in effluent processing procedures might affect phototrophic abundance and community composition (Carey & Migliaccio, 2009; Hamdhani et al., 2020; Holeton et al., 2011).

Here GAM analysis of lotic phytoplankton assemblages and physico-chemical conditions over six years revealed that a shift from tertiary (P removal) to BNR (NH_4^+ and P) processes reduced phytoplankton abundance relative to headwater values and favoured growth of siliceous algae and secondarily chlorophytes over cyanobacteria, in part due to a shift from NH_4^+ to NO_3^- (Glibert et al., 2016). Alongside urban N effects, temporal and spatial variation in discharge and pH influenced phytoplankton abundance in all reaches of first order Wascana Creek, but these effects were obscured after a confluence with the larger Qu'Appelle River, likely reflecting effects of regional land use (Andrus et al., 2015; Black, Morgan, & Frankforter, 2011), edaphic and geological conditions (Christiansen, 1960), QR (Reynolds & Descy, 1996), influx of phytoplankton from upstream lentic sources (Qu, Wu, Guse, & Fohrer, 2019), and already-elevated nutrient content (Dodds & Smith, 2017). Diminished effects of effluent on QR water

526   chemistry was also shown in the sharp decline in $\delta^{15}N$ following river confluence (Figure

527   2). Instead, effects of temperature on the seasonal succession of phytoplankton from

528   diatoms to chlorophytes and cyanobacteria (Breuer et al. 2016, 2017; Moorhouse et al.,

529   2018) were apparent in all years and stream reaches, except for effluent-impacted reaches

530   where BNR allowed siliceous algae and chlorophytes to remain paramount in most

531   seasons. Together, these patterns show that WWTP upgrade to BNR processes reduced

532   phytoplankton biomass, largely eliminated toxic NH_4^+, and favoured growth of mainly

533   siliceous algae and chlorophytes over cyanobacteria, although effects were limited to the

534   first-order ecosystem.

535   **4.1 Effect of a WWTP on lotic phytoplankton assemblages**

536   Influx of urban NH_4^+ increased lotic phytoplankton abundance in Wascana Creek

537   (Figure 3a), particularly prior to WWTP upgrade. In many freshwater systems the supply

538   of P controls primary production and the Chl a content of freshwaters (Schindler, 1977,

539   2006; Schindler et al., 2016); however, findings here suggest that phototrophs in some P-

540   rich prairie streams can be regulated by N influx, similar to patterns seen in neighbouring

541   lakes (Bogard et al., 2020; Donald et al., 2011, 2013; Hall et al., 1999; Leavitt et al.,

542   2006). Elsewhere, regulation of lotic phytoplankton by P is well-documented (Van

543   Nieuwenhuyse & Jones, 1996), whereas the effects of N on flowing waters are only

544   beginning to be understood (Dodds & Smith, 2017). In general, nutrient control of

545   phytoplankton is paramount when light is not limiting and river flow is slow (Reynolds &

546   Descy, 1996), conditions common in shallow prairie and lowland streams (Breuer et al.,

547   2017; Wu et al., 2011) and which may have been exacerbated by inputs of transparent

548   effluent (see below). Here urban NH_4^+ was associated with elevated Chl a for data from

549 2010-2012 despite TDN:SRP mass ratios suggesting P limitation (> 23; (Guildford &

550 Hecky, 2000), but consistent with results of nutrient-amendment experiments

551 demonstrating N limitation in headwater WC (Bergbusch, 2020).

552   The WWTP upgrade during 2016 reduced effluent NH_4^+ from as much as 30 mg

553 N-NH_4^+/L to < 1 mg N-NH_4^+/L to meet national guidelines and regulations (Canada 2012;

554 CCME, 2012; Chambers et al., 2012). In contrast, in-stream levels of NO_3^- varied little as

555 a result of BNR (~5 mg N/L) partly because of diminished dilution by lower baseflow

556 discharge (Figure 2) and partly due to rapid nitrification during pre-upgrade years

557 (Waiser et al., 2011). However, unlike the immediate stimulation of phytoplankton by

558 added NH_4^+, likely due to its ready use in amino acid synthesis (Flores & Herrero, 2005;

559 Harvey, 1953; Raven, Wollenweber, & Handley, 1992), elevated NO_3^- concentrations

560 were associated with lower overall biomass (Figures 5c, 6c) and changes in community

561 composition were noted only ~30 km downstream of effluent outfall (Figures 3, 4) when

562 N concentrations were reduced to intermediate levels due to in-stream denitrification

563 (Dylla, 2019).

564   Phosphorus from urban sources did not appear to stimulate growth of algae or

565 cyanobacteria in Wascana Creek (Figures 5, 6) unlike expectations from temperate

566 streams (Van Nieuwenhuyse & Jones, 1996) and large rivers (Descy, Leitao, Everbecq,

567 Smitz, & Deliege, 2011; Greene et al., 1975; Kim et al., 2020). Lack of response to P

568 influx is consistent with month-long mesocosm experiments in upstream Wascana Lake

569 demonstrating that N, but not P, limits primary production throughout the ice-free season

570 (Swarbrick, Quiñones-Rivera, & Leavitt, 2020), as well as bottle bioassays from WC

571 showing only N limitation in headwaters during 2017-2019 (Bergbusch, 2020). Further,

572   the effect of effluent P in phytoplankton may also reflect the fact that P levels changed

573   little in WC following influx of urban P (~ 0.5 mg P-SRP/L) or commissioning of the

574   BNR processes (Figure 2), despite marked temporal and spatial changes in phytoplankton

575   abundance (Figures 3, 4). Taken together, these findings suggest that the chemical form

576   of N in effluent was an important control of phytoplankton abundance and community

577   composition in fertilized reaches of Wascana Creek but that, in this instance, P supply

578   was not a critical control factor.

579   NH_4^+-rich discharge from a tertiary WWTP prior to a BNR upgrade supported

580   chlorophytes, moderately suppressed siliceous algae and cryptophytes, and did not affect

581   cyanobacteria (Figures 3, 6). Although NH_4^+ is expected to stimulate both cyanobacteria

582   and chlorophytes (Glibert et al., 2016; Swarbrick et al., 2019), a marked response was

583   only noted for green algae. Several mechanisms could have mediated NH_4^+ effects on

584   cyanobacteria. First, both the transcriptional activator and membrane transporter of NH_4^+

585   in cyanobacteria are repressed by the presence of NH_4^+ (Glibert et al., 2016; Post,

586   Rihtman, & Wang, 2011), therefore extreme levels of NH_4^+ such as observed here (>30

587   mg $N-NH_4^+$/L) may suppress prokaryotic phototrophs such as seen in Wascana Lake

588   (Bogard et al., 2020). Further, colonial cyanobacteria, including those capable of fixing

589   atmospheric N_2, were common in headwater assemblages (Supporting Information Figure

590   S4) and growth of diazotrophic cyanobacteria can be depressed by elevated

591   concentrations of most forms of N (NH_4^+, NO_3^-, urea) (Donald et al., 2011, 2013;

592   Swarbrick et al., 2020). In contrast, chlorophytes can use NH_4^+ uptake pathways that are

593   not repressed by ambient NH_4^+ supplies (Franco, Cárdenas, & Fernández, 1988; Glibert et

594   al., 2016). Similarly, the comparatively modest response of siliceous algae and

595 cryptophytes to urban NH_4^+ may in part reflect the ability of that molecule to block NO_3^-

596 uptake by these taxa, leading to photo-oxidative cell damage, particularly in cool waters

597 (Collos & Harrison, 2014; Glibert et al., 2016; Swarbrick et al., 2019). Together, this

598 study suggests that NH_4^+-rich WWTP effluent can selectively stimulate chlorophytes in

599 some small, P-rich, prairie streams.

600 BNR-treated wastewater (moderate NO_3^-, little NH_4^+, low P) supported

601 development of siliceous algae and chlorophytes in spring but suppressed cyanobacteria,

602 in contrast to tertiary-treated effluent (high NH_4^+, low P). Siliceous algae, in particular

603 diatoms, prefer NO_3^- even when NH_4^+ is present at much higher concentrations (Lomas &

604 Glibert, 1999a, 1999b). Nitrate stimulates diatom growth in flowing (Breuer et al., 2017;

605 Solomon et al., 2019; Varol & Sen, 2018) and lentic ecosystems (Bogard et al., 2020;

606 Donald et al, 2011, 2013; Swarbrick et al., 2019). Diatoms are physiologically adapted to

607 NO_3^- assimilation, rather than that of NH_4^+, for several reasons: diatoms have more

608 substrate-inducing NO_3^- membrane transporters than NH_4^+ transporters (Ambrust, Berges,

609 Bowler, Green, & Martinez, 2004); NO_3^- balances the redox state of a diatom cell via

610 NO_3^- and NO_2^- reductase pathways (Glibert et al., 2016), and; these NO_3^- pathways favour

611 protective mechanisms that reduce oxidative cell damage (Lomas & Glibert, 1999a,

612 1999b). Similarly, chlorophytes exhibit diverse N uptake pathways, including NO_3^-

613 assimilation, whereas cyanobacteria can have unique, NH_4^+-repressable NO_3^- transporters

614 or are incapable of any NO_3^- uptake (Glibert et al., 2016). Although further physiological

615 experiments are warranted, it appears that the BNR upgrade favoured siliceous algae and

616 chlorophytes because of the pronounced shift in N from chemically-reduced toxic NH_4^+

617 to oxidized NO_3^-.

618   Urban wastewater treatment likely affected stream phytoplankton by diluting

619   turbid productive waters with transparent effluent in all years (Supporting Information

620   Figure S6). Wascana Creek discharge was much greater in 2010-2011 than in other years

621   (Figure 2), resulting in effluent generally composing < 50 % of total discharge ('effluent-

622   impacted'; Schultz et al., 2010), whereas WC was 'effluent-dominated' (effluent ~100%

623   of Q; Du, Haddad, Scott, Chambliss, & Brooks, 2015) during much of the interval

624   following BNR upgrade (Supporting Information Figure S3b). Overall, phytoplankton

625   abundance declined with discharge during high flow years to a minimum at about 80 m^3/s

626   (Figure 5a, b, Figure 6a, b), values characteristic of discharge within the mainstem of the

627   QR (Figure 2), whereas biomass increased modestly with flow to about 9 m^3/s during

628   both intervals. Such an increase in biomass during low discharge intervals may reflect

629   the effects of modestly higher flow on rates of nutrient supply (see below).

630   **4.2 Effects of climate and regional characteristics**

631   Rising temperatures during the growing season appear to have altered community

632   composition, with spring diatoms giving way to more abundant cyanobacteria by late

633   summer as seen in previous studies (Breuer et al., 2017; Moorhouse et al., 2018;

634   Stevenson & White, 1995). In general, siliceous algae such as diatoms prefer cooler

635   water ($T_{optimum}$17-22°C) than do chlorophytes (27-32°C) or cyanobacteria (27-37°C),

636   although there is substantial variation among species within groups (Visser et al., 2016).

637   Overall, small cryptophytes exhibited few marked seasonal peaks, possibly because

638   selective grazers on this taxon tend to be less common in shallow streams than in lakes

639   (Walks & Cyr, 2004). Because of the variable timing of peaks, and reproducible annual

640   phenology, GAMs suggested that temperature exerted little unique effect on

641   phytoplankton abundance beyond that associated with seasonal change as captured in the

642   DOY parameter (Figure 5), except possibly in the case of siliceous algae (Figure 6).

643   Similarly, while maximum temperatures of ~25°C occurred during July of all years and

644   thermal optimum are expect to result in growth peaks (Breuer et al., 2017; Moorhouse et

645   al., 2018; Paerl & Huisman, 2008), this did not always result in a Chl a maximum due to

646   the combined effects of urban effluent and confluence of first- and second-order

647   ecosystems.

648   High inter-annual variation in hydrological flow may have affected phytoplankton

649   abundance as seen elsewhere (Reynolds & Descy, 1996; Stevenson & White, 1995).

650   While greatly heightened flow (shorter residence time) reduced phytoplankton biomass in

651   these (Figures 5, 6) and other streams (Soballe & Kimmel, 1987), there is no universal

652   threshold velocity above which Q reduces suspended Chl a in lotic ecosystems (Li,

653   Zhang, Zhu, Xiao, & Chen, 2013). In our study, phytoplankton biomass was stable or

654   increased slightly with discharge until Q exceeded ~9 m^3/s, after which Chl a

655   concentrations declined. While modest discharge, such as that observed in mid-stream

656   reaches of WC, can contribute nutrients to support new biomass (Baker & Baker, 1979),

657   high flows limit the ability of phytoplankton to assimilate and retain nutrients (Doyle,

658   2005), due to cellular damage or reduced irradiance as a result of sediment resuspension

659   (Reynolds, 1994). Although speculative, we suggest that such conditions may apply to

660   WC during high runoff years (2010, 2011), as well as to QR reaches downstream of the

661   WC confluence. While high variability in hydrologic flow may have masked nutrient

662   effects in our study, Waiser et al. (2011) reported that phytoplankton abundance in these

663   streams was negatively related to NH_4^+. Taken together, these patterns suggest that

664 nutrients may only control lotic phytoplankton until a critical flow velocity is reached,

665 resulting in a strong interaction between pollution with urban effluent and natural

666 variation in stream discharge. These findings are also consistent with recent findings that

667 release of high-quality effluent can benefit streams and rivers by augmenting flow

668 (Arnon, Avni, & Gafny, 2015; Halaburka et al., 2013; Plumlee, Gurr, & Reinhard, 2012)

669 and may serve to prevent excessive phytoplankton development.

670 **5 Conclusion**

671   Nutrient pollution from human-influenced landscapes impacts the ecological,

672 social, and economic values of freshwaters worldwide (Allan, 2004; Dodds et al., 2009;

673 Grimm et al., 2008). Unfortunately, N inputs to freshwaters are expected to increase by

674 ~75% by 2050 because of greater urbanization and agriculture needed to meet a growing

675 human population (Bogard et al., 2020; Peñuelas et al., 2013; Schlesinger, 2008; Tilman,

676 Cassman, Matson, Naylor, & Polasky, 2002). Upgrading cities' WWTPs to BNR has

677 been suggested as an effective strategy to reduce both N and P pollution of freshwaters

678 (Carey & Migliaccio, 2009), but little is known about BNR effects on receiving streams.

679 Here we used phytoplankton assemblages as indices of stream ecosystem health, wherein

680 a shift to lower biomass and beneficial diatoms records an improved environmental state

681 (Lobo et al., 2016; Mischke et al., 2011; Stevenson et al., 2009). We found that a BNR

682 upgrade reduced TDN from ~30 mg N/L to < 5 mg N/L, largely eliminated toxic levels of

683 NH_4^+, decreased phytoplankton abundance relative to headwaters, and favoured growth of

684 beneficial diatoms. In comparison, WWTP-impacted reaches prior to the upgrade

685 supported a greater diversity of phytoplankton, including chlorophytes and potentially-

686 harmful cyanobacteria, as well as generally higher phytoplankton abundance relative to

687   headwaters, as seen in other lotic ecosystems impacted by tertiary-treated effluent

688   (Hamdhani et al., 2020; Solomon et al., 2019; Wager & Schumacher, 1970). We

689   conclude that BNR technologies can improve the health of stream ecosystems,

690   particularly in regions with nutrient-rich soils such as the NGPs (Hall et al., 1999; Leavitt

691   et al., 2006).

692   **Acknowledgements**

705   **Conflict of interest**

706   The authors declare no conflict of interest

707   **Data availability statement**

708 The data that support the findings of this study are available from the corresponding

709 author upon reasonable request.

710 **ORCID**

711 *Nathanael T. Bergbusch,* nbergbusch@gmail.com, ORCID 0000-0002-8062-6876
712
713 *Nicole M. Hayes,* hayesn@uwstout.edu; ORCID 0000-0002-5664-9939
714
715 *Gavin L. Simpson,* Gavin.Simpson@uregina.ca, ORCID 0000-0002-9084-8413
716
717 *Vanessa J. Swarbrick,* vanessa.swarbrick@gmail.com, ORCID 0000-0002-9323-6172
718
719 *Zoraida J. Quiñones-Rivera,* Zoraida.Quinones-Rivera@uregina.ca, ORCID 0000-0001-
720   8418-8255
721
722 *Peter R. Leavitt,* Peter.Leavitt@uregina.ca, P.Leavitt@QUB.ac.uk, ORCID 0000-0001-

723   9805-9307

724

References

726 Affourtit, J., Zehr, J. P., & Paerl, H. W. (2001). Distribution of nitrogen-fixing
727   microorganisms along the Neuse River Estuary, North Carolina. *Microbial*
728   *Ecology, 41,* 114–123. https://doi.org/10.1007/s002480000090

729 Agriculture and Agri-Food Canada (AAFC). (2008). Watershed boundaries of the Prairie
730   Farm Rehabilitation Administration project. Government of Canada. Online data
731   available at: http://www4.agr.gc.ca/AAFC-AAC/displayafficher.do?
732   id=1228407260356&lang=eng.

733 Allan, J. D. (2004). Landscapes and riverscapes: The influence of land use on stream
734   ecosystems. *Annual Review of Ecology, Evolution and Systematics, 35,* 257–284.
735   https://doi.org/10.1146/annurev.ecolsys.35.120202.110122

736 Andrus, M. J., Winter, D., Scanlan, M., Sullivan, S., Bollman, W., Waggoner, J. B.
737   Hosmer, A. J. & Brain, R. A. (2015). Spatial and temporal variation of algal
738   assemblages in six Midwest agricultural streams having varying levels of atrazine
739   and other physicochemical attributes. *Science of the Total Environment, 505,* 65–
740   89. https://doi.org/10.1016/j.scitotenv.2014.09.033

741 APHA-AWWA/WEF. (1998). Standard Methods for the Examination of Water and
742   Wastewater, 20th Edition. American Public Health Association, Washington DC,
743   USA.

744 Armbrust, E. V., Berges, J. A., Bowler, C., Green, B. R., & Martinez, D., (2004). The
745   genome of the diatom *Thalassiosira pseudonana*: Ecology, evolution, and
746   metabolism. *Science, 306,* 79–86. https://doi.org/10.1126/science.1101156

747 Arnon, S., Avni, N., & Gafny, S. (2015). Nutrient uptake and macroinvertebrate
748   community structure in a highly regulated Mediterranean stream receiving treated
749   wastewater. *Aquatic Sciences, 77,* 623–637. https://doi.org/10.1007/s00027-015-
750   0407-6

751 Baker, A. L., & Baker, K. K. (1979). Effects of temperature and current discharge on the
752   concentration and photosynthetic activity of the phytoplankton in the upper
753   Mississippi River. *Freshwater Biology, 9,* 191–198. https://doi.org/10.1111/j.1365-
754   2427.1979.tb01502.x

755   Baker, P., & Humpage, A. (1994). Toxicity associated with commonly occurring

756   cyanobacteria in surface waters of the Murray-Darling Basin, Australia. Mar.

757   *Freshwater Research, 45,* 773-786. https://doi.org/10.1071/mf9940773

758   Bennett, E. M., Carpenter, S. R., & Caraco, N F. (2001). Human impact on erodable

759   phosphorus and eutrophication: A global perspective. *BioScience, 51,* 227-234.

760   https://doi.org/10.1641/0006-3568(2001)051[0227:hioepa]2.0.co;2

761   Bergbusch, N. T. (2020). The Impact of a Nitrogen-removal Wastewater Upgrade on

762   Primary Producers in Phosphorous-rich Streams of the Northern Great Plains. MSc

763   Thesis, University of Regina, Regina, Saskatchewan Canada.

764   Black, R. W., Moran, P. W., & Frankforter, J. D. (2010). Response of algal metrics to

765   nutrients and physical factors and identification of nutrient thresholds in

766   agricultural streams. *Environmental Monitoring and Assessment, 175,* 397–417.

767   https://doi.org/10.1007/s10661-010-1539-8

768   Bodirsky, B. L., Popp, A., Lotze-Campen, H., Dietrich, J. P., & Rolinski, S. (2014).

769   Reactive nitrogen requirements to feed the world in 2050 and potential to mitigate

770   nitrogen pollution. *Nature Communications 5,* 3858.

771   https://doi.org/10.1038/ncomms4858

772   Bogard, M. J., Vogt, R. J., Hayes, N. M., & Leavitt, P. R. (2020). Unabated nitrogen

773   pollution favors growth of toxic cyanobacteria over chlorophytes in most

774   hypereutrophic lakes. *Environmental Science & Technology, 54,* 3219–3227.

775   https://doi.org/0.1021/acs.est.9b06299

776   Breuer, F., Janz, P., Farrelly, E., & Ebke, K-P. (2016). Seasonality of algal communities

777   in small streams and ditches in temperate regions using delayed fluorescence.

778   *Journal of Freshwater Ecology, 31,* 393-406,

779   https://doi.org.10.1080/02705060.2016.1160846

780   Breuer, F., Janz, P., Farrelly, E., & Ebke, K-P. (2017). Environmental and structural

781   factors influencing algal communities in small streams and ditches in central

782   Germany. *Journal of Freshwater Ecology, 32,* 65-83.

783   https://doi.org/10.1080/02705060.2016.1241954

784   Buchanan, T. J., & Somers, W. P. (1976). Discharge measurements at gauging stations, p.

785   71. In Techniques of Water-Resources Investigations of the United States

786   Geological Survey - Book 3: Applications of Hydraulics. United States

787   Government Printing Office.

788   Bürkner, P. (2018). Advanced Bayesian Multilevel Modeling with the R Package brms. *R*

789   *Journal, 10,* 395–411. https://doi.org/10.32614/RJ-2018-017.

790   Canada, Government of (2012). Wastewater Systems Effluent Regulations. Fisheries Act

791   2012. Ottawa, Canada. https://laws-lois.justice.gc.ca/eng/regulations/sor-2012-139/

792   fulltext.html.

793   Canadian Council of Ministers of the Environment (CCME). (2012). Canadian Water

794   Quality Guidelines for the Protection of Aquatic Life: Canadian Environmental

795   Quality Guidelines. Government of Canada, Ottawa, Canada.

796   Carey, R. O., & Migliaccio, K. W. (2009). Contribution of wastewater treatment plant

797   effluents to nutrient dynamics in aquatic systems: a review. *Environmental*

798   *Management, 44,* 205–17. https://doi.org/10.1007/s00267-009-9309-5

799   Carpenter, S. R., Caraco, N. F., Correll, D. L., Howarth, R. W., Sharpley, A. N., & Smith,

800   V. H. (1998). Nonpoint pollution of surface waters with phosphorus and nitrogen.

801   *Ecological Applications, 8,* 559–568. https://doi.org/10.1890/1051-

802   0761(1998)008[0559:nposww]2.0.co;2

803   Chambers, P. A., McGoldrick, D. J., Brua, R. B., Vis, C., Culp, J. M., & Benoy, G. A.

804   (2012). Development of environmental thresholds for nitrogen and phosphorus in

805   streams. *Journal of Environmental Quality, 41,* 7-20.

806   https://doi.org/10.2134/jeq2010.0273

807   Chételat, J., Pick, F. R., & Hamilton, P. B. (2006). Potamoplankton size structure and

808   taxonomic composition: Influence of river size and nutrient concentrations.

809   *Limnology and Oceanography, 51,* 681–689.

810   https://doi.org/10.4319/lo.2006.51.1_part_2.0681

811   Christiansen, E. A. (1960). Geology and ground-water resources of the Qu'Appelle area,

812   Saskatchewan. Geology Division Report No. 1, Saskatchewan Research Council,

813   Saskatoon, Canada.

814   Collos, Y., & Harrison, P. J. (2014). Acclimation and toxicity of high ammonium

815   concentrations to unicellular algae. *Marine Pollution Bulletin, 80,* 8–23.

816   https://doi.org/10.1016/j.marpolbul.2014.01.006

817 Cooper, P., Upton, J. E., Smith, M., & Churchley, J. (1995). Biological nutrient removal:
818   Design snags, operational problems and costs. *Water and Environment, Journal, 9,*
819   7–17. https://doi.org/10.1111/j.1747-6593.1995.tb00921.x

820 del Giorgio, P. A., Vinocur, A. L., Lombardo, R. J., & Tell, H. G. (1991). Progressive
821   changes in the structure and dynamics of the phytoplankton community along a
822   pollution gradient in a lowland river — a multivariate approach. *Hydrobiologia,*
823   *224,* 129–154. doi:10.1007/bf00008464

824 Descy, J.-P., Leitao, M., Everbecq, E., Smitz, J. S., & Deliege, J.-F. (2011).
825   Phytoplankton of the River Loire, France: A biodiversity and modelling study.
826   *Journal of Plankton Research, 34*, 120–135. https://doi.org/10.1093/plankt/fbr085

827 Dodds, W.K. (1991). Factors associated with dominance of the filamentous green alga
828   *Cladophora glomerata*. *Water Research, 25,* 1325–1332.

829 Dodds, W. K., Bouska, W. W., Eitzmann, J. L., Pilger, T. J., Pitts, K. L., Riley, A. J.,
830   Schloesser, J. T., & Thornbrugh, D. J. (2009). Eutrophication of U.S. freshwaters:
831   Analysis of potential economic damages. *Environmental Science & Technology,*
832   *43,* 12–19. https://doi.org/10.1021/es801217q

833 Dodds, W. K., & Smith, V. H. (2017). Nitrogen, phosphorus, and eutrophication in
834   streams. *Inland Waters, 6,* 155–164. https://doi.org/10.5268/IW-6.2.909

835 Donald, D. B., Bogard, M. J., Finlay, K., & Leavitt, P. R. (2011). Comparative effects of
836   urea, ammonium, and nitrate on phytoplankton abundance, community
837   composition, and toxicity in hypereutrophic freshwaters. *Limnology and*
838   *Oceanography, 56,* 2161–2175. https://doi.org/10.4319/lo.2011.56.6.2161

839 Donald, D. B., Bogard, M. J., Finlay, K., Bunting, L., & Leavitt, P. R. (2013).
840   Phytoplankton-specific response to enrichment of phosphorus-rich surface waters
841   with ammonium, nitrate, and urea. *PLoS One, 8,* e53277.
842   https://doi.org/10.1371/journal.pone.0053277

843 Doyle, M. W. (2005). Incorporating hydrologic variability into nutrient spiraling. *Journal*
844   *of Geophysical Research: Biogeosciences, 110,* G01003.
845   https://doi.org/10.1029/2005jg000015

846 Du, B., Haddad, S. P., Scott, W. C., Chambliss, C. K., & Brooks, B. W. (2014).
847   Pharmaceutical bioaccumulation by periphyton and snails in an effluent-dependent

848   stream during an extreme drought. *Chemosphere, 119,* 927–34.

849   https://doi.org10.1016/j.chemosphere.2014.08.044

850   Dylla, N. P. (2019). Downstream effects on denitrification and nitrous oxide from an

851   advanced wastewater treatment plant upgrade. MSc thesis. University of

852   Saskatchewan, Saskatoon, Canada. 124 pp.

853   Elser, J. J., Bracken, M. E. S., Cleland, E. E., Gruner, D. S., Harpole, W. S., Hilleband,

854   H., ...& Smith, J. E. (2007). Global analysis of nitrogen and phosphorus limitation

855   of primary producers in freshwater, marine and terrestrial ecosystems. *Ecology*

856   *Letters, 10,* 1135–1142. https://doi.org/10.1111/j.1461-0248.2007.01113.x

857   Environmental Protection Agency. (2007). Biological Nutrient Removal Processes and

858   Costs. Office of Water Washington, DC, USA.

859   https://www.epa.gov/sites/production/files/documents/criteria_nutrient_bioremoval.

860   pdf

861   Finlay, K., Patoine, A., Donald, D. B., Bogard, M. J., & Leavitt, P. R. (2010).

862   Experimental evidence that pollution with urea can degrade water quality in

863   phosphorus-rich lakes of the Northern Great Plains. *Limnology and Oceanography*,

864   *55,* 1213–1230. https://doi.org/10.4319/lo.2010.55.3.1213

865   Flores, E., & Herrero, A. (2005). Nitrogen assimilation and nitrogen control in

866   cyanobacteria. *Biochemical Society Transactions, 33,* 164–167.

867   https://doi.org/10.1042/bst0330164

868   Franco, A. R., Cárdenas, J., & Fernández, E. (1988). Two different carriers transport both

869   ammonium and methylammonium in *Chlamydomonas reinhardtii*. *Journal of*

870   *Biological Chemistry, 263,* 14039–43.

871   Gianfagna, C. C., Johnson, C. E., Chandler, D. G., & Hofmann, C. (2015). Watershed

872   area ratio accurately predicts daily streamflow in nested catchments in the Catskills,

873   New York. *Journal of Hydrology: Regional Studies, 4,* 583-594.

874   https://doi.org/10.1016/j.ejrh.2015.09.002

875   Glibert, P. M., Wilkerson, F. P., Dugdale, R. C., Raven, J. A., Dupont, C., Leavitt, P. R.,

876   … & Kana, T. M. (2016). Pluses and minuses of ammonium and nitrate uptake and

877   assimilation by phytoplankton and implications for productivity and community

878   composition, with emphasis on nitrogen-enriched conditions. *Limnology and*

879   *Oceanography, 61,* 165–197. https://doi.org/10.1002/lno.10203

880 Greene, J. C., Miller, W. E., Shiroyama, T., & Maloney, T. E. (1975). Utilization of algal

881   assays to assess the effects of municipal, industrial, and agricultural wastewater

882   effluents upon phytoplankton production in the Snake River system. *Water Air and*

883   *Soil Pollution, 4,* 415–434. https://doi.org/10.1007/bf00280726

884 Grimm, N. B., Faeth, S. H., Golubiewski, N. E., Redman, C. L., Wu, J., Bai, X., &

885   Briggs, J. M. (2008). Global change and the ecology of cities. *Science, 319,* 756–

886   760. https://doi.org/10.1126/science.1150195

887 Guildford, S. J., & Hecky, R. E. (2000). Total nitrogen, total phosphorus, and nutrient

888   limitation in lakes and oceans: Is there a common relationship? *Limnology and*

889   *Oceanography, 45,* 1213–1223. https://doi.org/10.4319/lo.2000.45.6.1213

890 Haig, H. A., Hayes, N. M., Simpson, G. L., Yi, Y., Wissel, B., Hodder, K. R., & Leavitt,

891   P. R. (2020). Comparison of isotopic mass balance and instrumental techniques as

892   estimates of basin hydrology in seven connected lakes over 12 years. *Journal of*

893   *Hydrology X, 6, 100046.* https://doi.org/10.1016/ j.hydroa.2019.100046

894 Halaburka, B. J., Lawrence, J. E., Bischel, H. N., Hsiao, J., Plumlee, M. H., Resh, V. H.,

895   & Luthy, R. G. (2013). Economic and ecological costs and benefits of streamflow

896   augmentation using recycled water in a California coastal stream. *Environmental*

897   *Science & Technology, 47,* 10735–10743. https://doi.org/10.1021/es305011z

898 Hall, R. I., Leavitt, P. R., Quinlan, R., Dixit, A. S., & Smol, J P. (1999). Effects of

899   agriculture, urbanization, and climate on water quality in the northern Great Plains.

900   *Limnology and Oceanography, 44,* 739–756.

901   https://doi.org/10.4319/lo.1999.44.3_part_2.0739

902 Hamdhani, H., Eppehimer, D. E., & Bogan, M. T. (2020). Release of treated effluent into

903   streams: A global review of ecological impacts with a consideration of its potential

904   use for environmental flows. Freshwater Biology,

905   https://doi.org/10.1111/fwb.13519

906 Hardenbicker, P., Rolinski, S., Weitere, M., & Fischer, H. (2014). Contrasting long-term

907   trends and shifts in phytoplankton dynamics in two large rivers. *International*

908   *Review of Hydrobiology, 99,* 287–299. https://doi.org/10.1002/iroh.201301680

909   Harris, T. D., Smith, V. S., Graham, J. L., Van de Waal, D. B., Tedesco, L. P., & Clercin,

910   N. (2016). Combined effects of nitrogen to phosphorus and nitrate to ammonia

911   ratios on cyanobacterial metabolite concentrations in eutrophic Midwestern USA

912   reservoirs. *Inland Waters, 6,* 199-210. doi: 10.5268/IW-6.2.938.

913   Harvey, H. W. (1953). Synthesis of organic nitrogen and chlorophyll by *Nitzschia*

914   *closterium. Journal of the Marine Biological Association, 31,* 477–487.

915   https://doi.org/10.1017/s0025315400011632

916   Holeton, C., Chambers, P. A., Grace, L., & Kidd, K. A. (2011). Wastewater release and

917   its impacts on Canadian waters. *Canadian Journal of Fisheries and Aquatic*

918   *Sciences, 68,* 1836–1859. https://doi.org/10.1139/f2011-096

919   Houlton, B. Z., Boyer, E., Finzi, A., Galloway, J., & Leach, A. (2013). Intentional versus

920   unintentional nitrogen use in the United States: Trends, efficiency and implications.

921   *Biogeochemistry, 114,* 11–23. https://doi.org/10.1007/s10533-012-9801-5

922   Hutchins, M. G., Johnson, A. C., Deflandre-Vlandas, A., Comber, S., Posen, P., &

923   Boorman, D. (2010). Which offers more scope to suppress river phytoplankton

924   blooms: Reducing nutrient pollution or riparian shading? *Science of the Total*

925   *Environment, 408,* 5065–5077. https://doi.org/10.1016/j.scitotenv.2010.07.033

926   Jeffrey, S. W., & Humphrey, G. F. (1975). New spectrophotometric equations for

927   determining chlorophylls a, b, c1 and c2 in higher plants, algae and natural

928   phytoplankton. *Biochemie und Physiologie der Pflanzen, 167,* 191–194.

929   https://doi.org/10.1016/s0015-3796(17)30778-3

930   Kim, K., Mun, H., Shin, H., Park, S., Yu, C., Lee, J., …& Rhew, D. (2020). Nitrogen

931   stimulates *Microcystis*-dominated blooms more than phosphorus in river conditions

932   that favor non-nitrogen-fixing genera. *Environmental Science & Technology, 54,*

933   7185–7193. https://doi.org/10.1021/acs.est.9b07528

934   Leavitt, P. R., Brock, C. S., Ebel, C., & Patoine, A. (2006). Landscape-scale effects of

935   urban nitrogen on a chain of freshwater lakes in central North America. *Limnology*

936   *and Oceanography, 51,* 2262–2277. https://doi.org/10.4319/lo.2006.51.5.2262

937   Leavitt, P. R., & Hodgson, D. A. (2001). Sedimentary pigments, p. 295-325. *In* Smol, J.

938   P., Birks, H. J. B, & Last, W. M. (eds.), Tracking Environmental Change using

939   Lake Sediments. Volume 3: Terrestrial, Algal and Siliceous Indicators. Kluwer.

940   https://doi.org/10.1007/0-306-47668-1_15

941 Leland, H. V. (2003). The influence of water depth and flow regime on phytoplankton

942   biomass and community structure in a shallow, lowland river. *Hydrobiologia, 506–*

943   *509,* 247–255. https://doi.org/10.1023/b:hydr.0000008596.00382.56

944 Li, F., Zhang, H., Zhu, Y., Xiao, Y., & Chen, L. (2013). Effect of flow velocity on

945   phytoplankton biomass and composition in a freshwater lake. *Science of the Total*

946   *Environment, 447,* 64–71. https://doi.org/10.1016/j.scitotenv.2012.12.066

947 Lobo, E. A., Heinrich, C. G., Schuch, M., Wetzel, C. E., & Ector, L. (2016). Diatoms as

948   bioindicators in rivers, p. 245-271. *In* Necchi, J. R. [ed.], River Algae. Springer.

949   https://doi.org/10.1007/978-3-319-31984-1_11

950 Lomas, M. W., & Glibert, P. M. (1999a). Temperature regulation of nitrate uptake: A

951   novel hypothesis about nitrate uptake and reduction in cool-water diatoms.

952   *Limnology and Oceanography, 44,* 556–572.

953   https://doi.org/10.4319/lo.1999.44.3.0556

954 Lomas, M. W., & Glibert, P. M. (1999b). Interactions between NH_4^+ and NO_3^- uptake and

955   assimilation: Comparison of diatoms and dinoflagellates at several growth

956   temperatures. *Marine Biology, 133,* 541–551.

957   https://doi.org/10.1007/s002270050494

958 Marra, G., & Wood, S. N. (2011). Practical variable selection for generalized additive

959   models. *Computational Statistics & Data Analysis, 55,* 2372–2387.

960   https://doi.org/10.1016/j.csda.2011.02.004

961 Mischke, U., Venohr, M., & Behrendt, H. (2011). Using phytoplankton to assess the

962   trophic status of German rivers. *International Review of Hydrobiology, 96,* 578–

963   598. https://doi.org/10.1002/iroh.201111304

964 Moorhouse, H. L., Read, D. S., McGowan, S., Wagner, M., Roberts, C., Armstrong, L.

965   K., Nicholls, D. J. E., Wickham, H. D., Hutchins, M. G., & Bowes, M. J. (2018).

966   Characterisation of a major phytoplankton bloom in the River Thames (UK) using

967   flow cytometry and high performance liquid chromatography. *Science of the Total*

968   *Environment, 624,* 366–376. https://doi.org/10.1016/j.scitotenv.2017.12.128

969   Mowchenko, M., & Meid, P. O. (1983). The determination of gross and effective

970   drainage areas in the Prairie Provinces. Hydrology report #104. Prairie Farm

971   Rehabilitation Administration, Engineering Branch, Regina, Saskatchewan,

972   Canada.

973   Natural Resources Canada. (2016). CanVec (Topographic Data of Canada). Online data

974   available at: http://open.canada.ca/data/en/dataset/23387971-b6d3-4ded-a40b-

975   c8e832b4ea08.

976   Opute, F. I. (1974). Lipid and fatty-acid composition of diatoms. *Journal of Experimental*

977   *Botany, 25,* 823–835. https://doi.org/10.1093/jxb/25.4.823

978   Organization for Economic Cooperation and Development. (2020).

979   Variable:''Wastewater Treatment''. Available from http://stats.oecd.org/Index.aspx?

980   DataSetCode=WATER_TREAT

981   Paerl, H. W., & Huisman, J. (2008). Climate: Blooms like it hot. *Science, 320,* 57–58.

982   https://doi.org/10.1126/science.1155398

983   Paerl, H. W., & Otten, T. G. (2013). Harmful cyanobacterial blooms: Causes,

984   consequences, and controls. *Microbial Ecology, 65,* 995–1010.

985   https://doi.org/10.1007/s00248-012-0159-y

986   Paerl, H. W., Scott, T. J., McCarthy, M. J., Newell, S. E., & Gardner, W. S. (2016). It

987   takes two to tango: When and where dual nutrient (N & P) reductions are needed to

988   protect lakes and downstream ecosystems. *Environmental Science & Technology,*

989   *50,* 10805–10813. https://doi.org/10.1021/acs.est.6b02575

990   Paterson, M. J., Schindler, D. W., Hecky, R. E., Findlay, D. E., & Rondeau, K. J. (2011).

991   Comment: Lake 227 shows clearly that controlling inputs of nitrogen will not

992   reduce or prevent eutrophication of lakes. *Limnology and Oceanography, 56,*

993   1545-1547. doi:10.4319/lo.2011.56.4.1545

994   Paul, M. J., & Meyer, J. L. (2001). Streams in the urban landscape. *Annual Review of*

995   *Ecology, Evolution and Systematics, 32, 333*–365.

996   https://doi.org/10.1146/annurev.ecolsys.32.081501.114040

997   Pedersen, E. J., Miller, D. L., Simpson, G. L., & Ross, N. (2019). Hierarchical

998   generalized additive models in ecology: An introduction with mgcv. *Peerj, 7,*

999   e6876. https://doi.org/10.7717/peerj.6876

1000 Peñuelas, J., Poulter, B., Sardans, J., Ciais, P., van der Velde, M., Bopp, L., & Janssens, I.
1001   A. 2013. Human-induced nitrogen–phosphorus imbalances alter natural and
1002   managed ecosystems across the globe. *Nature Communications, 4,* 2934.
1003   https://doi.org/10.1038/ncomms3934

1004 Peterson, C. G., & Grimm, N. B. (1992). Temporal variation in enrichment effects during
1005   periphyton succession in a nitrogen limited desert stream ecosystem. *Journal of the*
1006   *North American Benthological Society, 11,* 20–36.

1007 Plumlee, M. H., Gurr, C. J., & Reinhard, M. (2012). Recycled water for stream flow
1008   augmentation: Benefits, challenges, and the presence of wastewater-derived organic
1009   compounds. *Science of the Total Environment, 438,* 541–548.
1010   https://doi.org/10.1016/j.scitotenv.2012.08.062

1011 Post, A. F., Rihtman, B., & Wang, Q. (2011). Decoupling of ammonium regulation and
1012   ntcA transcription in the diazotrophic marine cyanobacterium *Trichodesmium* sp.
1013   IMS101. *ISME Journal, 6,* 629–637. https://doi.org/10.1038/ismej.2011.121

1014 Qu, Y., Wu, N., Guse, B., & Fohrer, N. (2018). Riverine phytoplankton shifting along a
1015   lentic-lotic continuum under hydrological, physiochemical conditions and species
1016   dispersal. *Science of the Total Environment, 619–620,* 628-636
1017   https://doi.org/10.1016/j.scitotenv.2017.10.139

1018 R Core Team. (2018). R: A Language and Environment for Statistical Computing; https://
1019   www.R-project.org/

1020 Raven, J. A., Wollenweber, B., & Handley, L. L. (1992). A comparison of ammonium
1021   and nitrate as nitrogen sources for photolithotrophs. *New Phytologist, 121,* 19–32.
1022   https://doi.org/10.1111/j.1469-8137.1992.tb01088.x

1023 Reynolds, C. S. (1988). Potamoplankton: Paradigms, paradoxes, prognoses. *In* Round, F.
1024   E. [ed.], p. 285–311, Algae and the Aquatic Environment. Biopress Ltd., Bristol,
1025   UK.

1026 Reynolds, C. S. (1994). The long, the short and the stalled: On the attributes of
1027   phytoplankton selected by physical mixing in lakes and rivers. *Hydrobiologia, 289,*
1028   9–21. https://doi.org/10.1007/bf00007405

1029   Reynolds, C. S., & Descy, J.-P. (1996). The production, biomass and structure of

1030   phytoplankton in large rivers. *River Systems, 10,* 161–187.

1031   https://doi.org/10.1127/lr/10/1996/161

1032   Schindler, D. W. (1977). Evolution of phosphorus limitation in lakes. *Science, 195,* 260–

1033   262. https://doi.org/10.1126/science.195.4275.260

1034   Schindler, D. W. (2006). Recent advances in the understanding and management of

1035   eutrophication. *Limnology and Oceanography, 51,* 356–363.

1036   https://doi.org/10.4319/lo.2006.51.1_part_2.0356

1037   Schindler, D. W., Carpenter, S. R., Chapra, S. C., Hecky, R. E., & Orihel, D. M. (2016).

1038   Reducing phosphorus to curb lake eutrophication is a success. *Environmental*

1039   *Science & Technology, 50,* 8923–9. https://doi.org/10.1021/acs.est.6b02204

1040   Schlesinger, W. H. (2008). On the fate of anthropogenic nitrogen. *Proceedings of the*

1041   *National Academy of Science USA, **106**, 203*–208.

1042   https://doi.org/10.1073/pnas.0810193105

1043   Schultz, M. M., Furlong, E. T., Kolpin, D. W., Werner, S. L., Schoenfuss, H. L., Barber,

1044   M. B., …Vajda, A.. (2010). Antidepressant pharmaceuticals in two U.S. effluent-

1045   impacted streams: Occurrence and fate in water and sediment, and selective uptake

1046   in fish neural tissue. *Environmental Science & Technology, 44,* 1918–1925. https://

1047   doi.org/10.1021/es9022706

1048   Shatwell, T., & Köhler, J. (2019). Decreased nitrogen loading controls summer

1049   cyanobacterial blooms without promoting nitrogen-fixing taxa: Long-term response

1050   of a shallow lake. *Limnology and Oceanography, 64,* S166-S178.

1051   https://doi.org/10.1002/lno.11002

1052   Soballe, D. M., & Kimmel, B. L. (1987). A large-scale comparison of factors influencing

1053   phytoplankton abundance in rivers, lakes, and impoundments. *Ecology, 68,* 1943–

1054   1954. https://doi.org/10.2307/1939885

1055   Solomon, C. M., Jackson, M., & Glibert, P. M. (2019). Chesapeake Bay's "forgotten"

1056   Anacostia River: Eutrophication and nutrient reduction measures. *Environmental*

1057   *Monitoring and Assessment, **191,*** 265. https://doi.org/10.1007/s10661-019-7437-9

1058   Stevenson, R. J., Pan, Y., & van Dam, H. (2009). Assessing environmental conditions in

1059   rivers and streams with diatoms, p. 57-85. *In* Smol, J.P., and Stoermer, E. F. [eds.],

1060 The Diatoms. Cambridge University Press.

1061 https://doi.org/10.1017/cbo9780511763175.005

1062 Stevenson, R. J., & White, K. D. (1995). A comparison of natural and human

1063 determinants of phytoplankton communities in the Kentucky River basin, USA.

1064 *Hydrobiologia, 297,* 201–216. https://doi.org/10.1007/bf00019285

1065 Swarbrick, V. J. (2017). Season impacts and regulation of nitrogen pollutions in the

1066 Northern Great Plains: Insights from microcosm, mesocosm, and mensurative-scale

1067 studies. PhD thesis. University of Regina, Regina, Saskatchewan, Canada. 217 pp.

1068 Swarbrick, V. J., Simpson, G. L., Glibert, P. M., & Leavitt, P. R. (2019). Differential

1069 stimulation and suppression of phytoplankton growth by ammonium enrichment in

1070 eutrophic hardwater lakes over 16 years. *Limnology and Oceanography, 64,* S130–

1071 S149. https://doi.org/10.1002/lno.11093

1072 Swarbrick, V. J., Quiñones-Rivera, Z., & Leavitt, P. R. (2020). Seasonal variability in

1073 effects of urea and phosphorous on phytoplankton abundance and composition in a

1074 hypereutrophic hardwater lake. Freshwater Biology,

1075 https://doi.org/10.1111/fwb.13580

1076 Taipale, S., Strandberg, U., Peltomaa, E., Galloway, A., Ojala, A., & Brett, M. T. (2013).

1077 Fatty acid composition as biomarkers of freshwater microalgae: Analysis of 37

1078 strains of microalgae in 22 genera and in seven classes. *Aquatic Microbial Ecology,*

1079 *71,* 165–178. https://doi.org/10.3354/ame01671

1080 Tchobanoglous G., Burton F. L., & Stensel H. D. (2003). Wastewater Engineering:

1081 Treatment and Reuse, 4th Ed. McGraw-Hill Education, Metcalf and Eddy, Inc.

1082 Tilman, D., Cassman, K. G., Matson, P. A., Naylor, R., & Polasky, S. (2002).

1083 Agricultural sustainability and intensive production practices. *Nature, 418,* 671–

1084 677. https://doi.org/10.1038/nature01014

1085 Van Nieuwenhuyse, E. E., & Jones, J. R. (1996). Phosphorus–chlorophyll relationship in

1086 temperate streams and its variation with stream catchment area. *Canadian Journal*

1087 *of Fisheries and Aquatic Sciences, 53,* 99–105.

1088 Vannote, R. L., Minshall, G. W., Cummins, K. W., Sedell, J. R., & Cushing, C. E.
1089   (1980). The river continuum concept. *Canadian Journal of Fisheries and Aquatic*
1090   *Sciences, 37,* 130–137. https://doi.org/10.1139/f80-017

1091 Varol, M., & Şen, B. (2018). Abiotic factors controlling the seasonal and spatial patterns
1092   of phytoplankton community in the Tigris River, Turkey. *River Research and*
1093   *Applications, 34,* 13–23. https://doi.org/10.1002/rra.3223

1094 Visser, P. M., Verspagen, J. M. H., Sandrini, G., Stal, L. J., Matthijs, H. P. C., Davis, T.
1095   W., Paerl, H. W., & Huisman, J. (2016). How rising CO_2 and global warming may
1096   stimulate harmful cyanobacterial blooms. *Harmful Algae, 54,* 145–159.
1097   https://doi.org/10.1016/j.hal.2015.12.006

1098 Wager, D. B., & Schumacher, G. J. (1970). Phytoplankton of the Susquehanna River near
1099   Binghamton, New York: Seasonal variations, effect of sewage effluents 2. *Journal*
1100   *of Phycology, 6,* 110–117. https://doi.org/10.1111/j.1529-8817.1970.tb02367.x

1101 Waiser, M. J., Tumber, V., & Holm, J. (2011). Effluent-dominated streams. Part 1:
1102   Presence and effects of excess nitrogen and phosphorus in Wascana Creek,
1103   Saskatchewan, Canada. *Environmental Toxicology and Chemistry, 30,* 496-507.
1104   https://doi.org/10.1002/etc.399

1105 Walks, D. J., & Cyr, H. (2004). Movement of plankton through lake-stream systems.
1106   *Freshwater Biology, 49,* 745–759. https://doi.org/10.1111/j.1365-
1107   2427.2004.01220.x

1108 Walsh, C. J., Roy, A. H., Feminella, J. W., Cottingham, P. D., Groffman, P. M., &
1109   Morgan, R. P. (2005). The urban stream syndrome: Current knowledge and the
1110   search for a cure. *Journal of the North American Benthological Society, 24,* 706–
1111   723. https://doi.org/10.1899/04-028.1

1112 Walsh, G. W., & Wepener, V. (2009). The influence of land use on water quality and
1113   diatom community structures in urban and agriculturally stressed rivers. *Water*
1114   *South Africa, 35,* https://doi.org/10.4314/wsa.v35i5.49184

1115 Wehr, J. D., & Descy, J. P. (1998). Use of phytoplankton in large river management.
1116   *Journal of Phycology, 34,* 741–749.

1117 Wickham H. (2016). *ggplot2:* Elegant Graphics for Data Analysis. Springer-Verlag New
1118   York. ISBN 978-3-319-24277-4, https://ggplot2.tidyverse.org.

1119   Wood, S. N. (2011). Fast stable restricted maximum likelihood and marginal likelihood

1120   estimation of semiparametric generalized linear models. *Journal of the Royal*

1121   *Statistical Society Series B: Statistical Methodology, 73,* 3–36.

1122   https://doi.org/10.1111/j.1467-9868.2010.00749.x

1123   Wood, S. N. (2017). Generalized Additive Models: An Introduction with R, 2 edition,

1124   Chapman and Hall/CRC.

1125   Wood, S. N., Pya, N., & Säfken, B. (2016). Smoothing parameter and model selection for

1126   general smooth models. *Journal of the American Statistical Association, 111,*

1127   1548–1563. https://doi.org/10.1080/01621459.2016.1180986

1128   Wu, N., Schmalz, B., & Fohrer, N. (2011). Distribution of phytoplankton in a German

1129   lowland river in relation to environmental factors. *Journal of Plankton Research,*

1130   *33,* 807–820. https://doi.org/10.1093/plankt/fbq139

1131   Wurtsbaugh, W. A., Paerl, H. W., & Dodds, W. K. (2019). Nutrients, eutrophication and

1132   harmful algal blooms along the freshwater to marine continuum. *Wiley*

1133   *Interdisciplinary Review Water, 6,* e1373. https://doi.org/10.1002/wat2.1373

1134   Zurawell, R. W., Chen, H., Burke, J. M., & Prepas, E. E. (2005). Hepatotoxic

1135   cyanobacteria: A review of the biological importance of microcystins in freshwater

1136   environments. *Journal of Toxicology and Environmental Health Part B, 8,* 1–37.

1137   https://doi.org/10.1080/10937400590889412

1138

1139 Table 1. Mean (± standard error) of physico-chemical conditions at four sites along

1140 Wascana Creek (WC) and the Qu'Appelle River (QR) when receiving tertiary treated

1141 wastewater (Tertiary; 2010-2012) or biological nutrient removal (BNR; 2017-2019)

1142 treated effluent. Values are means of one to three sites sampled May-September.

1143 Locations include a headwater site within WC, WC sites receiving wastewater treatment

1144 plant (WWTP) effluent, and subsequent sites in the QR both above (control) and below

1145 (downstream) of the confluence of WC and the QR. Soluble reactive phosphorous =

1146 SRP, total dissolved nitrogen = TDN, and N stable isotope ratios = δ^{15}N.

1147

Treatment process (Sampling years)	Tertiary (2010-2012)				Biological nutrient removal (2017-2019)			
Location	WC Headwaters	Impacted WC	QR control	Downstream QR	WC Headwaters	Impacted WC	QR control	Downstream QR
Site	2	3-5	6	7-9	2	3-5	6	7-9
Distance to WWTP (km)	-7	5-60	-	65-140	-7	5-60	-	65-140
Chl a (µg/L)	28.52 ± 3.74	41.49 ± 4.77	56.25 ± 8.08	49.01 ± 4.09	67.30 ± 5.60	44.15 ±8.01	22.68 ± 3.71	65.72 ± 5.39
Discharge (m³/s)	4.08 ± 1.28	5.49 ± 0.86	15.66 ± 5.64	27.64 ± 2.73	0.41 ± 0.17	1.20 ± 0.16	2.99 ± 0.54	3.46 ± 0.22
pH	8.50 ± 0.08	8.06 ± 0.05	8.43 ± 0.11	8.51 ± 0.05	8.37 ± 0.06	8.33 ± 0.07	8.50 ± 0.04	8.64 ± 0.03
Sp. conductance (µS/cm)	942.48 ± 47.64	1292.53 ± 35.15	917.88 ± 51.83	1235.95 ± 43.31	900.06 ± 34.90	1481.91 ± 33.14	872.76 ± 53.90	1193.64 ± 38.58
Temperature (°C)	18.50 ± 0.85	18.56 ± 0.48	19.49 ± 1.25	19.36 ± 0.44	17.22 ± 0.68	17.82 ± 0.39	17.81 ± 0.65	18.00 ± 0.36
NO3$^-$ (mg N/L)	0.22 ± 0.05	1.98 ± 0.16	0.27 ± 0.13	1.17 ± 0.11	0.05 ± 0.01	1.70 ± 0.16	0.01 ± 0.002	0.19 ± 0.03
NH4$^+$ (mg N/L)	0.13 ± 0.03	6.64 ± 0.96	0.07 ± 0.01	0.19 ± 0.03	0.09 ± 0.02	0.33 ± 0.07	0.04 ± 0.02	0.04 ± 0.02
δ^{15}N (‰)	5.70 ± 0.49	18.98 ± 0.75	11.14 ± 1.13	10.71 ± 0.89	5.73 ± 0.52	14.71 ± 0.45	5.35 ± 0.65	8.14 ± 0.39
SRP (mg P/L)	0.36 ± 0.03	0.36 ± 0.02	0.13 ± 0.03	0.17 ± 0.01	0.04 ± 0.01	0.07 ± 0.01	0.02 ± 0.005	0.02 ± 0.003
TDN:SRP	3.73 ± 0.81	22.02 ± 2.52	9.06 ± 2.37	13.24 ± 1.40	54.57 ± 9.04	90.85 ± 10.03	68.07 ± 8.97	160.83 ± 54.39

1148

1149 **Figure 1.** Map of sampling locations within Wascana Creek and Qu'Appelle River (main

1150 panel), within the Qu'Appelle River catchment (lower right) in Saskatchewan, Canada

1151 (upper right).

1152 **Figure 2.** The modelled spatial and temporal distribution of physico-chemical covariates

1153 in 2010-2012 (pre-upgrade) and 2017-2019 (BNR upgrade) in Wascana Creek (WC) and

1154 Qu'Appelle River (QR) along a continuum in reference to Regina's wastewater treatment

1155 plant (WWTP). Modelled physical-chemical variables and their explained deviance

1156 included: discharge (92.9%), pH (79.5%), specific conductance (78.1%), temperature

1157 (89.3%), NO_3^- (75.3%), NH_4^+ (86.2%), $\delta^{15}N$ (84.3%), soluble reactive phosphorus (SRP)

1158 (85.7%), and total dissolved nitrogen (TDN):SRP (87.0%). Coloured lines are predicted

1159 means at four days of year, 136, 170, 200, 236, in that correspond to summer months in

1160 the growing season. Shaded areas are 95% confidence intervals. Square boxes and error

1161 bars are the monthly mean and confidence intervals, respectively, of a control site in QR

1162 ~5 km upstream of the WC-QR continuum that was not modelled. Red and black dotted

1163 lines represent the inflow of WWTP effluent into WC and the confluence of WC with

1164 QR, respectively.

1165 **Figure 3.** The modelled spatial distribution of phytoplankton trichromatic Chl a and

1166 HPLC-derived biomarker pigments in 2010-2012 (pre-upgrade) and 2017-2019

1167 (wastewater treatment upgrade) in Wascana Creek (WC) and Qu'Appelle River (QR) as a

1168 function of distance to Regina's wastewater treatment plant (WWTP). Modelled

1169 phytoplankton (pigments) and deviance explained include total phytoplankton (Chl a;

1170 71.3% dev. explained), cryptophytes (alloxanthin; 65.7% dev. explained), siliceous algae

1171 (fucoxanthin; 74.0% dev. explained), chlorophytes (Chl b; 76.7% dev. explained), and

1172 total cyanobacteria (echinenone; 79.6% dev. explained). Coloured lines are predicted

1173 means from 10,000 model simulations for 100 distance values and six years. Shaded

1174 areas are 95% confidence intervals. Square boxes and error bars are the yearly mean and

1175 confidence intervals, respectively, of a control site in QR ~5 km upstream of the WC-QR

1176 continuum that was not modelled. Red and black dotted lines represent the inflow of

1177 WWTP effluent into WC and the confluence of WC with QR, respectively.

1178 **Figure 4.** The modelled seasonal pattern of phytoplankton trichromatic Chl a and HPLC-

1179 derived biomarker pigments in pre-upgrade (2010-2012) and post upgrade years (2017-

1180 2019) in Wascana Creek (WC) and Qu'Appelle River (QR) as a function of distance to

1181 Regina's wastewater treatment plant (WWTP) at headwaters (7 km upstream of the

1182 WWTP [WC]), WWTP-impacted (36 km downstream of the WWTP [WC]), and

1183 downstream (137 km downstream of the WWTP [QR]) locations. Modelled pigments

1184 with deviance explained include total phytoplankton (Chl a; 71.3% dev. explained),

1185 siliceous algae (fucoxanthin; 74% dev. explained), cryptophytes (alloxanthin; 65.7% dev.

1186 explained), chlorophytes (Chl b; 76.7% dev. explained), and total cyanobacteria

1187 (echinenone; 79.6% dev. explained). Each line is the predicted mean for one distance

1188 and day of year for periods before or after the WWTP BNR upgrade. Shaded areas are

1189 95% confidence intervals.

1190 **Figure 5.** The modelled marginal smooth effects of physico-chemical variables on total

1191 phytoplankton biomass (centred trichromatic Chl a) in Wascana Creek (WC) and the

1192 Qu'Appelle River (QR) in 2010-2012 and 2017-2019. Models include a) all years

1193 (39.8% dev. explained), b) pre-upgrade years only (2010-2012; 56.3% dev. explained)

1194 and, c) post-upgrade years only (2017-2019; 42.7% dev. explained). Red lines represent

1195 the mean effect, while blue dotted lines are 95% credible intervals. Variables that were

1196 not significant for any model (TDN:SRP) are not presented. Significance of model fit is

1197 presented as P-values in panels.

1198 **Figure 6.** The modelled marginal smooth effects of physico-chemical variables on

1199 centred phytoplankton pigments in Wascana Creek (WC) and the Qu'Appelle River (QR)

1200 before (2010-2012) and after (2017-2019) upgrade to biological nutrient removal

1201 technology. For the global pigment smooths (all pigments included) red lines represent

1202 the mean effect and blue dotted lines are 95% credible intervals. The taxa-specific

1203 effects (how the specific pigments vary over the global effect) are to the right of the

1204 global effect panels and shaded areas are 95% credible intervals. Cryptophytes

1205 (alloxanthin) are in yellow, chlorophytes (chl b) are in green, cyanobacteria (echinenone)

1206 are in blue, and siliceous algae (fucoxanthin) are in orange. Deviance explained for the

1207 phytoplankton models was 68.8% for all years, 78.2% for pre-upgrade years (2010-

1208 2012), and 74.1% for BNR upgrade years (2017-2019). P-values are presented at the top

1209 panels.

1210

1211

1212
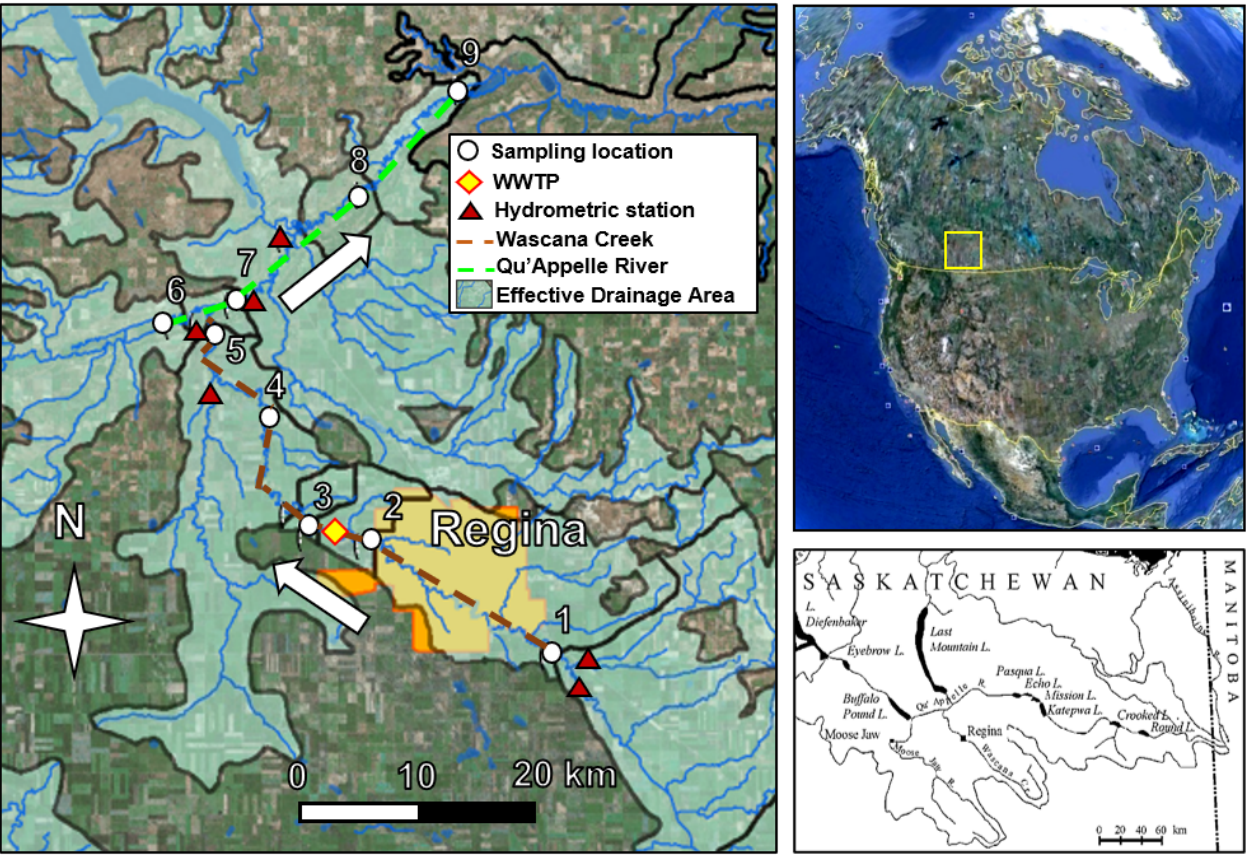
1214
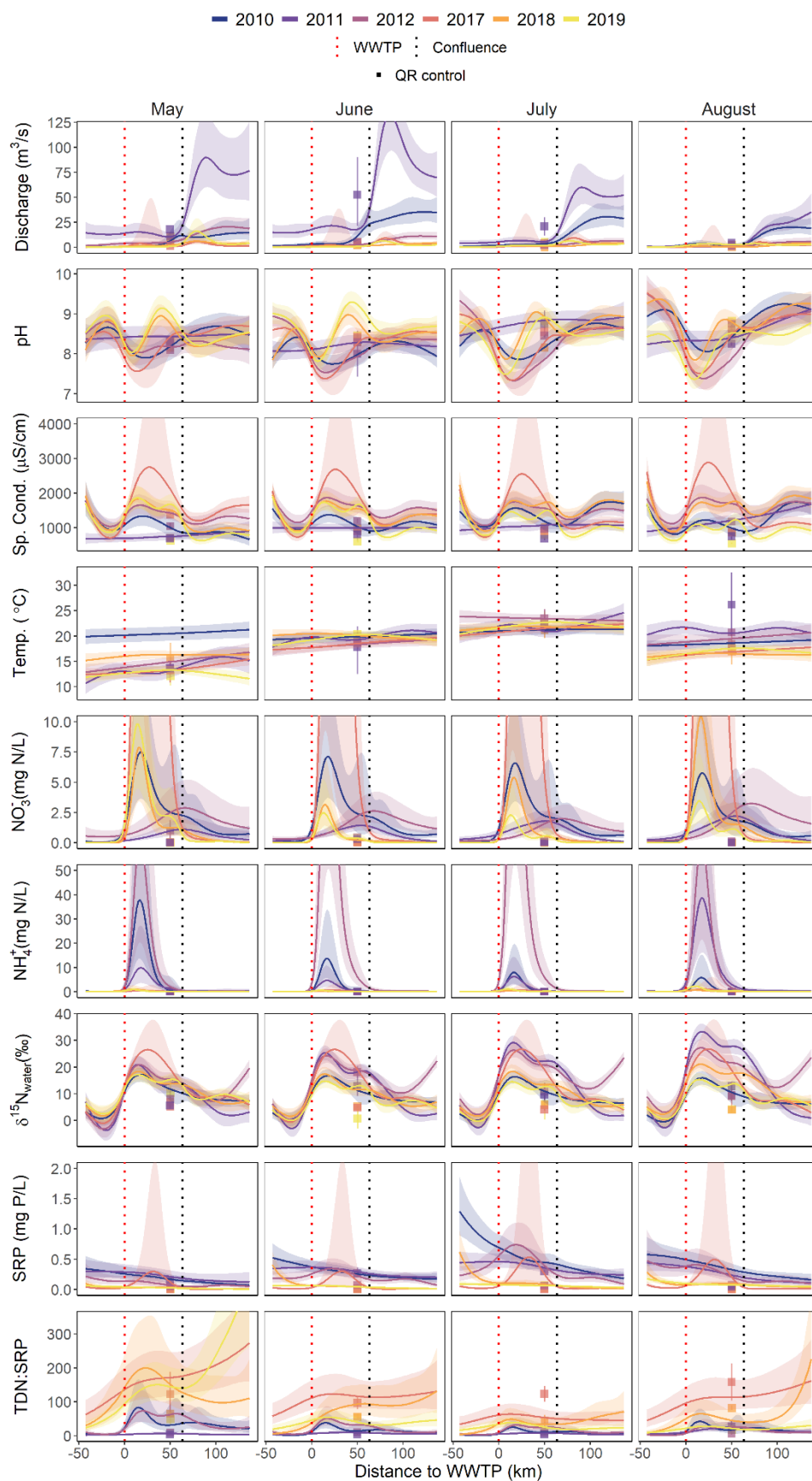
Figure 1.

Figure 2.

1219 **Figure 3.**

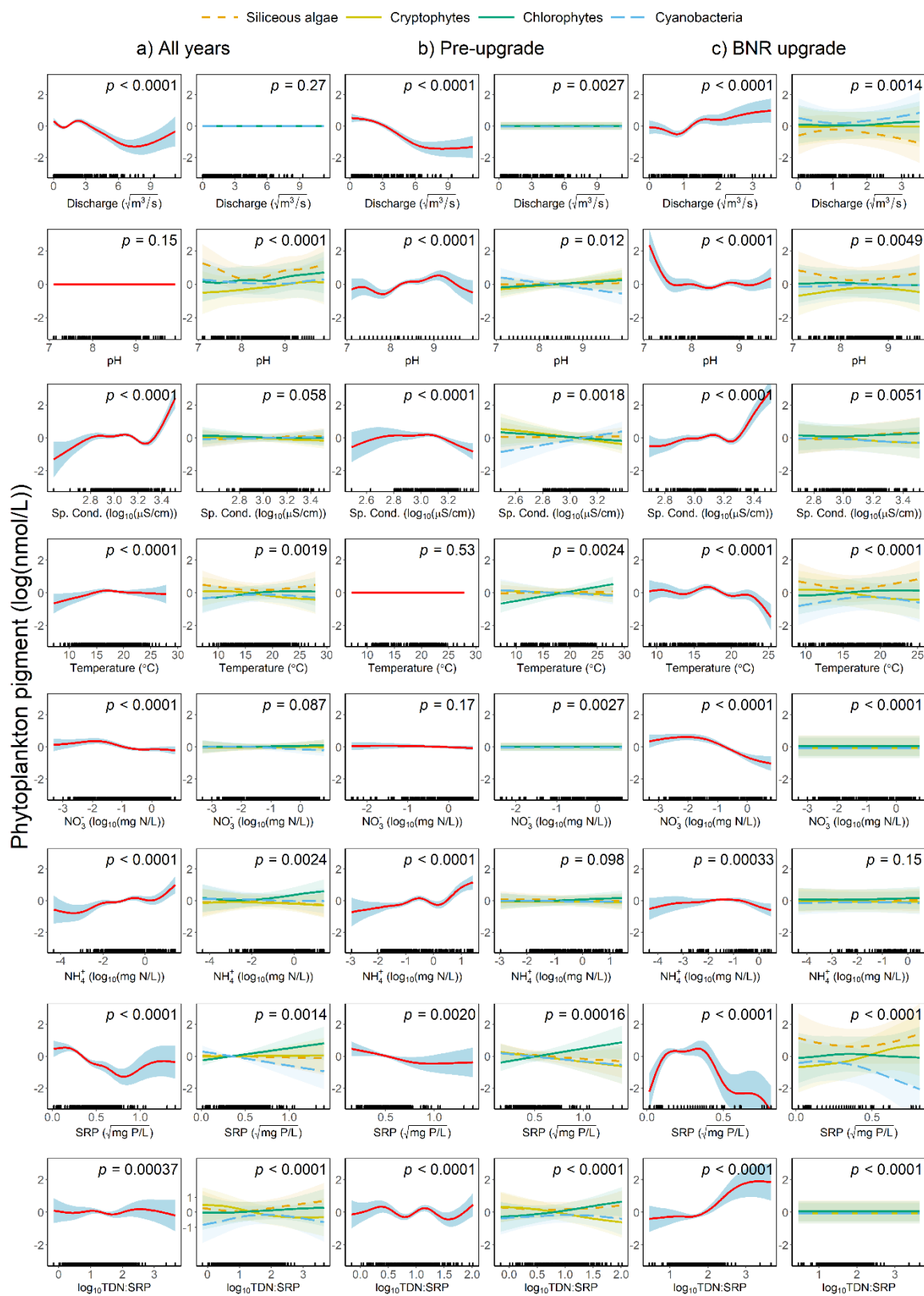**a) All years**   **b) Pre-upgrade**   **c) BNR upgrade**

1224   **Figure 5.**

Figure 6.

58
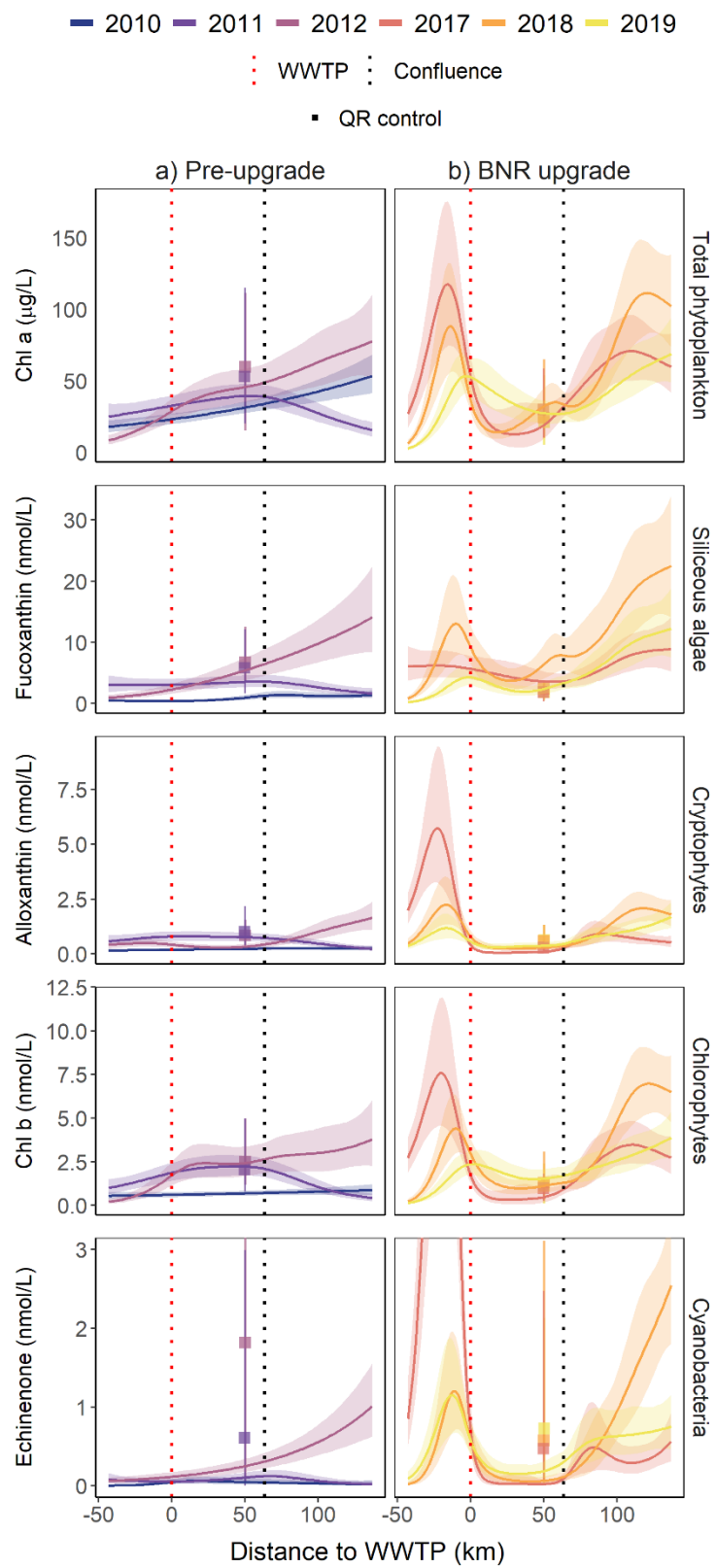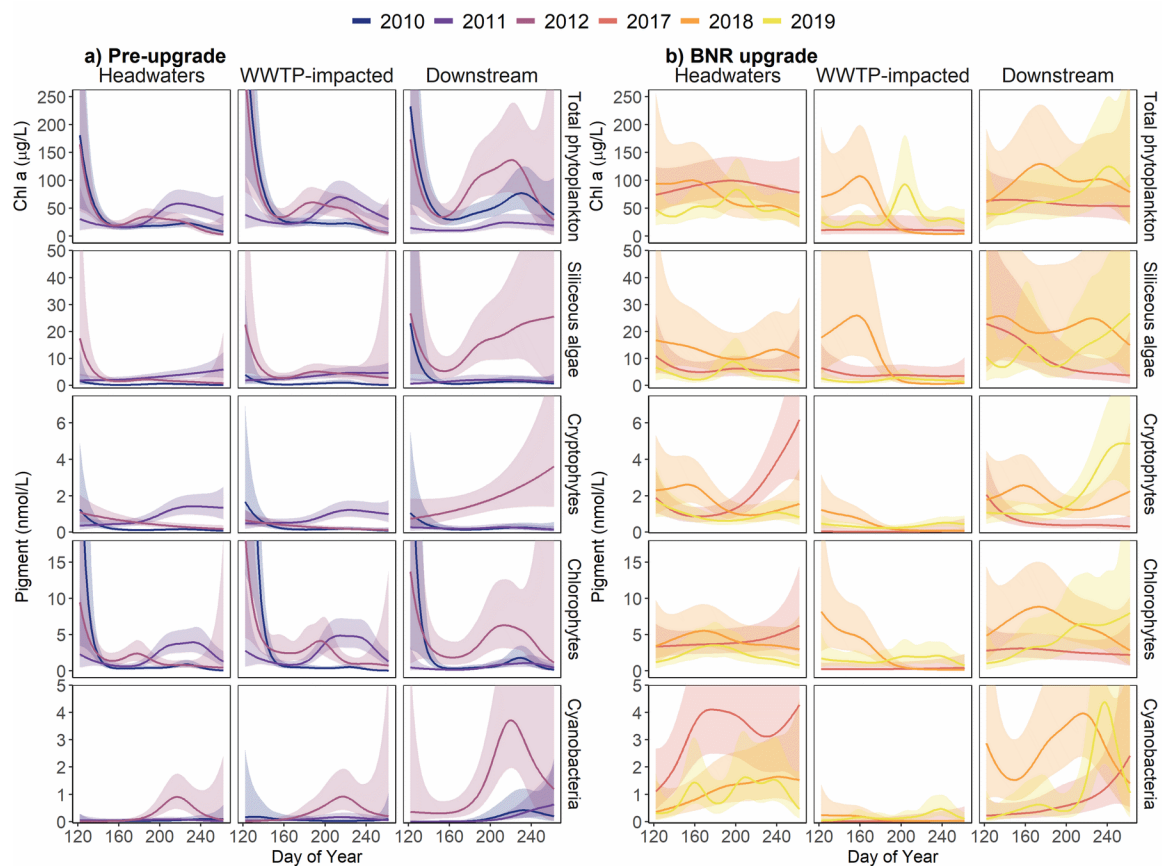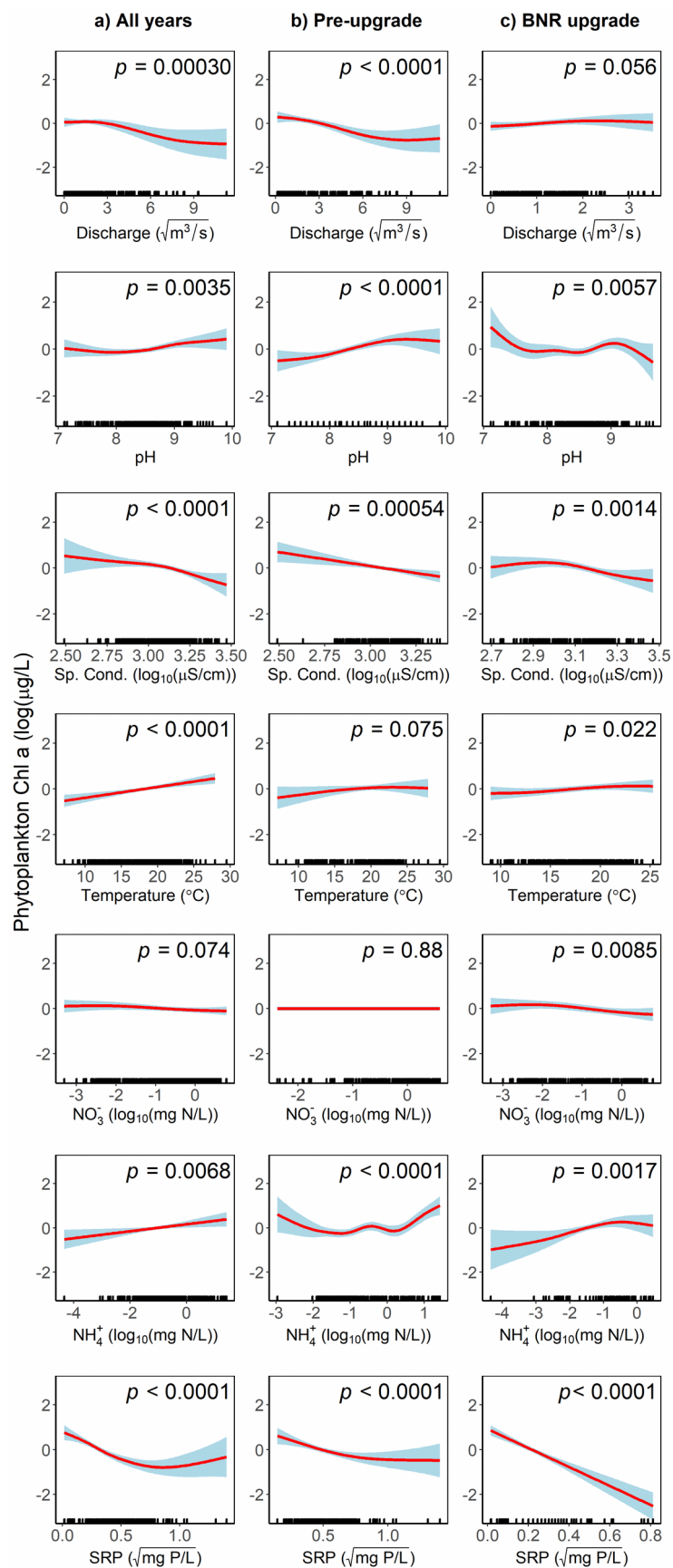
Supporting Information

1227

1228

Adjusted R-squared: 0.709
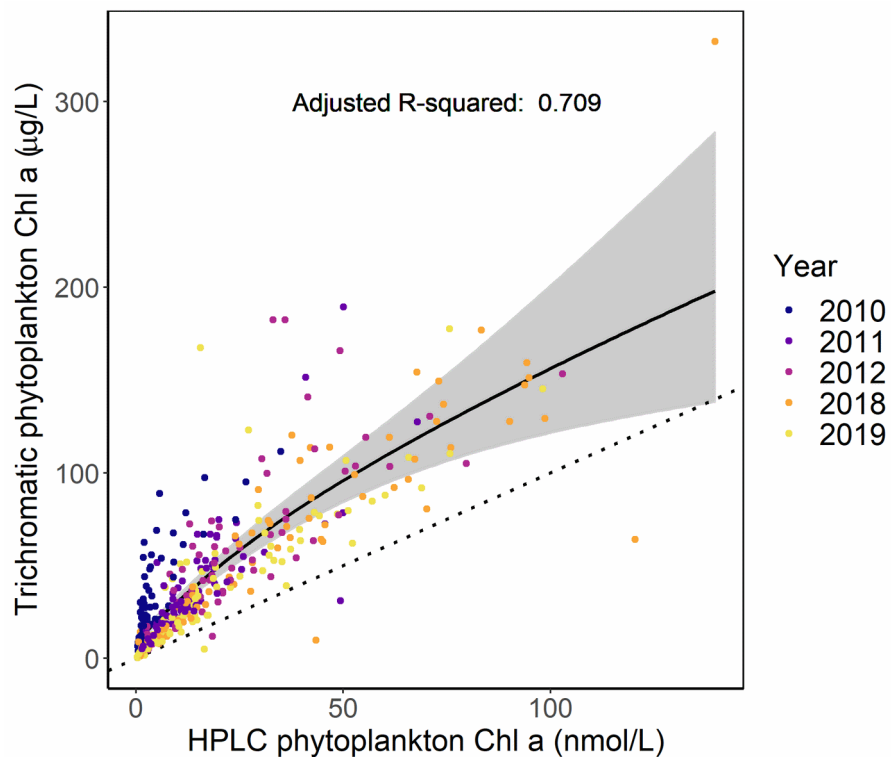
1230 **Figure S1.** Comparison of Chl a measured by trichromatic and high performance liquid

1231 chromatographic methods in 2010-2012, 2018 and 2019. The black line is the mean

1232 estimate and the shaded area is the confidence interval from a generalized additive

1233 models (GAM). This relationship was used to estimate trichromatic Chl a from HPLC

1234 Chl a in 2017.

1235
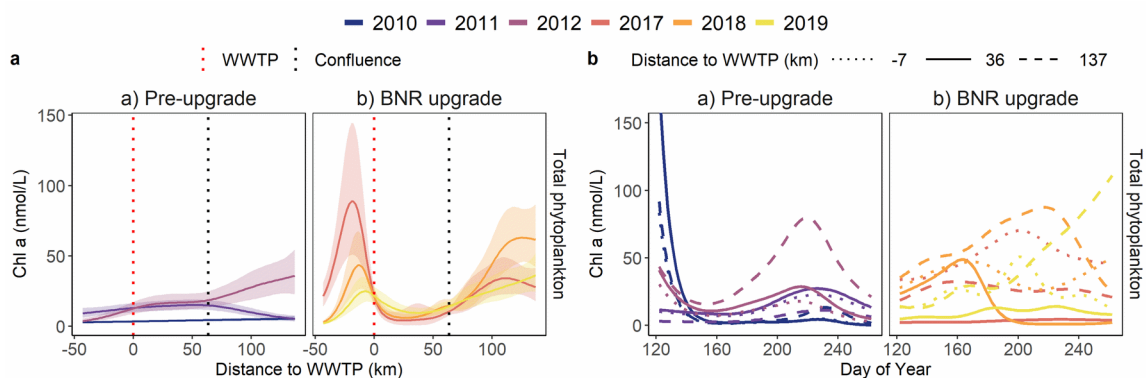
Figure S2. Modelled spatial (a) and temporal (b) variation in phytoplankton abundance estimated by high performance liquid chromatography analysis Chl a during 2010-2012 (pre-upgrade) and 2017-2019 (BNR upgrade) in Wascana Creek (WC) and the Qu'Appelle River (QR) as a function of (a) distance from the City of Regina's wastewater treatment plant (WWTP) and (b) day of year for three distances from the WWTP (-7 km, 36 km, 137 km). Overall deviance explained by generalized additive models was 76.8%. Coloured lines are predicted means from 10,000 model simulations for 100 distance values and six years. Shaded areas are 95% confidence intervals. Coloured lines are predicted means from 10,000 model simulations for 100 distance values and six years. Shaded areas are 95% confidence intervals. Red and black dotted lines in a represent the position of effluent influx into WC and the location of the confluence of WC with QR, respectively.
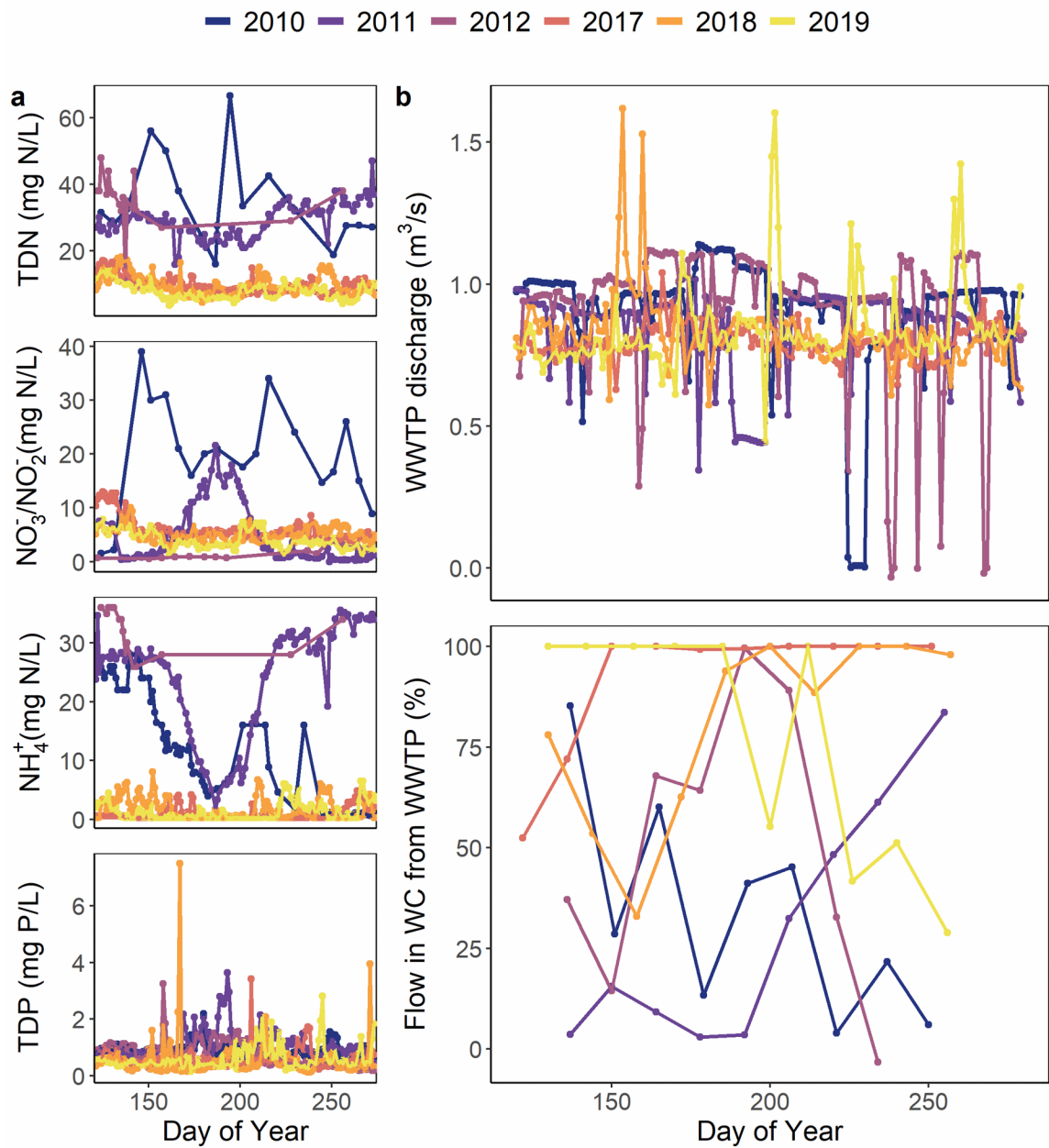
Figure S3. Final effluent nutrient concentrations and outflow from the City of Regina wastewater treatment plant (WWTP). Effluent discharge and contribution of Wascana Creek flow (WC) during the May to September growing season in (a) 2010-2012 and (b) 2017-2019
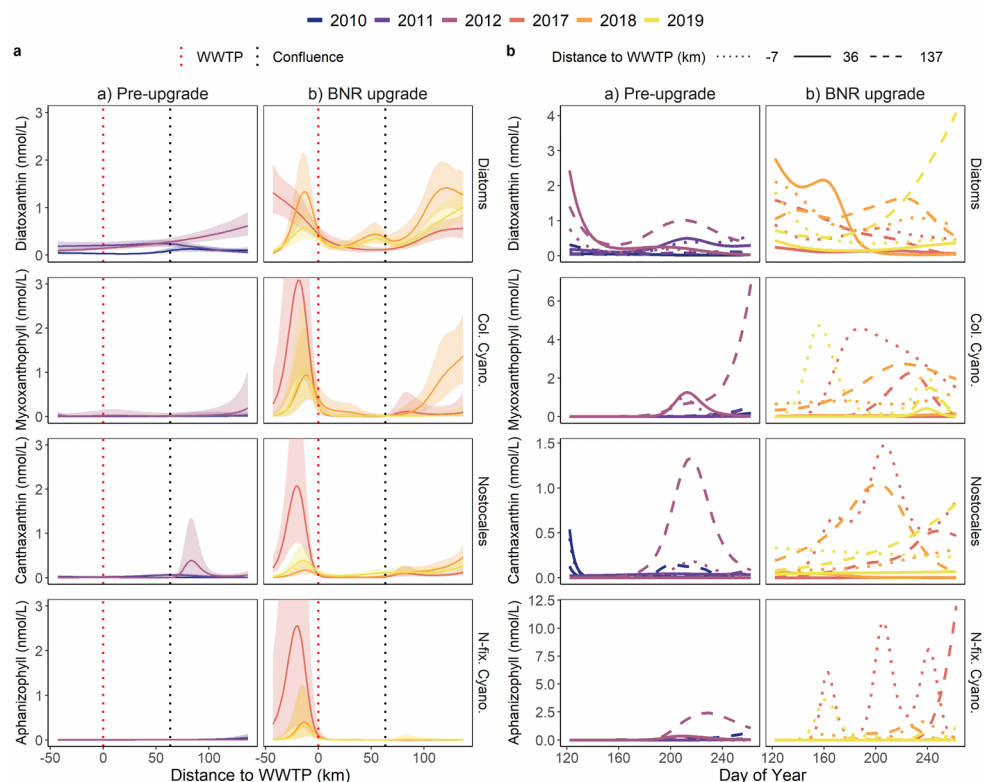
Figure S4. Modelled spatial (a) and temporal (b) distribution of auxiliary biomarker
pigments analyzed using high performance liquid chromatography during 2010-2012
(pre-upgrade) and 2017-2019 (BNR upgrade) for samples from Wascana Creek (WC)
and the Qu'Appelle River (QR) as a function of distance from Regina's wastewater
treatment plant (WWTP). Modelled pigments with deviance explained include diatoms
(diatoxanthin; 74.8% dev. explained), colonial cyanobacteria (myxoxanthophyll; 82.5%
dev. explained), Nostocales (81.7% dev. explained), and N_2-fixing cyanobacteria
(aphanizophyll; 90.4% dev. explained). Dinoflagellates (diadinoxanthin) was removed
because of poor model fit, but values were within 1-3 nmol/L. Coloured lines for the
spatial model are predicted means from 10,000 model simulations for 100 distance values
and six years. Shaded areas are 95% confidence intervals. Red and black dotted lines
represent the inflow of WWTP effluent into WC and the confluence of WC with QR,
respectively. Coloured lines for the temporal model are predicted Day of Year means for
six years at three distances from the WWTP (-7, 36, 137 km) represented with dotted
lines.

1271
1272
1273
1274
1275
1276
1277
1278
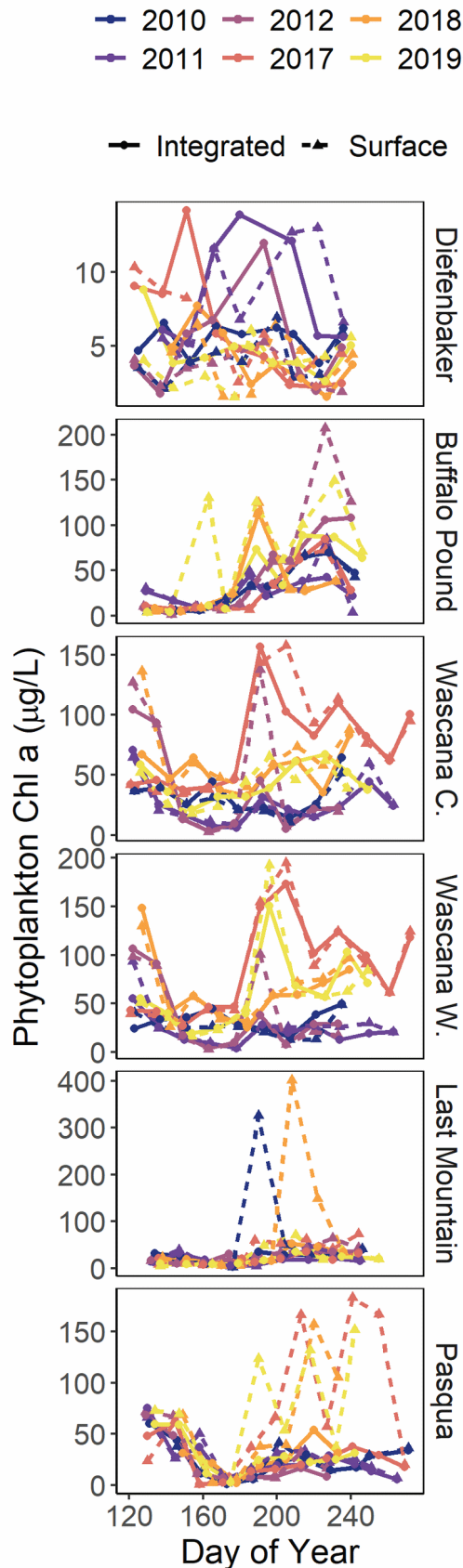1279
1280
1281
1282
1283
1284
1285

Figure S5. Surface and integrated phytoplankton Chl a values in five Qu'Appelle Valley prairie lakes over the growing season (May to September). Basins are geographically ordered from headwater western sites to eastern basins; Diefenbaker, Buffalo Pound, Wascana., Last Mountain, and Pasqua lakes in 2010-2012 (pre-WWTP-upgrade) and 2017-2019 (post-BNR-upgrade). Coloured lines and points are years, and dotted and straight lines represent surface and integrated values, respectively.
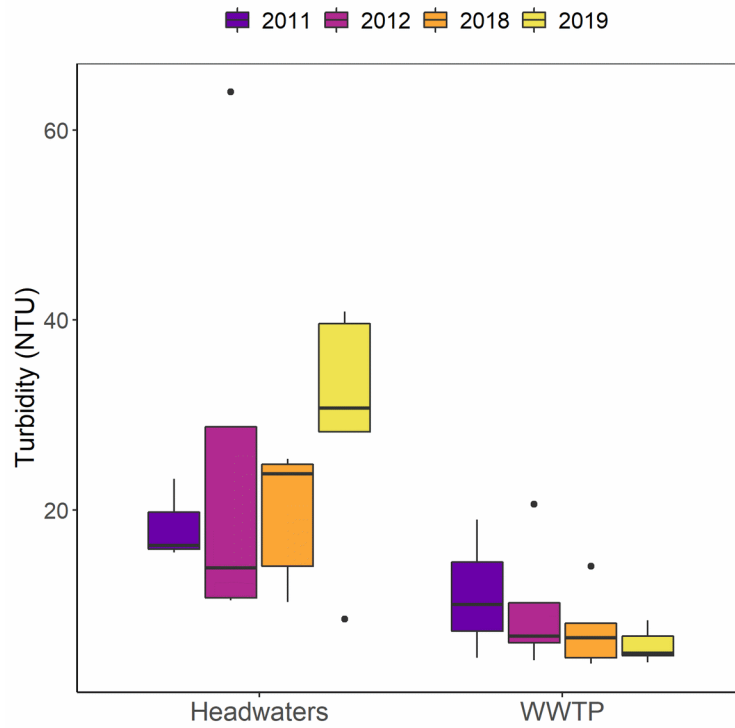
1287 **Figure S6.** Boxplots of stream turbidity (NTU) at sites directly upstream (headwaters)

1288 and downstream (WWTP) of urban wastewater inputs in 2011, 2012, 2018, and 2019.