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Title: Spatial and seasonal variability in surface water chemistry in the Okavango Delta, Botswana: a multivariate approach

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Response to Reviewers: I have added a title page to the Supplementary material and saved all the excel worksheets into one PDF file.

I have also turned-off track changes on the revised manuscript and tables. Sorry about that!

Anson

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1 Abstract

2 The annual flood pulse in the Okavango Delta (Botswana), has a major influence on water 3 chemistry and habitat. We explore spatial and temporal patterns in a suite of chemical variables, 4 analysed from 98 sample points, across four regions, taken at different stages of the flood cycle. 5 The major pattern in water chemistry is characterised by an increasing gradient in ionic 6 concentration from deep-water sites in the Panhandle to more shallow, distal regions to the south. 7 Concentrations of cations, anions, dissolved organic carbon, and SiO₂ are significantly higher in 8 the seasonally inundated floodplains than in permanently flooded regions. Several variables 9 (including Na and total nitrogen) significantly increase from low flood to high flood, while others 10 (including HCO₃, SiO₂, and Cl) increase in concentration, initially between low flood and flood 11 expansion, before declining at maximum flood extent. Redundancy analysis (RDA) revealed that 12 hydrological variables (water depth, flow velocity, flood frequency, and hydroperiod class) 13 significantly explain 17% variation in surface water chemistry. Predictions of increasing flood 14 volume in the near future may result in a decline in alkalinity and dilution of DOC. Our study 15 provides an important baseline from which to monitor future change in the Delta.

16 Keywords: floodplains; multivariate techniques; water chemistry; wetlands

17

18 Introduction

Wetlands provide vital ecosystem services for human populations worldwide through the provision of freshwater, food and biodiversity, and the mitigation of climate change (Millennium Ecosystem Assessment 2005). Yet these ecosystems are particularly vulnerable to threats from human impact through increasing economic development and population growth. For example, over the last 100 years, biodiversity of inland waters has been especially impacted by habitat change and excessive nutrient loading (Jenkins 2003). Over the next 100 years, climate change is

- 1 expected to exacerbate these problems, although in some regions, such as southwest Africa,
- 2 climate change scenarios are still highly uncertain (Hughes et al. 2010).

3 The Okavango Delta (hereafter referred to as the Delta) in northern Botswana is one of the 4 largest wetlands in Africa, and one of the few where human impact is minimal. The Delta is a low-5 gradient alluvial fan, and occupies the lowest region of the Kalahari Basin. The Delta is fed by 6 rivers with their source in the highlands of Angola, and both the rivers and the Delta together form 7 the Okavango River Basin (Fig. 1). The Delta is a flood-pulse ecosystem (Junk et al. 1989), 8 controlled mainly by an annual pulse of water, turning dry floodplains into productive aquatic 9 environments (Mendelsohn et al. 2010). Every year, precipitation falling in the highlands of 10 Angola during October, flow down the Cubango and Cuito rivers, which join to form the 11 Okavango River at the border between Angola and Namibia. This flood pulse reaches the northern 12 part of the Delta (in Botswana) in February–May, before slowly expanding out, and reaching the 13 distal regions by July-August. Maximum flood extent occurs towards the end of the dry season 14 between July–September, and declines again during the summer, between October–March. 15 The flood pulse is ultimately forced by climate, although as it expands out in the Delta 16 there is strong spatial variability, due to the Delta's flat topography and vegetation-controlled 17 channel-floodplain interactions (Wolski and Murray-Hudson 2006a). Currently, technological 18 modifications of the Delta are few, but threats are real. For example, dam building upstream for 19 hydroelectricity and agriculture in Angola and Namibia have the potential to alter the volume of 20 water flowing into the Delta (Mendelsohn et al. 2010). Increasing tourism, on the other hand, may 21 increase habitat disturbance, while population growth in villages and towns brings with it the 22 possibility of deteriorating water quality (Masamba and Mazvimavi 2008). There is substantial 23 seasonal, inter-annual, and decadal variation in size of the flood and the extent of inundated area 24 (Mazvimavi and Wolski 2006). This variation is mainly due to variation in flow of the Okavango River (9 x $10^9 \text{m}^3/\text{yr}$), but also to local summer rainfall (6 x $10^9 \text{m}^3/\text{yr}$) and to a lesser extent the 25

1 nature of the previous year's flood (McCarthy et al. 2003). Rainfall falls mainly during December, 2 January, and February and is higher in the north (c. 550 mm/yr) than in the south (450 mm/yr). 3 Approximately 2% of water leaves the Delta through rivers in the south; the remainder is lost 4 through evaporation (74%) and transpiration processes (24%) (Mendelsohn et al. 2010). 5 Hydrology and wetland chemistry are closely linked (Mitsch and Gosselink 2000). However, 6 while the hydrology of the Delta has been comprehensively studied (see Milzow et al. 2009 for a 7 review), there have been considerably fewer investigations into how the flood pulse in the Delta 8 impacts nutrients (Mubyana et al. 2003; Lindholm et al. 2007) and inorganic chemistry (Sawula 9 and Martins 1991). Previous studies focussed only on very short time periods (Ashton et al. 2003), 10 or were restricted to the Jao / Boro river system (Sawula and Martins 1991; Cronberg et al. 11 1996a,b). Here we report on a large, comprehensive, surface water chemistry dataset that covers 12 four important regions in the Delta, sampled over a complete flood cycle. Given the importance of 13 the Delta in terms of biodiversity (Ramberg et al. 2006) and ecosystem services (Mendelsohn et 14 al. 2010) it is important to characterise baseline information on surface water chemistry across 15 different regions, and at different stages, of the hydrological cycle. This information is 16 fundamental for the detection and monitoring of future ecosystem change. The first aim of this 17 study was to characterise variation in surface water chemistry in relation to (i) the different regions 18 of the Delta, (ii) different stages of the flood cycle over a period of one year, and (iii) between 19 habitats which were either seasonally or permanently flooded. The second aim was to explore the 20 influence of four hydrological variables linked to the flood pulse (water depth, flow velocity, flood 21 frequency and hydroperiod class) on surface water chemistry, using multiple regression 22 techniques.

23 Methods

24 <u>Regional descriptions and site selection</u>

1	Study sites were selected across four regions in the Delta (Fig. 1). The upper Panhandle
2	(UPH) in the north is a fault-bounded, flat-bottomed valley dominated by fluvial input from the
3	Okavango River. Downstream from the UPH is the region known as the lower Panhandle (LPH),
4	where the Okavango River meanders across the floodplain. South of the LPH, the Okavango River
5	spreads out into channels, which get progressively smaller with distance. The main distributary
6	(Maunachira / Khwai) is an eastern extension of the Okavango River, with many large, flow-
7	through lagoons such as Xakanaxa (XAK). Several secondary distributaries branch off from the
8	Okavango River, including the Jao / Boro (BOR) river system to the west of Chief's Island (Fig.
9	1). Fifty sites were selected to capture a strong hydrological gradient, and built on previous
10	aquatic research undertaken in the Delta (Ashton et al. 2003; Dallas and Mosepele 2007).
11	Sample collection and analysis of chemical response variables
12	Ninety-eight sample points were analysed for water chemistry. These consisted of 23
13	sample points visited only once in the study. The remaining 75 sample points were from 27 sites
14	visited on more than one occasion, during different stages of the flood-pulse cycle: low flood (28
15	Nov-10 Dec 2006; n=28); flood expansion (23 Apr-6 May 2007; n=23); high flood (24 Jul-7 Aug
16	2007; n=26); and flood recession (2 Oct-12 Oct 2007; n=21). Across the regions, 15 sample
17	points were analysed from UPH, 17 from LPH, 31 from XAK, and 35 from BOR. Sample points
18	were further characterised by the dominant habitat, including marginal vegetation in channels
19	(MV-IC), marginal vegetation in lagoons (MV-L), floating vegetation (FV), inundated floodplains
20	(IF), and isolated, seasonally flooded pools (SP) (after Dallas and Mosepele 2007). The first three
21	habitat types are permanently wet (WET), while IF and SP experience wet-dry cycles
22	(WET/DRY). Sample point co-ordinates are given in Online Resource 1(i).
23	pH, conductivity (μ S/cm), and dissolved oxygen (DO) (mg/L) were analysed in situ. pH
24	and conductivity were measured using a portable Fisher Scientific accumet AP85 portable
25	waterproof pH/conductivity meter. A YSI 550 dissolved oxygen instrument was used to measure

1 DO and water temperature (temp) (°C). Field equipment was calibrated before each trip. Total 2 suspended solids (TSS) were determined by filtering Delta water (at least 1L) and measuring the 3 residue weight on GF/C filter papers after being dried at 105°C for 24 h (mg/L). 250 ml of water 4 was filtered through a GF/C filter into acid-washed bottles for non-metal (HCO₃, Cl, NO₃, SO₄, 5 dissolved organic carbon (DOC)) analyses. A second 250 ml of water was also filtered using 6 cellulose nitrate papers for cation analyses (Na, K, Mg, Ca) and immediately acidified with three 7 drops of concentrated HNO₃. A final 250 ml of water was filtered using cellulose nitrate filter 8 papers for silica analysis. All filtered samples were stored on ice in acid-washed plastic sample 9 bottles, then transported to the Okavango Research Institute where they were either analysed 10 immediately on their return, or frozen until analyses could be carried out. HCO₃ was determined 11 within 48 h of sample collection. DOC and anions were analysed within 7 days, while TN and TP 12 were determined within three weeks of collection. Metals were analysed within 2 months after 13 collection. 14 Mg (mg/L) and Ca (mg/L) levels were determined by flame atomic absorption 15 spectrometry using a Varion Spectra 220 instrument. Lanthanum was used as a releasing agent to reduce interferences during Ca analysis (Eaton et al. 1995). Na (mg/L) and K (mg/L) were 16 17 determined by flame photometry using a Sherwood Flame Photometer 410 instrument. HCO₃ 18 (mg/L) was analysed using an auto-titrator (Mettler Toledo model DL 50). Cl (mg/L) and SO₄ 19 (mg/L) were analysed by ion chromatography using a DX-120 ion chromatograph (Eaton et al. 20 1995). An approximation of DOC (mg/L) was obtained by measuring the absorbance of the water 21 samples at 280 nm using a Perkin Elmer Lamda 20 UV/Vis spectrophotometer (Mladenov et al. 22 2005). Total phosphorus (TP (mg/L)), total nitrogen (TN (mg/L)), and nitrates (NO₃ (mg/L)) were 23 analysed by an air segmented flow analyser (Bran + Luebbe AA3) after persulphate digestion.

24 SiO₂ (mg/L) was measured using the heteropoly blue method at 815nm.

25 Collation of hydrological explanatory variables

1 Water depth (m) and velocity (m/s) variables were measured in situ at each sample point. 2 Water depth was usually measured using a *Plastimo Echotest II* handheld depth sounder. In 3 locations with dense, submerged vegetation, water depths were instead measured using a 4 graduated metal pole. Water flow velocities were estimated using an OTT Nautilus C 2000 5 *Electromagnetic Flow Sensor*, which was designed to measure water currents in the marginal 6 zones of river banks, shallow water, and waters with low flow velocities. Annual flood frequency 7 and hydroperiod class variables were derived from remotely sensed images, hydrometric data, and 8 general understanding of hydrological variability in the system. We used 16 data layers depicting 9 maximum annual inundation extent during years 1989–2006 (excluding 1991 and 2003). The 10 layers were obtained from Landsat (5 and ETM) images, using a classification procedure 11 involving spectral clustering and contextual classification (Wolski and Murray-Hudson 2006a). To 12 depict the Delta, four Landsat scenes had to be mosaicked for each coverage. Inundation layers 13 had spatial resolution of 30 by 30 m, and 97% accuracy of determination of inundation extent 14 (Wolski and Murray-Hudson 2006a). Dates of the imagery were selected to coincide with the 15 period of maximum, annual inundation. The flooding in the Delta takes the form of a single, 16 annual event with progressive transition between low inundation and high inundation taking place 17 throughout a year. Earlier work, with weekly NOAA AVHRR images, revealed that the crest of 18 the flood lasts approximately 1-2 months in distal parts, but 3-4 months in the proximal parts of 19 the Delta proper, and fast recession occurs only in October (Wolski and Murray-Hudson 2006b). 20 Based on these findings, Landsat scenes from end of August to mid-September were selected for 21 high-resolution inundation mapping. Number of years inundation occurred was calculated for the 22 entire Delta on a pixel-by-pixel basis, by stacking up all individual layers. Flood frequency was 23 then obtained by dividing number of years with inundation by the total number of flood maps. 24 Flood frequency for each of the sampling sites was obtained by averaging flood frequency from 25 the nine surrounding pixels, corresponding to the sampling point, using neighbourhood statistics

1 procedure. This was done to reduce possible influence of errors resulting from image 2 misclassification and misregistration. In the seasonally inundated parts of the Delta, annual 3 inundation frequency can be considered as a proxy for mean duration of inundation. This results 4 from the nature of the flood event where parts of the system are likely to be inundated longer only 5 in high flood years, for a short term only in moderate years, and not inundated at all in low flood 6 years. However, the relationship between the amplitude of annual water level fluctuations and 7 flood frequency varies for different parts of the system. Available hydrometric data were not 8 enough to formalize these relationships in strict mathematical form. Instead, a set of seven 9 hydroperiod/amplitude classes was defined, and a hydroperiod class was attached to each of the 10 sample points. The classification was based on flood frequency maps and hydrometric data from 11 hydrometric stations in the vicinity of sampling points.

12 Statistical Analyses

13 Prior to all analyses, chemical and hydrological variables were tested for normality on the 14 basis of frequency histograms, quantile-quantile (Q-Q) plots, and Shapiro-Wilk tests using SPSS 15 Statistics 17.0. Differences in chemistry and hydrology were determined among (i) sample points 16 in different regions; (ii) sample points at different stages of the flood cycle; and (iii) permanently 17 or seasonally inundated sample points. Initially, Levene's tests for equality of variances were 18 undertaken to ensure that appropriate parametric and non-parametric tests were used. Variables 19 with equal variance were analysed using either analysis of variance (ANOVA) or 2-tailed t-tests. 20 Regional and flood cycle differences were further investigated using post-hoc tests with 21 Bonferroni corrections. Variables with unequal variances were analysed using Kruskal-Wallis 22 (KW) tests. KW has no equivalent post-hoc test, so where the KW test did reveal significant 23 differences among regions, and within the flood cycle, pairwise Mann-Whitney U tests were 24 undertaken to determine significant relationships between pairs of regions and between pairs of 25 flood cycle stages. Pearson product moment correlations (PPMC; 2-tailed) using SPSS Statistics

1 17.0 revealed substantial inter-correlation among water chemistry variables (47% of variables 2 were significantly correlated at the p=0.05 level; Online Resource 1(ii)). Main gradients in the 3 chemistry dataset were therefore explored using principal components analysis (PCA), with 4 symmetric scaling of the ordination scores, to both summarise our multivariate data, and to 5 determine any underlying structure. Variables were not log transformed, but were centred and 6 standardised as they were measured in different units (Lepš and Šmilauer 2003). Like Cronberg et 7 al. (1996b), we have chosen to include conductivity and alkalinity because not all ionic species 8 were measured in our study. However, we have chosen to exclude DO and temp variables from 9 multivariate analyses because they have such a strong diurnal component, which we did not 10 systematically take account of during our sampling. As measurements for Ca, NO₃, and TSS were 11 incomplete, these variables are also omitted (Table 1). A broken stick model was used to test the 12 significance of PCA axes (Joliffer 1986) using BSTICK v1.0 (Line and Birks 1996). In order to assess the influence of the four hydrological, explanatory variables, we used the linear, direct 13 14 gradient technique of redundancy analysis (RDA), which constrains ordination axes as linear 15 combinations of explanatory variables. Initially, the explanatory power and significance of each 16 hydrological variable was determined through a series of single constrained RDAs, together with 17 Monte Carlo permutation tests (p = 0.002; n = 499). The unique contribution of each variable was 18 then assessed through a series of partial RDAs with the remaining hydrological variables as 19 covariables. A further, partial RDA determined the influence of flood cycle stage. To assess the 20 influence of habitat, a similar set of analyses were undertaken, this time grouping permanently wet 21 habitats (MV-IC+MV-L+FV; n=77) and seasonally flooded habitats (IF+SP; n=21). A major 22 feature of spatial datasets such as ours is the influence of site proximity (e.g., Legendre 1993), and 23 here we partial out sample point co-ordinates using RDA (ter Braak 1987). Ordination analyses 24 were undertaken using Canoco v. 4.5 (Lepš and Šmilauer 2003).

25 **Results**

1 Summary statistical analyses for hydrochemistry data are given in Table 1. Output from all 2 statistical tests is given in Online Resource 1(iii-v). Unfortunately, data are not available for NO₃ 3 and TSS during low flood, nor for Ca during flood expansion. In Figure 2, boxplots are shown for 4 variables that demonstrated significant regional variability. Only conductivity showed significant 5 differences among all the regions, with values increasing along a north-south gradient. DOC also 6 shows significant regional variability along a north-south gradient, except between LPH and 7 XAK. Ca concentrations were only not significant among sites in the Panhandle, and between 8 UPH and XAK. HCO₃ and SiO₂ show a significant increase between Panhandle and distal regions, 9 although there is no significant difference between UPH and LPH, nor between XAK and BOR. 10 The reverse pattern is true for water depth. The pH of surface water is significantly higher at XAK 11 than at BOR or LPH (Fig. 2). DO was similar across most of the Delta, except in the BOR region, 12 where values are significantly lower. Na was significantly lower in UPH than in the other three 13 regions. Cl was significantly lower only between LPH and BOR, and between LPH and XAK. 14 Velocity, SO₄, TSS, and nutrients (TP, TN, and NO₃) did not show any significant regional 15 differences. In Figure 3, boxplots are shown only for variables that demonstrated significant 16 variability among different stages of the flood cycle. Only two variables (Na and Mg) differed 17 significantly among all stages; both had highest concentrations during flood recession, and lowest 18 concentrations during low flood. Pair-wise comparisons for Ca are limited because of 19 measurement problems during flood expansion for this variable. However, these data indicate that 20 Ca concentrations are significantly higher during low flood than during either high flood or flood 21 recession. All other variables shown, exhibited some form of pair-wise significant variation. 22 Surface water temperatures were only not different between flood expansion and flood recession, 23 while pH was significantly different during flood expansion. K and Cl were significantly lower, 24 and TN significantly higher, during high flood. Peak concentrations of SiO₂ during flood 25 expansion, were significantly higher than during high flood and flood recession. DO was

1 significantly higher during high flood than during either low flood or flood expansion. HCO_3 was 2 significantly higher was during flood recession than either low or high flood, while TP was 3 significantly higher during flood recession than either low flood or flood expansion. Six variables 4 exhibited no significant variability with stage of the flood cycle: conductivity, DOC, NO_3 , SO_4 , 5 velocity, and water depth (Table 1; Online Resource 1(iv)). In Figure 4, boxplots are shown only 6 for variables that demonstrated significant variability between either seasonally or permanently 7 inundated habitats. Many variables exhibited no significant difference between the two habitat 8 types, including pH, temp, DO, TP, TN, Cl, Ca, and SO₄. The remaining variables, however, were 9 significantly higher in seasonally inundated habitats, including conductivity, DOC, K, SiO₂, Mg, 10 HCO_3 , Na, and NO_3 (Table 1; Online Resource 1(v)). In contrast, velocity and water depth were 11 significantly lower in seasonally inundated habitats.

12 PCA axes 1 and 2 capture 30% and 16% of variation in the chemistry dataset, respectively. Broken stick reveals axis 1 to be significant, and although the eigenvalue of axis 2 is the same as 13 14 its broken stick variance, here we exercise caution and treat axis 2 as being not significant (Table 15 2). Main patterns in our dataset are shown in the form of a PCA biplot (Fig. 5). Axis 1 represents a 16 significant gradient in surface water chemistry of increasing conductivity, HCO₃, K, Mg, DOC, 17 SiO₂, TN, and Cl, with highest values in sample points mainly in BOR and XAK. These variables 18 have lowest concentrations in the Panhandle region of the Delta. PPMC analyses show that most 19 of these correlations are significant (Online Resource 1(ii)). Axis 2 is associated mainly with TP 20 and SO_4 (Fig. 5). RDA reveals that the four hydrological variables (depth, velocity, flood 21 frequency, and hydroperiod class) significantly account for 17.0% of variation in the chemistry 22 data (Table 3i), with axis 1 being by far the most important gradient (Fig. 6). The RDA triplot 23 shows that axis one is dominated by a strong gradient of positively correlated variables, including 24 conductivity, alkalinity, SiO₂, DOC, nutrients, and cations, all of which are negatively correlated 25 with hydroperiod class and water depth (Fig. 6). Measured chemical variables are generally lowest

in concentration in UPH and LPH sample points, associated with greatest water depths and
 hydroperiod class. Hydroperiod class and water depth are the most significant variables
 influencing water chemistry, even after other hydrological variables, flood extent, and site
 proximity are partialled out (Table 4).

5 Discussion

6 Interpreting the datasets collected is complicated by several factors. Not only are there 7 regional and habitat influences on water chemistry, but the slow moving flood pulse adds an extra 8 dimension to the complexity of the system. For example, as the flood expands in northern part of 9 the Delta, the previous year's flood is still receding in the south. Cronberg et al. (1996b) correlated 10 solute concentrations with discharge measurements to account for some of this hydrological 11 variability. Here, we took a multivariate approach, and used four explanatory variables linked to 12 the flood pulse to model impacts on hydrochemistry across different regions of the Delta. 13 Do significant differences exist within the surface water chemistry dataset among the different 14 regions of the Delta, among different stages of the flood cycle, and between seasonally or 15 *permanently flooded habitats?*

Water depth and duration of inundation were found to influence surface water chemistry in the seasonally inundated floodplains of the Jao/Boro river system (Cronberg et al. 1996a). For that reason, we have decompiled our datasets, so that average values for each of the chemical constituents can be determined for each of the four regions during each stage of the flood cycle (Online Resource 1(vi)). Much of the comparative work, therefore, can only be done with previous work on the BOR region, although significant differences among regions in this study are also highlighted.

Conductivity of the Okavango River is very low, dominated by silica and bicarbonates of
Ca, Mg, Na, and K (McCarthy and Ellery 1994). These cations are generally very dilute, but show
a significant increase between the Panhandle and distal regions of the Delta (Table 1; Fig. 2). The

increase in SiO₂ and cations is due to evapo-transpiration processes (Dincer et al. 1978) and an
increase in area of seasonally inundated floodplains (Sawula and Martins 1991; Krah et al. 2006).
Yet total concentration of dissolved ions even in the distal regions of the Delta is still very low,
despite evaporation being 2–3 times greater than precipitation (Ramberg and Wolski 2008). This
is because of a unique combination of processes that allows the formation of geochemical islands,
and density-driven sinking of saline waters beneath them, removing solutes permanently from the
surface waters.

8 Concentrations of Mg are highest during flood expansion and recession phases, but decline 9 during peak flood, which can be attributed to the removal and dissolution of precipitated 10 magnesium salts (Cronberg et al. 1996a). Na however, is lowest during low flood and increases 11 steadily throughout the year (Fig. 3). At these concentrations, it is unlikely that Na is co-12 precipitating out of solution, and further work needs to be done to understand these trends in 13 relation to evaporation. Ca concentrations were significantly higher during low flood, similar to 14 trends reported by Cronberg et al. (1996a). Ca is linked mainly to reactions of the carbonate 15 system and dissolution of silicate materials (Sawula and Martins 1991), and it is the most 16 abundant cation throughout the Delta: Ca >K >Na>Mg. When concentrations are converted to 17 milliequivalents/litre (mequiv/L), the abundance order changes to Ca>Na >Mg>K, identical to the 18 relative proportions determined by Cronberg et al. (1996b). Similar to both Sawula and Martins 19 (1991) and Cronberg et al. (1996b), HCO₃ is by far the most dominant anion (over 90%) 20 throughout the Delta because of circumneutral pH (Wetzel 2001). 21 The Okavango River itself is oligotrophic, and nutrients brought in by surface flow are 22 quickly taken up by fringing vegetation along the channels in the Upper Panhandle. The seasonal 23 floodplains link the aquatic habitats in the Delta to terrestrial habitats. Several chemical 24 constituents were significant higher in seasonally inundated habitats, including conductivity,

25 DOC, K, SiO₂, Mg, HCO₃, Na, and NO₃ (Table 1; Fig. 4). Floodplains contain greater

1 concentrations of chemical constituents because flooding acts to mobilise ions and nutrients from 2 previously dry soil surfaces into solution (Cronberg et al. 1996b). Nutrients in the floodplains are 3 derived from a number of sources, including river flow (Cronberg et al. 1996b), in situ from 4 accumulated sedimentary stores over many thousands of years (Mendelesohn et al. 2010), from 5 wind-blown dust (Garstang et al. 1998; Krah et al. 2006), and animal faeces (Lindholm et al. 6 2007). Lindholm et al. (2007) measured highly elevated nutrient concentrations during a period of 7 low flood extent in a BOR floodplain, which they attributed to low dilution of stored nutrients. 8 Further work needs to be done, however, to determine if such observations are more widely 9 applicable, or are particular to that floodplain. Nitrogen and phosphorus are two of the most 10 important macronutrients in wetlands, dependent on a number of factors, including oxygen 11 content, redox potential, temperature, pH, and microbiological processes (Mitsch and Gosselink 12 2000). Our measurements of macronutrients are rather incomplete; nitrate was not measured 13 during low flood, and there were technical problems with analyses of phosphate, nitrite, and 14 ammonium, and so those are not presented here. However, TN and TP have been measured in 15 every region during each stage of the flood cycle, and these still serve as useful macronutrient 16 indicators. Inorganic NO₃ concentrations did not vary significantly with seasonal changes in 17 hydrology (Table 1), perhaps confirming observations by Krah et al. (2006) who also did not 18 detect any nitrate "boost" as the flood arrived and expanded. Garstang et al. (1998) suggests that 19 this may be because most of the nitrogen has been taken up by plant growth at the end of the 20 previous flood period or has undergone denitrification. However, TN concentrations are 21 significantly higher during the period of high flood, perhaps because the floodwaters contain 22 slightly higher nitrogen concentrations than wetland soils (Cronberg et al. 1996a). Mean TP 23 concentrations are low (<0.04 mg/L), similar to levels determined by Krah et al. (2006) for a Boro 24 floodplain, but lower than values obtained by Lindholm et al. (2007). Statistically, however, TP 25 shows no significant regional variation (Table 1). TP concentrations do vary significantly with

1 stages of the flood cycle (Table 1, Fig. 3), especially during flood recession, when concentrations 2 are highest. These findings are in contrast to previous studies, which reported the highest 3 concentrations of TP occurred during the initial flood stage at the Boro floodplain (e.g., Krah et al. 4 2006; Lindholm et al. 2007), and as the flood expanded, concentrations declined. Lindholm et al. 5 (2007) undertook a comparison of two years of nutrient data with different flood sizes, and 6 concluded that local differences are overwhelmed when flood sizes are large because connectivity 7 between river channels and floodplains is increased. It may be that our TP data are a reflection of 8 overall flood size; however, more work needs to be done on the regional and long-term changes of 9 this important macronutrient. TN/TP ratios are useful in determining whether P and/or N is 10 limiting growth in freshwater ecosystems, and how susceptible that ecosystem is to developing 11 algal blooms. For example, high levels of TP might suggest impacted ecosystems through elevated 12 phosphorus supplies (and will give a smaller ratio, e.g., less than 10), while ratios above 15 13 suggest potential P-limitation (Abell et al. 2010). The TN/TP ratio for BOR was 20.4, a figure 14 very similar to that determined by Cronberg et al. (1996b), which suggests potential P limitation in 15 this region. Ratios for the other regions were lower: 13.6 (UPH), 11.8 (LPH), 15.7 (XAK), and it 16 is likely that the availability of N and P in these regions is close to that needed for balanced 17 growth of primary producers, i.e., these nutrients are co-limiting (Abell et al. 2010). Threats of 18 nutrient enrichment from increasing economic activity (e.g., tourism and aquaculture) pose serious 19 management issues for these delicately balanced ecosystems.

Dissolved organic matter (of which DOC is an important component) is formed mainly on the seasonal floodplains, with the arrival of the annual flood (Cronberg et al. 1996b), and from the decomposition of vascular plant material (Mladenov et al. 2007). Few studies have investigated DOC in the Delta, and again, these are restricted to the Jao/Boro river system and associated floodplains. Our data show that there is substantial variation in DOC concentrations throughout the Delta, which are significantly higher in BOR and significantly lower in UPH than elsewhere in

the Delta (Fig. 2). Mladenov et al. (2005) found that DOC concentrations declined just before peak flood, which may be due to a dilution effect from the increased volume of water, together with decomposition by bacterial degradation and UV light (Mladenov et al. 2007). Our study, however, shows no significant differences linked to different stages of the flood cycle, although this may be due to the differences in temporal resolution between our studies.

6 How do hydrological variables, linked to the flood-pulse, influence variation in surface water7 chemistry?

8 Cronberg et al. (1996a) concluded that spatial heterogeneity and seasonal flow was 9 responsible for most of the variation of water chemistry in the Jao/Boro river system. Here we 10 provide the first quantitative estimates that seasonal flow and spatial heterogeneity significantly 11 account for 19% and 17% variation in Delta chemistry, respectively (Table 3). The influence of 12 biogeochemical processes that take place in seasonally inundated floodplains on surface water 13 chemistry is very important (e.g., Cronberg et al. 1996a,b; Mladenov et al. 2005; Krah et al. 2006). 14 By grouping the sites as seasonally or permanently flooded, we show that this habitat distinction 15 accounts for 8% significant variation in the chemistry data (Table 3iii). This lower value may be a 16 reflection of larger flood sizes experienced at the time of the study, diluting nutrients, and other 17 chemical constituents in the floodplains (Lindholm et al. 2007), and the balance between the 18 numbers of permanently and seasonally inundated sites visited in the study.

The four hydrological variables significantly account for 17% of variation in the chemistry data (Table 3i). Even after taking into account variation in the flood cycle, depth and hydroperiod class are still highly important variables (Table 4iii). Water depth is likely to act as a proxy for inundation (Cronberg et al. 1996a), and chemical constituents in shallow water sample points are present in higher concentrations than deep-water sample points (Fig. 6). However, even taking into account whether sites are permanently or seasonally flooded, still leaves depth and hydroperiod class as significant explanatory variables (Table 4iv). It is to be expected that our

1 dataset will be subject to spatial autocorrelation, especially given the proximity of many sample 2 points to each other (Fig. 1; Legendre 1993). This can lead to over-estimation of the interactions 3 between the chemical variables and hydrological predictors. Variation captured by each 4 hydrological variable taking into account proximity, declines by approximately two-thirds (Table 5 4v). It is notable, however, that depth and hydroperiod class variables are still highly significant. 6 The size of the flood pulse in the Delta has increased over the last five years, and is likely 7 to continue to increase into the near future due to a quasi, 80-year, climatic oscillation (Gumbricht 8 et al. 2004). A key question therefore remains as to how an increase in volume of water in the 9 flood pulse will influence surface water chemistry. Here we use t-value biplots to further explore 10 significant relationships between hydroperiod class and water depth with chemical variables. 11 Variables that fall within the Van Dobben circles in the t-value biplot are significantly correlated 12 either positively (solid circle) or negatively (dashed circle) with the variable under investigation 13 (Fig. 7). Hydroperiod class has a significant, positive relationship with pH values in the Delta, but 14 a significant, negative relationship with conductivity, HCO_3 , Mg, and DOC. Therefore, an 15 increasing area of sites with higher hydroperiod class may result in a decline in DOC, 16 conductivity, HCO₃, and certain cations such as Mg, through dissolution of precipitated salts 17 (Cronberg et al. 1996a). An increase in water depth would not have a positive effect on any of the 18 variables that we measured, but would result in a significant decline in concentration of many 19 variables (conductivity, HCO₃, DOC, Mg, K, and SiO₂). Water depth likely represents a proxy for 20 volume of water, and so in shallow water regions, concentrations of solutes are greater. Therefore, 21 in sites that currently experience flooding, an increase in flood size is likely to result in an overall 22 dilution of chemical constituents (Cronberg et al. 1996a). Other factors not measured here are also 23 likely to have a strong influence on water chemistry in the Delta, especially history of the water 24 prior to sampling, and vegetation composition at any one location. Moreover, an increase in flood 25 size will also result in new, dryland regions becoming inundated for the first time in many

1 decades, resulting in new, productive floodplains. This is a large and important uncertainty and

2 requires much more intensive spatial and temporal monitoring in key regions.

3 Conclusions

4 We provide, for the first time, a quantitative assessment of factors that influence surface water 5 chemistry in the Okavango Delta. Significant variation in surface water chemistry exists, 6 especially between the Panhandle and distal regions of the Delta. Seasonal variation linked to 7 different stages in the flood pulse is also very important, although influence on different chemical 8 variables appears to be quite individualistic. It is apparent that hydroperiod class and water depth 9 are important variables in influencing surface water chemistry across the Delta, even when taking 10 into account influences from other hydrological variables, stage of the flood cycle, habitat, and 11 proximity of the sites to each other. Our analyses ignore the likely increase in extent of new 12 floodplains being wetted by expanding floodwaters, a process which is likely to continue into the 13 near future. However, the work presented here provides an important baseline study for spatial 14 variability in water chemistry of this internationally important wetland, from which to monitor 15 future change associated with both natural variability and human impact.

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13 Figure Legends

14 Figure 1: Map of the Okavango Delta with site locations indicated on aerial photographs from

- 15 each of the four study regions
- 16 Figure 2: Boxplots for hydrochemical variables that show significant regional variation (see text
- 17 for details). The central line is the median value, while the bottom and top of the box indicate 25th

18 and 75th percentiles. T-bars extend to 1.5 times the height of the box. Outliers are given as points,

19 whereas extreme outliers (i.e. values that are more than 3x the height of the box) are given as

20 asterisks.

- Figure 3: Boxplots for hydrochemical variables, that show significant variation among different
 stages of the flood cycle.
- 23 Figure 4: Boxplots for hydrochemical variables that show significant variation between sites that
- are either permanently or seasonally flooded.

1 Figure 5: PCA biplot of surface water chemistry in the Okavango Delta. Sample points have been 2 coded according to their location in each of one of the four principal regions. Broken stick reveals 3 that that first axis significantly explained 30% variation. 4 Figure 6: RDA triplot showing sample points (coded according to their location), surface water 5 chemistry variables, and 4 hydrological explanatory variables (depth, velocity, flood frequency, 6 and hydroperiod class). 7 Online Resource 1: PDF consisting of a number of worksheets: (i) sample point co-ordinates; (ii) 8 PPMC analyses of chemistry data; (iii-v) statistical analyses of data showing main results from 9 Levene'stests for equality, ANOVA, 2-tailed t-tests, Kruskal-Wallis tests, pairwise Mann-Whitney 10 tests for (iii) regions; (iv) different stages of the flood cycle; (v) between permanently and 11 seasonally inundated sites; and (vi) decompiled mean data for regions at different stages of the 12 flood cycle.

table

Click here to download table: WELA279 Tables.doc Table 1: Summary statistical analyses for hydrochemical data measured from 98 sample points. Mean values are also given for (i) each of the 4 regions, (ii) each stage of the flood cycle, and (iii) whether sample points were permanently inundated or not. * = no significant difference; ND = not determined. _

		Min	Max	Mean	SD	UPH	LPH	XAK	BOR	low	expand	high	recede	WET-DRY	WET
Depth	m	0.00	5.00	1.43	0.96	2.09	2.24	0.93	1.17	1.27*	1.35*	1.88*	1.15*	0.77	1.61
Velocity	m/S	0.00	1.56	0.15	0.26	0.25*	0.11*	0.16*	0.10*	0.11*	0.20*	0.14*	0.14*	0.05	0.17
рН	pH units	4.97	7.89	6.64	0.45	6.60	6.52	6.98	6.49	6.82	6.61	6.48	6.66	6.70*	6.62*
Temp	°C	13.60	35.00	23.47	4.65	22.46*	21.83*	23.92*	24.76*	28.12	23.92	17.24	24.87	22.83*	23.65*
DO	mg/L	0.39	10.37	3.49	2.13	4.19	3.70	4.59	2.15	3.10	2.74	4.63	3.37	3.63*	3.45*
Conductivity	µS/cm	19.30	119.10	61.72	23.59	36.27	43.64	70.77	80.14	67.90*	62.12*	52.16*	65.50*	84.91	55.39
TSS	mg/L	0.00	10.00	2.34	2.11	3.26*	2.17*	2.11*	1.06*	ND*	3.37*	3.51*	1.99*	2.38*	2.34*
DOC	mg/L	2.73	23.62	8.03	4.28	4.14	7.56	8.36	10.31	8.06*	9.08*	6.45*	8.92*	9.93	7.51
HCO ₃	mg/L	3.50	168.30	57.30	22.57	38.70	43.92	62.74	67.46	50.36	63.71	50.84	68.37	72.28	53.21
K	mg/L	0.33	6.90	2.28	1.34	0.94	1.37	3.29	2.81	2.64	2.93	1.26	2.42	3.08	2.07
Na	mg/L	0.10	7.19	2.01	1.43	1.03	1.57	2.19	2.41	0.97	1.28	2.36	3.86	2.87	1.78
SiO ₂	mg/L	3.00	32.34	11.35	6.48	8.18	8.93	11.09	14.00	11.42	15.74	9.44	8.76	13.90	10.65
Cl	mg/L	0.11	4.24	0.66	0.61	0.51	0.42	1.38	0.86	0.64	1.12	0.40	0.53	0.71*	0.65*
ТР	mg/L	0.00	0.25	0.04	0.03	0.04*	0.05*	0.04*	0.03*	0.03	0.03	0.04	0.06	0.04*	0.04*
TN	mg/L	0.08	1.94	0.62	0.34	0.53*	0.63*	0.56*	0.71*	0.52	0.61	0.77	0.56	0.74*	0.58*
NO ₃	mg/L	0.00	0.71	0.20	0.15	0.16*	0.19*	0.13*	0.27*	ND*	0.21*	0.20*	0.19*	0.17	0.32
SO_4	mg/L	0.01	2.58	0.35	0.60	0.28*	0.21*	1.01*	0.25*	0.58*	0.38*	0.19*	0.23*	0.55*	0.30*
Mg	mg/L	0.14	2.00	0.92	0.47	0.56	0.70	1.02	1.14	0.44	1.13	0.85	1.43	1.24	0.83
Ca	mg/L	3.20	15.46	7.68	3.37	5.27	5.24	7.23	10.24	10.42	ND	5.67	6.44	8.53*	7.36*
						I				1				1	

					Total
Axes	1	2	3	4	variance
Eigenvalues (EVs)	0.304	0.155	0.105	0.094	1
Cum. % var.	30.4	46.0	56.5	65.9	
Broken stick					
variances	0.221	0.155	0.121	0.099	

Table 2: Principal components analysis (PCA) of surface water chemistry.

Table 3: Redundancy analysis (RDA) of surface water chemistry with (i) four hydrological variables; (ii) flood extent; (iii) habitat type; (iv) site locations as explanatory variables.

Table 3i: 4 hydrological variables	Axes	1	2	3	4	Total variance
Eigenvalues:		0.134	0.027	0.007	0.002	1
Cum. % variance of species data:		13.4	16.1	16.8	17.0	
\sum canonical EVs; p = 0.002						0.17
Table 3ii: flood extent						
Eigenvalues:		0.110	0.058	0.024	0.265	1
Cum. % var. spp data:		11.0	16.8	19.1	45.9	
\sum canonical EVs; p = 0.002						0.19
Table 3iii: habitat						
Eigenvalues:		0.085	0.239	0.155	0.103	1
Cum. % variance of species data:		8.5	32.4	48.0	58.2	
\sum canonical EVs; p = 0.002						0.085
Table 3iv: location co-ordinates						
Eigenvalues:		0.147	0.026	0.180	0.155	1
Cum. % variance of species data:		14.7	17.3	35.3	50.8	
\sum canonical EVs; p = 0.002						0.173

Table 4: (i) % variation explained by each of the four hydrological variables; (ii) unique variation explained by each variable with the remaining three variables as co-variables; (iii) variation explained by hydrological variables with flood extent as co-variables; (iv) variation explained by hydrological variables with habitat as co-variables; (v) variation explained by hydrological variables with locational co-ordinates as co-variables. $p \le 0.05$; n=499 permutations).

Hydrological Variables	(i)		(ii)		(iii)		(iv)		(v)	
	% var	р	% var	р						
Depth	9.6	0.002	5.5	0.002	8.2	0.002	4.6	0.002	3.0	0.006
Velocity	1.7	0.088	1.7	0.056	1.9	0.030	1.1	0.310	1.5	0.072
Flood Frequency	3.6	0.010	2.6	0.012	4.3	0.002	1.1	0.332	2.0	0.036
Hydroperiod Class	7.9	0.002	5.5	0.002	8.8	0.002	3.3	0.002	3.6	0.002



Fig 2 Click here to download line figure: Fig 2 Regions combin cs2.eps













Spatial and seasonal variability in surface water chemistry in the Okavango Delta, Botswana: a multivariate approach

Journal: Wetlands

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Online Resource 1(i)

Code	lat	long	Code	lat	long	Code	lat	long	Code	lat	long
UPH1-2	18.3391	21.8371	LPH1-2	18.8425	22.4043	XAK1-2	19.1830	23.3974	BOR1-2	19.6116	23.2188
UPH1-3	18.3391	21.8371	LPH2-2	18.8791	22.3912	XAK1-3	19.1831	23.3977	BOR10-2	19.5333	23.1829
UPH1-4	18.3391	21.8371	LPH2-4	18.8791	22.3912	XAK1-5	19.1827	23.3983	BOR10-3	19.5333	23.1829
UPH3-3	18.4097	21.8870	LPH2-5	18.8791	22.3912	XAK10-2	19.1253	23.3791	BOR10-4	19.5334	23.1831
UPH4-4	18.4109	21.8811	LPH3-2	18.8790	22.3911	XAK12-2	19.2401	23.3569	BOR10-5	19.5334	23.1831
UPH4-2	18.4109	21.8811	LPH3-5	18.8790	22.3911	XAK12-3	19.2401	23.3569	BOR11-2	19.5289	23.1825
UPH4-3	18.4109	21.8811	LPH3-4	18.8788	22.3913	XAK12-4	19.2401	23.3569	BOR13-3	19.5385	23.1181
UPH5-2	18.4275	21.9812	LPH4-2	18.9555	22.3763	XAK12-5	19.2401	23.3569	BOR13-5	19.5385	23.1181
UPH5-3	18.4275	21.9812	LPH4-4	18.9555	22.3763	XAK13-3	19.1915	23.4520	BOR14-3	19.5269	23.1506
UPH5-4	18.4275	21.9812	LPH4-5	18.9555	22.3763	XAK13-4	19.1915	23.4520	BOR14-4	19.5271	23.1507
UPH7-3	18.4270	21.9714	LPH5-2	18.9606	22.3826	XAK13-5	19.1915	23.4520	BOR14-5	19.5271	23.1507
UPH7-4	18.4270	21.9714	LPH5-4	18.9606	22.3826	XAK14-3	19.1830	23.4409	BOR15-3	19.5380	23.1841
UPH8-3	18.4118	21.8891	LPH5-5	18.9606	22.3826	XAK15-3	19.1773	23.4380	BOR16-3	19.5759	23.2016
UPH8-4	18.4118	21.8891	LPH6-4	18.9176	22.4095	XAK16-3	19.1726	23.4408	BOR17-4	19.5390	23.1133
UPH9-3	18.4284	21.9194	LPH6-5	18.9176	22.4095	XAK18-4	19.1957	23.4413	BOR17-5	19.5390	23.1133
			LPH8-4	18.8653	22.4198	XAK19-4	19.1870	23.4313	BOR18-4	19.5700	23.2045
			LPH9-4	18.9611	22.4069	XAK19-5	19.1870	23.4313	BOR19-4	19.5465	23.1867
						XAK2-2	19.1460	23.3836	BOR19-5	19.5465	23.1867
						XAK2-5	19.1454	23.3835	BOR2-2	19.5660	23.2032
						XAK3-2	19.1866	23.3966	BOR2-3	19.5660	23.2032
						XAK4-3	19.1753	23.4199	BOR2-4	19.5660	23.2032
						XAK4-5	19.1753	23.4199	BOR2-5	19.5660	23.2032
						XAK5-2	19.1878	23.4337	BOR20-5	19.5454	23.1847
						XAK5-4	19.1878	23.4337	BOR3-2	19.5558	23.2002
						XAK5-5	19.1878	23.4337	BOR4-2	19.5500	23.1794
						XAK6-2	19.1919	23.4321	BOR5-2	19.5344	23.1948
						XAK7-2	19.1889	23.4524	BOR6-2	19.5394	23.0474
						XAK7-3	19.1889	23.4524	BOR7-2	19.5437	23.0478
						XAK7-4	19.1889	23.4524	BOR8-2	19.5397	23.0896
						XAK9-2	19.2019	23.4607	BOR8-3	19.5397	23.0896
						XAK9-4	19.2019	23.4607	BOR8-4	19.5397	23.0896
									BOR9-2	19.5494	23.1775
									BOR9-3	19.5494	23.1775
									BOR9-4	19.5494	23.1775
									BOR9-5	19.5494	23.1775

Online Resource 1(ii)

Significant Pearson Product Moment Correlations between measured hydrochemical variables and location co-ordinates. * correlation is significant at the 0.05 level (2-tailed); ** correlation is significant at the 0.01 level (2-tailed).

	pН	Temp	DO	Conduct	DOC	HCO3	K	Na	SiO2	Cl	Mg	Ca	Depth	Veloc
Temp	0.225*													
DO	0.369**													
Conductivity		0.313**	-0.395**											
DOC		0.209*	-0.361**	0.546**										
HCO3			-0.247*	0.607**	0.445**									
K		0.454**	-0.297**	0.589**	0.411**	0.390**								
Na						0.311**								
SiO2		0.207*	-0.233*	0.378**	0.391**		0.511**							
Cl		0.216*	-0.286**	0.389**	0.294**	0.238*	0.498**		0.372**					
ТР								0.222*		-0.229*				
TN	-0.292**			0.229*	0.221*	0.286**			0.241*					
SO4	0.234*	0.206*						-0.200*						
Mg	-0.207*		-0.251*	0.456**	0.290**	0.553**	0.349**	0.687**	0.268**	0.201*				
Ca		0.504**	-0.395**	0.651**			0.549**			0.397**	-0.257*			
Depth		-0.295**		-0.522**	-0.316**	-0.379**	-0.527**	-0.230*	-0.226*		-0.274**			
Veloc														

Online Resource 1(iii)

Resurce (iii)
Test of Itamogenetics of Variances for Regions
Interview of 1 and 2 an Sig. pH Temp DO Conductivity DOC HCO3 K Na SiO2 Cl TP .495 .003 .190 .015 .326 .219 .000 .001 .007 .290 2.096 4.894 6.329 21.348 2.234 18.685 13.611 5.749 TN NO3 TSS SO4 Mg Ca Depth Velocity 94 57 83 94 94 71 94 .106 .004 .001 .000 .089 .000 .000 .001

	A	NOVA of var	iables with co	qual variances	4
	Sum of		Mean		
	Squares	df	Square	F	Sig.
pН	3.172	3	1.057	5.942	.001
DO	105.939	3	35.313	9.969	.000
HCO3	12577.021	3	4192.340	10.699	.000
К	60.424	3	20.141	16.712	.000
TP	0.005	3	.002	1.611	.192
TN	0.522	3	.174	1.499	.220
Mg	4.477	3	1.492	8.171	.000

Multiple Comparisons with	

Multi	ple Comparis	ons with		-	_		
						95%	
Dependent			Mean Diff (I-	e	<i>a</i> .	Confidence	
variable	(I) REGION	(J) REGIUN	1)	Std. Error	SIG.	interval	
						Lower	Upper
ald	000	LOW	0.020	0.125	1.000	0.265	Bouliu 0.20
рн	BOR	- LPH	+0.029	0.125	1.000	-0.365	0.30
		YAK	-0.109	0.130	0.001	-0.400	0.24
	1 PM	ROR	0.029	0.104	1,000	-0.307	0.13
			-0.029	0.149	1.000	-0.507	0.30
		XAK	-0.382	0.147	0.021	-0.725	-0.02
	UPH	BOR	0.109	0.130	1.000	-0.242	0.46
		LPH	0.079	0.149	1.000	.0.323	0.48
		XAK	-0.302	0.133	0.150	-0.660	0.05
	XAK	BOR	0.411	0.104	0.001	0.131	0.69
	-	LPH	0.382	0.127	0.021	0.039	0.72
		UPH	0.302	0.133	0.150	-0.055	0.66
DO	BOR	LPH	-1.553	0.556	0.038	-3.053	-0.05
		UPH	-2.043	0.581	0.004	-3.608	-0.47
		XAK	-2.401	0.464	0.000	-3.652	-1.15
	LPH	BOR	1.553	0.556	0.038	0.054	3.05
		UPH	-0.489	0.667	1.000	-2.286	1.30
		XAK	-0.848	0.568	0.833	-2.379	0.68
	UPH	BOR	2.043	0.581	0.004	0.477	3.60
		LPH	0.489	0.667	1.000	-1.308	2.28
		XAK	+0.359	0.592	1.000	-1.954	1.23
	XAK	BOR	2.401	0.464	0.000	1.150	3.65
		LPH	0.848	0.568	0.833	-0.683	2.37
		UPH	0.359	0.592	1.000	-1.237	1.95
HCO3	BOR	LPH	23.537	5.852	0.001	7.765	39.30
		UPH	28.761	6.109	0.000	12.296	45.22
		XAK	5.300	4.882	1.000	-7.858	18.45
	LPH	BOR	-23.537	5.852	0.001	-39.309	-7.76
		UPH	5.224	7.012	1.000	-13.675	24.12
	11011	XAK	-18.236	5.974	0.018	-34.338	-2.13
	UPH	-BOK	-28.761	6.109	0.000	-45.220	-12.29
		LPH	-5.224	7.012	1.000	-24.124	13.67
	YAK	ROR	-5 300	4 882	1.000	-18 459	7.85
	2200		18 236	5.974	0.018	2 135	24 32
		LIPH	23.461	6 226	0.002	6.680	40.24
Mg	BOR	LPH	0.439	0.126	0.002	0.000	0.78
		LIPH	0.579	0.132	0.000	0.224	0.93
		XAK	0.189	0.105	0.456	.0.095	0.47
	LPH	BOR	-0.439	0.126	0.005	-0.780	-0.09
		UPH	0.140	0.151	1.000	-0.268	0.54
		XAK	+0.250	0.129	0.332	-0.598	0.09
	UPH	BOR	-0.579	0.132	0.000	-0.935	-0.22
		LPH	-0.140	0.151	1.000	-0.548	0.26
		XAK	-0.390	0.134	0.028	-0.753	-0.02
	XAK	BOR	-0.189	0.105	0.456	-0.473	0.09
		LPH	0.250	0.129	0.332	-0.097	0.59
		UPH	0.390	0.134	0.028	0.028	0.75

Kruskal Wallis Test												
	Temp	Conduct	DOC	Na	SiO2	a	NO3	TSS	S04	Ca	Depth	Veloc
Chi-Square	5.497	53.604	49.075	13.917	19.346	10.026	7.604	.604	5.012	29.798	25.834	.967
df	3	3	3	3	3	3	3	3	3	3	3	3
Asymp. Sig.	.139	.000	.000	.003	.000	.018	.055	.895	.171	.000	.000	.809

BOR / XAK	Conduct	DOC	Na	SiO2	ci	Ca	Depth
Mann-Whitney U	268	203	527	496	486	166	412
Sig. (2-tailed)	.000	.000	.842	.550	.464	.005	.092
BOR / LPH	Conduct	DOC	Na	SiO2	a	Ca	Depth
Mann-Whitney U	27	112	231	130	141	32	167
Sig. (2-tailed)	.000	.000	.195	.001	.002	.000	.011
BOR / UPH	Conduct	DOC	Na	SiO2	a	G	Death
Mann-Whitney U	9	0	131	122	192	38	94
Sig. (2-tailed)	.000	.000	.005	.003	.136	.006	.000
LPH / XAK	Conduct	000	Na	sint	<i>a</i>	0	Denth
Mann-Whitney II	90	184	103	121	161	61	115
Sig. (2-tailed)	.000	.087	.129	.002	.027	.000	.002
	<u> </u>	- 1	1	-	-		
LPH / UPH	Conduct	DOC	Na	SiO2	CI	Ca	Depth
Mann-Whitney U	72	71	59	89	90	53	127
Sig. (2-tailed)	.036	.031	.010	.141	.157	.336	.985
UPH / XAK	Conduct	DOC	Na	SiO2	CI	Ca	Depth
Mann-Whitney U	45	14	80	110	190	53	44

Online Resource 1(iv)

 Test of Humogeneity of Variances for Flowed extent

 Gaussian
 Gaussian

 Constant
 Aff
 Sig.

 6.155
 0.44
 0.04
 0.01

 1.216
 0.46
 0.04
 0.01

 1.234
 0.4
 0.01
 1.24

 0.468
 0.46
 0.01
 1.06

 1.699
 0.4
 0.01
 2.54
 0.4
 0.00

 2.542
 0.4
 0.00
 2.542
 0.4
 0.00

 2.542
 0.4
 0.00
 2.542
 0.4
 0.00

 2.545
 0.4
 0.01
 2.544
 0.4
 0.00

 2.745
 0.4
 0.02
 2.434
 0.4
 0.00

 2.745
 0.4
 0.00
 2.64
 0.00
 0.01
 0.40
 0.00

 2.64
 0.47
 0.00
 2.64
 0.00
 2.64
 0.00
 2.64
 0.00
 2.64
 0.00
 2.64
 0.00
 2.01
 0.01
 2.02
 0.01 pH Temp DO Conduct DOC HCO3 K Na SiO2 C1 TP TN NO3 TSS SO4 Mg Ca Depth Veloc Flood Hydrog

	1 6	ANOVA of va	riables with equ	al variances			
	Squares	df	Mean Square	F	Sig.		
ſemp	1697 574		565.858	133 778	0-0		
DO	52.939	3	17.646	4.298	.007		
DOC	108.391	3	36.130	2.041	.113		
Conductivity	3824.507	3	1274.836	2.390	.074		
HCO3	58/0.121	3	1956.707	4.224	.008		
155	16.111	2	8.058	1.904	0.158		
Mg	12.839	1	4 280	45.687	000		
Veloc	.115	3	.038	573	.634		
Post	hoc tests: mul	tiple compari	sons with Bonfe	rroni			
Dependent	(I) flood	(J) flood	Mean			95%	Upper
/ariable	extent	extent	Difference (I-J)	Std. Error	Sig.	Confidence	Bound
ſemp	low	expand	4.196	0.579	0.000	2.636	5.756
		high	10.881	0.555	0.000	9.386	12.376
		recede	3.250	0.602	0.000	5.756	4.873
	expand	high	-4.190	0.579	0.000	+5./50	+2.030
		recede	-0.945	0.629	0.816	-2.640	0.749
	high	low	-10.881	0.555	0.000	-12.376	-9.386
	-	expand	-6.685	0.584	0.000	-8.258	-5.112
		recede	-7.630	0.607	0.000	-9.266	-5.995
	recede	low	-3.250	0.602	0.000	-4.873	-1.627
		expand	0.945	0.629	0.816	-0.749	2.640
		high	7.630	0.607	0.000	5.995	9.266
00	low	expand	0.360	0.570	1.000	-1.177	1.897
		high	-1.536	0.547	0.036	-3.009	-0.063
	<u> </u>	recede	-0.270	0.593	1.000	-1.869	1.329
	expand	low	-0.360	0.570	1.000	-1.897	1.1//
		nacada	-1.890	0.575	1,000	-2 301	1 039
	high	low	1.536	0.547	0.036	0.063	3.009
		expand	1.896	0.575	0.008	0.346	3.446
		recede	1.265	0.598	0.222	-0.346	2.877
	recede	low	0.270	0.593	1 000	-1 329	1.869
		expand	0.631	0.620	1.000	-1.039	2.301
		high	-1.265	0.598	0.222	-2.877	0.346
4CO3	low	expand	-13.345	6.056	0.180	-29.668	2.979
		high	-0.475	5.805	1.000	-16.121	15.170
		recede	-18.007	6.301	0.032	-34.990	-1.025
	expand	low	13.345	6.056	0.180	-2.979	29.668
		high	12.869	6.107	0.226	-3.590	29.329
		recede	-4.663	6.580	1.000	-22.398	13.072
	high	low	0.475	5.805	1.000	-15.170	16.121
		expand	-12.869	6.107	0.226	-29.529	3.590
	nacada	low	-17.532	6.349	0.042	-54.645	-0.419
	recoue	avnand	4 663	6.580	1.000	-13.072	24.990
		high	17.532	6 3 4 9	0.042	0.419	34 645
Mø	low	expand	-0.688	0.086	0.042	-0.971	0.456
		high	-0.000	0.083	0.000	-0.631	-0.186
		recede	.0.993	0.090	0.000	-1 234	-0.751
	expand	low	0,688	0.086	0.000	0.456	0.971
	passa	high	0.279	0.087	0.011	0.045	0.514
		recede	-0.304	0.094	0.010	-0.556	-0.052
	high	low	0.409	0.083	0.000	0.186	0.631
	~	expand	-0.279	0.087	0.011	-0.514	-0.045
		recede	-0.584	0.090	0.000	-0.827	-0.340
	recede	low	0.993	0.090	0.000	0.751	1.234
		expand	0.304	0.094	0.010	0.052	0.556
		high	0.584	0.090	0.000	0.340	0.827

Kruskal Wallis Test										
	pH	к	Na	SiO2	a	TP	TN	TSS	SO4	Ca
Chi-Square	11.402	28.196	54.992	10.261	15.219	10.719	12.476	59.638	4.387	26.09
df	3	3	3	3	3	3	3	3	3	
Sig. (2-tailed)	.010	.000	.000	.016	.002	.013	.006	.000	.223	.00
Pair-wise MW-U compariso	as									
low/expand	pH	К	Na	SiO2	CI	TP	TN	TSS		
Mann-Whitney U	256	266	166	236	258	313	277	0		
Sig. (2-tailed)	.208	.284	.003	.104	.226	.864	.394	.000		
low/high	pH	к	Na	SiO2	a	TP	TN	TSS	Ca	
Mann-Whitney U	256	77	101	303	191	266	202	0	102	
Sig. (2-tailed)	.208	.000	.000	.207	.002	.058	.003	.000	.000	
low/recede	pH	К	Na	SiO2	CI	TP	TN	TSS	Ca	
Mann-Whitney U	177	211	3	208	224	137	227	0	102	
Sig. (2-tailed)	.031	.148	.000	.132	.242	.003	.263	.000a	.000	
	oli	ĸ	Na	502	0	тр	TN	TSS		
expand/nigh	pri 2/0		140	3/02	1.00		102	133		
Sig (2 tailed)	200	143	102	1/5	150	239	182	245		
sig. (2-tailed)	.320	.001	.004	.008	.002	.101	.012	.199		
expand/recede	pH	К	Na	SiO2	CI	TP	TN	TSS		
Mann Milsinger II										
maini-winuley o	229	176	0	119	160	144	224	117		
Sig. (2-tailed)	229 .971	176 .184	0 .000	119 .007	160 .088	144 .035	224 .884	.465		
Sig. (2-tailed)	229 .971	176 .184 K	0 .000 Na	119 .007 SiO2	160 .088	144 .035	224 .884	117 .465 TSS	Ca	
high/recede	229 .971 pH 200	176 .184 K	0 .000 Na	119 .007 SiO2 259	160 .088 Cl	144 .035 TP	224 .884 TN 145	117 .465 TSS 86	Ca 199	

Online Resource 1(iv)

	Levene Statistic	df	Sig.
pH	6.155	94	.001
Temp	2.219	94	.091
DO	1.284	94	.284
Conduct	6.403	94	.001
DOC	1.688	94	.175
HCO3	1.699	94	.173
K	9.524	94	.000
Na	25.342	94	.000
SiO2	9.980	94	.000
Cl	15.966	94	.000
TP	7.116	94	.000
TN	2.745	94	.047
NO3	0.103	58	.902
TSS	2.733	59	.073
SO4	9.277	94	.000
Mg	2.643	94	.054
Ca	26.486	71	.000
Depth	5.174	94	.002
Veloc	1.012	94	.391
Flood	0.703	94	.553
Hydroperiod	0.473	94	.702

	/	NOVA of va	riables with equ	al variances			
	Sum of Squares	df	Mean Square	F	Sig.		
Temp	1697.574	3	565.858	133.778	.000		
DO	52.939	3	17.646	4.298	.007		
DOC	108.391	3	36.130	2.041	.113		
Conductivity	3824.507	3	1274.836	2.390	.074		
HCO3	5870.121	3	1956.707	4.224	.008		
TSS	16.117	2	8.058	1.904	0.158		
NO3	.002	2	.001	.050	.951		
Mg	12.839	3	4.280	45.687	.000		
Veloc	.115	3	.038	.573	.634		
Post h	oc tests: mult	iple compari	sons with Bonfe	rroni			
Dependent	(I) 1100d	(J) nood	Mean			95%	Upper
Variable	extent	extent	Difference (I-J)	Std. Error	Sig.	Confidence	Bound
Temp	low	expand	4.196	0.579	0.000	2.636	5.75
		high	10.881	0.555	0.000	9.386	12.376
		recede	3.250	0.602	0.000	1.627	4.873
	expand	low	-4.196	0.579	0.000	-5.756	-2.636
		high	6.685	0.584	0.000	5.112	8.258
		recede	-0.945	0.629	0.816	-2.640	0.749
	high	low	-10.881	0.555	0.000	-12.376	-9.38
	-	expand	-6.685	0.584	0.000	-8.258	-5.113
		recede	-7.630	0.607	0.000	-9.266	-5.99
	recede	low	-3.250	0.602	0.000	-4.873	-1.62
		expand	0.945	0.629	0.816	-0.749	2.64
		high	7.620	0.607	0.000	6 006	0.26
00	low	avpand	0.360	0.607	1.000	-1.177	9.20
	1011	high	-1.536	0.547	0.036	-3.009	-0.06
		man	0.270	0.597	1.000	1 860	1 2 2
	exmand	low	-0.360	0.570	1.000	-1.807	1.32
	expand	high	-1.896	0.575	0.008	-3.446	-0.34
		recede	-0.631	0.670	1 000	-2 301	1.03
	high	low	1.536	0.547	0.036	0.063	3.00
	Bu	expand	1.896	0.575	0.008	0.346	3.44
		racada	1.265	0.598	0.222	-0.346	2.87
	·	iceede	1.100	0.570	0.222	-0.340	2.07
	recede	low	0.270	0.593	1.000	-1.329	1.869
		expand	0.631	0.620	1.000	-1.039	2.30
		high	-1.265	0.598	0.222	-2.877	0.34
HCO3	low	expand	-13.345	6.056	0.180	-29.668	2.97
		high	-0.475	5 805	1.000	-16 121	15 17
		racada	-18.007	6 301	0.032	-34 990	-1.02
	expand	low	13 246	6.054	0.052	-2 970	70.44
	expandu	high	13.343	6,107	0.180	-2.979	29.00
		mgn	12.809	6.580	1.000	-3.390	12.07
	high	low	-4.003	6.580	1.000	*22.398	16.12
	mgn	IOW	0.475	5.805	1.000	-15.170	10.12
		expand	-12.869	6.107	0.226	-29.329	3.59
	·	recede	-17.532	6.349	0.042	-34.645	-0.41
	recede	low	18.007	6.301	0.032	1.025	34.99
		expand	4.663	6.580	1.000	-13.072	22.39
		high	17.532	6.349	0.042	0.419	34.64
Mg	low	expand	-0.688	0.086	0.000	-0.921	-0.45
		high	-0.409	0.083	0.000	-0.631	-0.18
		recede	+0.993	0.090	0.000	-1.234	-0.75
	expand	low	0.688	0.086	0.000	0.456	0.92
		high	0.279	0.087	0.011	0.045	0.51
		recede	-0.304	0.094	0.010	-0.556	-0.05
	high	low	0.409	0.094	0.000	0.186	0.63
		expand	-0.279	0.087	0.000	-0.514	-0.04
		espanu	-0.279	0.087	0.000	-0.314	-0.04
		recoue	-0.284	0.090	0.000	-0.827	-0.34
	recede	low	0.993	0.090	0.000	0.751	1.23
		expand	0.304	0.094	0.010	0.052	0.556
		high	0.584	0.090	0.000	0.340	0.827

Kruskal Wallis Test

	pН	K	Na	SiO2	a	TP	TN	TSS	SO4	Ca
Chi-Square	11.402	28.196	54.992	10.261	15.219	10.719	12.476	59.638	4.387	26.092
df	3	3	3	3	3	3	1 3	3	3	2
Sig. (2-tailed)	.010	.000	.000	.016	.002	.013	.006	.000	.223	.000
Pair-wise MW-U comparisons										

low/expand	pH	к	Na	SiO2	Ci	TP	TN	TSS	
Mann-Whitney U	256	266	166	236	258	313	277	0	
Sig. (2-tailed)	.208	.284	.003	.104	.226	.864	.394	.000	
low/high	pH	K	Na	SiO2	CI	TP	TN	TSS	Ca
Mann-Whitney U	256	77	101	303	191	266	202	0	102
Sig. (2-tailed)	.208	.000	.000	.207	.002	.058	.003	.000	.000
low/recede	pH	K	Na	SiO2	CI	TP	TN	TSS	Ca
Mann-Whitney U	177	211	3	208	224	137	227	0	102
Sig. (2-tailed)	.031	.148	.000	.132	.242	.003	.263	.000a	.000
			N	6:00	<i>a</i> 1	70	71	****	
expand/high	рн	ĸ	Na	5102	u	IP	IN	122	
Mann-Whitney U	260	143	162	175	150	239	182	245	
Sig. (2-tailed)	.326	.001	.004	.008	.002	.161	.012	.199	
or non-d/meaning	oH	ĸ	Na	SiO2	all	тр	TN	755	
Mann-Whitney II	220	176	110	110	160	144	224	133	
Sig. (2-tailed)	.971	.184	.000	.007	.088	.035	.884	.465	
high/recede	pH	ĸ	Na	SiO2	CI	TP	TN	TSS	Ca
Mann-Whitney U	200	87	122	259	173	188	145	86	199
Sig (2-tailed)	129	000	001	813	037	078	007	021	127

Online Resource 1(v)

Independent Samples Test

	Levene's Test of Vari	t for Equality ances	t-test f	t-test for Equality of Means			
	F	Sig.	t	df	Sig. (2- tailed)		
рН	2.203	.141	.696	96	.488		
Temp	2.496	.117	716	96	.476		
DO	1.661	.201	.331	96	.741		
Conduct	.881	.350	5.903	96	.000		
DOC	.047	.830	2.353	96	.021		
нсоз	4.888	.029	3.642	96	.000		
к	.841	.361	3.221	96	.002		
Na	9.613	.003	3.233	96	.002		
SiO2	.415	.521	2.069	96	.041		
ТР	.143	.706	.113	96	.910		
TN	.924	.339	1.951	96	.054		
NO3	8.771	.004					
TSS	.106	.746	.006	85	.936		
CI	.003	.955	.398	96	.692		
SO4	11.355	.001	1.702	96	.092		
Mg	2.913	.091	3.747	96	.000		
Са	1.432	.235	1.219	73	.227		
Depth	3.454	.066	-3.802	96	.000		
Veloc	5.877	.017	-2.007	96	.048		

Mann-Whitney Test

Test Statistics

	HCO3	Na	SO4	Veloc
Mann-Whitney U	459	492	783	509
Sig. (2-tailed)	.002	.006	.825	.006

FOR NO3, TSS

	Levene Statistic	df1	df2	Sig.
NO3	8.771	1	59	.004
TSS	.106	1	85	.746

	HABITAT	N	Mean Rank
NO3	WetDry	13	43.46
	Wet	48	27.63
	Total	61	

	NO3
Chi-Square	8.144
df	1
Sig. (2-tailed)	.004

Online Resource 1(vi)

	Depth	Veloc	pН	Temp	DO	Conduct	DOC	нсоз	К	Na	SiO2	CI	ТР	TN	SO4	TSS	Mg	Ca
UPH-low	1.47	0.06	6.35	27.45	3.82	40.43	4.9	42.62	2.33	1.04	13.83	0.47	0.03	0.11	0.38	ND	0.16	8.02
UPH-expand	2.25	0.44	6.33	23.84	2.58	38.26	4.71	37.12	0.64	0.95	7.4	0.52	0.05	0.34	0.17	3.17	0.84	ND
UPH-high	2.25	0.09	6.49	17.53	6.67	30.98	2.9	38.55	0.53	1.15	5.88	0.51	0.03	1.06	0.36	5.34	0.41	3.62
UPH-recede	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
LPH-low	1.96	0.04	6.71	26.82	3.19	49.35	5.82	27.88	1.88	1.12	19.28	0.49	0.03	0.49	0.25	ND	0.3	6.95
LPH-expand	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
LPH-high	3.09	0.16	6.38	16.11	3.99	33.25	4.26	42.96	0.6	1.55	3.89	0.21	0.05	0.73	0.1	3.93	0.77	4.44
LPH-recede	1.32	0.13	6.54	24.85	3.81	52.47	13.94	61.31	1.94	2.03	5.63	0.65	0.08	0.62	0.32	2.34	1.01	4.67
XAK-low	1.21	0.22	7.15	29.74	4.36	63.14	8.41	50.77	2.69	1.15	10.76	0.58	0.03	0.61	1.21	ND	0.41	8.14
XAK-expand	0.96	0.1	7.05	21.45	4.43	53.9	7.51	73.95	3.49	1.28	12.22	1.01	0.03	0.44	0.67	3.76	1.11	ND
XAK-high	0.77	0.14	6.52	15.66	5.36	72.76	7.81	67.23	2.11	3.21	13.38	0.46	0.04	0.74	0.16	3.06	0.97	6.8
XAK-recede	0.89	0.23	6.8	25.29	4.11	64.1	6.38	58.26	3	4	8.45	0.49	0.04	0.4	0.12	2.11	1.45	6.47
BOR-low	0.95	0.07	6.73	27.57	1.82	87.72	9.64	62.35	3.02	0.73	7.72	0.79	0.03	0.56	0.27	ND	0.61	14.52
BOR-expand	0.97	0.11	6.02	26.47	1.18	91.23	14.46	76.72	4.38	1.56	26.57	1.76	0.02	1.03	0.28	2.15	1.39	ND
BOR-high	1.57	0.15	6.53	19.43	3.28	63.92	9.4	51.05	1.56	3.1	13.07	0.44	0.03	0.63	0.19	2.39	1.08	7.05
BOR-recede	1.26	0.07	6.61	24.51	2.43	74.85	8	81.63	2.22	4.88	10.99	0.49	0.07	0.65	0.26	1.65	1.68	7.53

Mean hydrochemical data for each of the 4 regions decomposed by field visit. ND = not determined