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Corresponding Author: Anson Mackay

Corresponding Author's Institution:

First Author: Anson Mackay

Order of Authors: Anson Mackay;Thomas Davidson, PhD;Piotr Wolski, PhD;Richard Mazebedi, MPhil;Wellington RL Masamba, PhD;Philippa Huntsman-Mapila, PhD;Martin Todd, PhD

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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

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

Anson W. Mackay[1]
Thomas Davidson[1,2]
Piotr Wolski[3]
Richard Mazebedi[3]
Wellington R.L. Masamba[3]
Philippa Huntsman-Mapila[3]
Martin Todd[1,4]

1ECRC, Department of Geography, UCL, Gower Street, London WC1E 6BT
2 Institute of Bioscience, NERI, Aarhus University, Vejlsøvej 25, DK-8600 Silkeborg, Denmark
3 ORI, University of Botswana, Private Bag 285, Maun, Botswana
4 Department of Geography, University of Sussex, Brighton, BN1 9RH UK

Corresponding author:
Anson W. Mackay
a.mackay@ucl.ac.uk
p: +44 (0)20 7679 0558; f: +44 (0)20 7679 0565

Abstract

The annual flood pulse in the Okavango Delta (Botswana), has a major influence on water chemistry and habitat. We explore spatial and temporal patterns in a suite of chemical variables, analysed from 98 sample points, across four regions, taken at different stages of the flood cycle. The major pattern in water chemistry is characterised by an increasing gradient in ionic concentration from deep-water sites in the Panhandle to more shallow, distal regions to the south. Concentrations of cations, anions, dissolved organic carbon, and SiO_2 are significantly higher in the seasonally inundated floodplains than in permanently flooded regions. Several variables (including Na and total nitrogen) significantly increase from low flood to high flood, while others (including HCO_3, SiO_2, and Cl) increase in concentration, initially between low flood and flood expansion, before declining at maximum flood extent. Redundancy analysis (RDA) revealed that hydrological variables (water depth, flow velocity, flood frequency, and hydroperiod class) significantly explain 17% variation in surface water chemistry. Predictions of increasing flood volume in the near future may result in a decline in alkalinity and dilution of DOC. Our study provides an important baseline from which to monitor future change in the Delta.

Keywords: floodplains; multivariate techniques; water chemistry; wetlands

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

The Okavango Delta (hereafter referred to as the Delta) in northern Botswana is one of the largest wetlands in Africa, and one of the few where human impact is minimal. The Delta is a low-gradient alluvial fan, and occupies the lowest region of the Kalahari Basin. The Delta is fed by rivers with their source in the highlands of Angola, and both the rivers and the Delta together form the Okavango River Basin (Fig. 1). The Delta is a flood-pulse ecosystem (Junk et al. 1989), controlled mainly by an annual pulse of water, turning dry floodplains into productive aquatic environments (Mendelsohn et al. 2010). Every year, precipitation falling in the highlands of Angola during October, flow down the Cubango and Cuito rivers, which join to form the Okavango River at the border between Angola and Namibia. This flood pulse reaches the northern part of the Delta (in Botswana) in February–May, before slowly expanding out, and reaching the distal regions by July–August. Maximum flood extent occurs towards the end of the dry season between July–September, and declines again during the summer, between October–March.

The flood pulse is ultimately forced by climate, although as it expands out in the Delta there is strong spatial variability, due to the Delta’s flat topography and vegetation-controlled channel-floodplain interactions (Wolski and Murray-Hudson 2006a). Currently, technological modifications of the Delta are few, but threats are real. For example, dam building upstream for hydroelectricity and agriculture in Angola and Namibia have the potential to alter the volume of water flowing into the Delta (Mendelsohn et al. 2010). Increasing tourism, on the other hand, may increase habitat disturbance, while population growth in villages and towns brings with it the possibility of deteriorating water quality (Masamba and Mazvimavi 2008). There is substantial seasonal, inter-annual, and decadal variation in size of the flood and the extent of inundated area (Mazvimavi and Wolski 2006). This variation is mainly due to variation in flow of the Okavango River (9 x $10^9 m^3$/yr), but also to local summer rainfall (6 x $10^9 m^3$/yr) and to a lesser extent the nature of the previous year's flood (McCarthy et al. 2003). Rainfall falls mainly during December, January, and February and is higher in the north (c. 550 mm/yr) than in the south (450 mm/yr). Approximately 2% of water leaves the Delta through rivers in the south; the remainder is lost through evaporation (74%) and transpiration processes (24%) (Mendelsohn et al. 2010). Hydrology and wetland chemistry are closely linked (Mitsch and Gosselink 2000). However, while the hydrology of the Delta has been comprehensively studied (see Milzow et al. 2009 for a review), there have been considerably fewer investigations into how the flood pulse in the Delta impacts nutrients (Mubyana et al. 2003; Lindholm et al. 2007) and inorganic chemistry (Sawula and Martins 1991). Previous studies focussed only on very short time periods (Ashton et al. 2003), or were restricted to the Jao / Boro river system (Sawula and Martins 1991; Cronberg et al. 1996a,b). Here we report on a large, comprehensive, surface water chemistry dataset that covers four important regions in the Delta, sampled over a complete flood cycle. Given the importance of the Delta in terms of biodiversity (Ramberg et al. 2006) and ecosystem services (Mendelsohn et al. 2010) it is important to characterise baseline information on surface water chemistry across different regions, and at different stages, of the hydrological cycle. This information is fundamental for the detection and monitoring of future ecosystem change. The first aim of this study was to characterise variation in surface water chemistry in relation to (i) the different regions of the Delta, (ii) different stages of the flood cycle over a period of one year, and (iii) between habitats which were either seasonally or permanently flooded. The second aim was to explore the influence of four hydrological variables linked to the flood pulse (water depth, flow velocity, flood frequency and hydroperiod class) on surface water chemistry, using multiple regression techniques.

Methods

Regional descriptions and site selection

Study sites were selected across four regions in the Delta (Fig. 1). The upper Panhandle (UPH) in the north is a fault-bounded, flat-bottomed valley dominated by fluvial input from the Okavango River. Downstream from the UPH is the region known as the lower Panhandle (LPH), where the Okavango River meanders across the floodplain. South of the LPH, the Okavango River spreads out into channels, which get progressively smaller with distance. The main distributary (Maunachira / Khwai) is an eastern extension of the Okavango River, with many large, flow-through lagoons such as Xakanaxa (XAK). Several secondary distributaries branch off from the Okavango River, including the Jao / Boro (BOR) river system to the west of Chief's Island (Fig. 1). Fifty sites were selected to capture a strong hydrological gradient, and built on previous aquatic research undertaken in the Delta (Ashton et al. 2003; Dallas and Mosepele 2007).

Sample collection and analysis of chemical response variables

Ninety-eight sample points were analysed for water chemistry. These consisted of 23 sample points visited only once in the study. The remaining 75 sample points were from 27 sites visited on more than one occasion, during different stages of the flood-pulse cycle: low flood (28 Nov–10 Dec 2006; n=28); flood expansion (23 Apr–6 May 2007; n=23); high flood (24 Jul–7 Aug 2007; n=26); and flood recession (2 Oct–12 Oct 2007; n=21). Across the regions, 15 sample points were analysed from UPH, 17 from LPH, 31 from XAK, and 35 from BOR. Sample points were further characterised by the dominant habitat, including marginal vegetation in channels (MV-IC), marginal vegetation in lagoons (MV-L), floating vegetation (FV), inundated floodplains (IF), and isolated, seasonally flooded pools (SP) (after Dallas and Mosepele 2007). The first three habitat types are permanently wet (WET), while IF and SP experience wet-dry cycles (WET/DRY). Sample point co-ordinates are given in Online Resource 1(i).

pH, conductivity (μS/cm), and dissolved oxygen (DO) (mg/L) were analysed in situ. pH and conductivity were measured using a portable *Fisher Scientific accumet* AP85 portable waterproof pH/conductivity meter. A *YSI 550* dissolved oxygen instrument was used to measure

DO and water temperature (temp) (°C). Field equipment was calibrated before each trip. Total suspended solids (TSS) were determined by filtering Delta water (at least 1L) and measuring the residue weight on GF/C filter papers after being dried at 105°C for 24 h (mg/L). 250 ml of water was filtered through a GF/C filter into acid-washed bottles for non-metal (HCO_3, Cl, NO_3, SO_4, dissolved organic carbon (DOC)) analyses. A second 250 ml of water was also filtered using cellulose nitrate papers for cation analyses (Na, K, Mg, Ca) and immediately acidified with three drops of concentrated HNO_3. A final 250 ml of water was filtered using cellulose nitrate filter papers for silica analysis. All filtered samples were stored on ice in acid-washed plastic sample bottles, then transported to the Okavango Research Institute where they were either analysed immediately on their return, or frozen until analyses could be carried out. HCO_3 was determined within 48 h of sample collection. DOC and anions were analysed within 7 days, while TN and TP were determined within three weeks of collection. Metals were analysed within 2 months after collection.

Mg (mg/L) and Ca (mg/L) levels were determined by flame atomic absorption 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 determined by flame photometry using a *Sherwood Flame Photometer 410* instrument. HCO_3 (mg/L) was analysed using an auto-titrator (Mettler Toledo model DL 50). Cl (mg/L) and SO_4 (mg/L) were analysed by ion chromatography using a *DX-120* ion chromatograph (Eaton et al. 1995). An approximation of DOC (mg/L) was obtained by measuring the absorbance of the water samples at 280 nm using a *Perkin Elmer Lamda 20* UV/Vis spectrophotometer (Mladenov et al. 2005). Total phosphorus (TP (mg/L)), total nitrogen (TN (mg/L)), and nitrates (NO_3 (mg/L)) were analysed by an air segmented flow analyser (*Bran + Luebbe* AA3) after persulphate digestion. SiO_2 (mg/L) was measured using the heteropoly blue method at 815nm.

Collation of hydrological explanatory variables

Water depth (m) and velocity (m/s) variables were measured in situ at each sample point. Water depth was usually measured using a *Plastimo Echotest II* handheld depth sounder. In locations with dense, submerged vegetation, water depths were instead measured using a graduated metal pole. Water flow velocities were estimated using an *OTT Nautilus C 2000 Electromagnetic Flow Sensor*, which was designed to measure water currents in the marginal zones of river banks, shallow water, and waters with low flow velocities. Annual flood frequency and hydroperiod class variables were derived from remotely sensed images, hydrometric data, and general understanding of hydrological variability in the system. We used 16 data layers depicting maximum annual inundation extent during years 1989–2006 (excluding 1991 and 2003). The layers were obtained from Landsat (5 and ETM) images, using a classification procedure involving spectral clustering and contextual classification (Wolski and Murray-Hudson 2006a). To depict the Delta, four Landsat scenes had to be mosaicked for each coverage. Inundation layers had spatial resolution of 30 by 30 m, and 97% accuracy of determination of inundation extent (Wolski and Murray-Hudson 2006a). Dates of the imagery were selected to coincide with the period of maximum, annual inundation. The flooding in the Delta takes the form of a single, annual event with progressive transition between low inundation and high inundation taking place throughout a year. Earlier work, with weekly NOAA AVHRR images, revealed that the crest of the flood lasts approximately 1–2 months in distal parts, but 3–4 months in the proximal parts of the Delta proper, and fast recession occurs only in October (Wolski and Murray-Hudson 2006b). Based on these findings, Landsat scenes from end of August to mid-September were selected for high-resolution inundation mapping. Number of years inundation occurred was calculated for the entire Delta on a pixel-by-pixel basis, by stacking up all individual layers. Flood frequency was then obtained by dividing number of years with inundation by the total number of flood maps. Flood frequency for each of the sampling sites was obtained by averaging flood frequency from the nine surrounding pixels, corresponding to the sampling point, using neighbourhood statistics procedure. This was done to reduce possible influence of errors resulting from image misclassification and misregistration. In the seasonally inundated parts of the Delta, annual inundation frequency can be considered as a proxy for mean duration of inundation. This results from the nature of the flood event where parts of the system are likely to be inundated longer only in high flood years, for a short term only in moderate years, and not inundated at all in low flood years. However, the relationship between the amplitude of annual water level fluctuations and flood frequency varies for different parts of the system. Available hydrometric data were not enough to formalize these relationships in strict mathematical form. Instead, a set of seven hydroperiod/amplitude classes was defined, and a hydroperiod class was attached to each of the sample points. The classification was based on flood frequency maps and hydrometric data from hydrometric stations in the vicinity of sampling points.

Statistical Analyses

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

17.0 revealed substantial inter-correlation among water chemistry variables (47% of variables were significantly correlated at the p=0.05 level; Online Resource 1(ii)). Main gradients in the chemistry dataset were therefore explored using principal components analysis (PCA), with symmetric scaling of the ordination scores, to both summarise our multivariate data, and to determine any underlying structure. Variables were not log transformed, but were centred and standardised as they were measured in different units (Lepš and Šmilauer 2003). Like Cronberg et al. (1996b), we have chosen to include conductivity and alkalinity because not all ionic species were measured in our study. However, we have chosen to exclude DO and temp variables from multivariate analyses because they have such a strong diurnal component, which we did not systematically take account of during our sampling. As measurements for Ca, NO_3, and TSS were incomplete, these variables are also omitted (Table 1). A broken stick model was used to test the 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 gradient technique of redundancy analysis (RDA), which constrains ordination axes as linear combinations of explanatory variables. Initially, the explanatory power and significance of each hydrological variable was determined through a series of single constrained RDAs, together with Monte Carlo permutation tests ($p = 0.002$; $n = 499$). The unique contribution of each variable was then assessed through a series of partial RDAs with the remaining hydrological variables as covariables. A further, partial RDA determined the influence of flood cycle stage. To assess the influence of habitat, a similar set of analyses were undertaken, this time grouping permanently wet habitats (MV-IC+MV-L+FV; n=77) and seasonally flooded habitats (IF+SP; n=21). A major feature of spatial datasets such as ours is the influence of site proximity (e.g., Legendre 1993), and here we partial out sample point co-ordinates using RDA (ter Braak 1987). Ordination analyses were undertaken using Canoco v. 4.5 (Lepš and Šmilauer 2003).

Results

Summary statistical analyses for hydrochemistry data are given in Table 1. Output from all statistical tests is given in Online Resource 1(iii-v). Unfortunately, data are not available for NO_3 and TSS during low flood, nor for Ca during flood expansion. In Figure 2, boxplots are shown for variables that demonstrated significant regional variability. Only conductivity showed significant differences among all the regions, with values increasing along a north-south gradient. DOC also shows significant regional variability along a north-south gradient, except between LPH and XAK. Ca concentrations were only not significant among sites in the Panhandle, and between UPH and XAK. HCO_3 and SiO_2 show a significant increase between Panhandle and distal regions, although there is no significant difference between UPH and LPH, nor between XAK and BOR. The reverse pattern is true for water depth. The pH of surface water is significantly higher at XAK than at BOR or LPH (Fig. 2). DO was similar across most of the Delta, except in the BOR region, where values are significantly lower. Na was significantly lower in UPH than in the other three regions. Cl was significantly lower only between LPH and BOR, and between LPH and XAK. Velocity, SO_4, TSS, and nutrients (TP, TN, and NO_3) did not show any significant regional differences. In Figure 3, boxplots are shown only for variables that demonstrated significant variability among different stages of the flood cycle. Only two variables (Na and Mg) differed significantly among all stages; both had highest concentrations during flood recession, and lowest concentrations during low flood. Pair-wise comparisons for Ca are limited because of measurement problems during flood expansion for this variable. However, these data indicate that Ca concentrations are significantly higher during low flood than during either high flood or flood recession. All other variables shown, exhibited some form of pair-wise significant variation. Surface water temperatures were only not different between flood expansion and flood recession, while pH was significantly different during flood expansion. K and Cl were significantly lower, and TN significantly higher, during high flood. Peak concentrations of SiO_2 during flood expansion, were significantly higher than during high flood and flood recession. DO was significantly higher during high flood than during either low flood or flood expansion. HCO_3 was significantly higher was during flood recession than either low or high flood, while TP was significantly higher during flood recession than either low flood or flood expansion. Six variables exhibited no significant variability with stage of the flood cycle: conductivity, DOC, NO_3, SO_4, velocity, and water depth (Table 1; Online Resource 1(iv)). In Figure 4, boxplots are shown only for variables that demonstrated significant variability between either seasonally or permanently inundated habitats. Many variables exhibited no significant difference between the two habitat types, including pH, temp, DO, TP, TN, Cl, Ca, and SO_4. The remaining variables, however, were significantly higher in seasonally inundated habitats, including conductivity, DOC, K, SiO_2, Mg, HCO_3, Na, and NO_3 (Table 1; Online Resource 1(v)). In contrast, velocity and water depth were significantly lower in seasonally inundated habitats.

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 its broken stick variance, here we exercise caution and treat axis 2 as being not significant (Table 2). Main patterns in our dataset are shown in the form of a PCA biplot (Fig. 5). Axis 1 represents a significant gradient in surface water chemistry of increasing conductivity, HCO_3, K, Mg, DOC, SiO_2, TN, and Cl, with highest values in sample points mainly in BOR and XAK. These variables have lowest concentrations in the Panhandle region of the Delta. PPMC analyses show that most of these correlations are significant (Online Resource 1(ii)). Axis 2 is associated mainly with TP and SO_4 (Fig. 5). RDA reveals that the four hydrological variables (depth, velocity, flood frequency, and hydroperiod class) significantly account for 17.0% of variation in the chemistry data (Table 3i), with axis 1 being by far the most important gradient (Fig. 6). The RDA triplot shows that axis one is dominated by a strong gradient of positively correlated variables, including conductivity, alkalinity, SiO_2, DOC, nutrients, and cations, all of which are negatively correlated 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).

Discussion

Interpreting the datasets collected is complicated by several factors. Not only are there regional and habitat influences on water chemistry, but the slow moving flood pulse adds an extra dimension to the complexity of the system. For example, as the flood expands in northern part of the Delta, the previous year's flood is still receding in the south. Cronberg et al. (1996b) correlated solute concentrations with discharge measurements to account for some of this hydrological variability. Here, we took a multivariate approach, and used four explanatory variables linked to the flood pulse to model impacts on hydrochemistry across different regions of the Delta.

Do significant differences exist within the surface water chemistry dataset among the different regions of the Delta, among different stages of the flood cycle, and between seasonally or 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 SiO_2 and cations is due to evapo-transpiration processes (Dinçer 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.

Concentrations of Mg are highest during flood expansion and recession phases, but decline during peak flood, which can be attributed to the removal and dissolution of precipitated magnesium salts (Cronberg et al. 1996a). Na however, is lowest during low flood and increases steadily throughout the year (Fig. 3). At these concentrations, it is unlikely that Na is co-precipitating out of solution, and further work needs to be done to understand these trends in relation to evaporation. Ca concentrations were significantly higher during low flood, similar to trends reported by Cronberg et al. (1996a). Ca is linked mainly to reactions of the carbonate system and dissolution of silicate materials (Sawula and Martins 1991), and it is the most abundant cation throughout the Delta: Ca >K >Na>Mg. When concentrations are converted to milliequivalents/litre (mequiv/L), the abundance order changes to Ca>Na >Mg>K, identical to the relative proportions determined by Cronberg et al. (1996b). Similar to both Sawula and Martins (1991) and Cronberg et al. (1996b), HCO_3 is by far the most dominant anion (over 90%) throughout the Delta because of circumneutral pH (Wetzel 2001).

The Okavango River itself is oligotrophic, and nutrients brought in by surface flow are quickly taken up by fringing vegetation along the channels in the Upper Panhandle. The seasonal floodplains link the aquatic habitats in the Delta to terrestrial habitats. Several chemical constituents were significant higher in seasonally inundated habitats, including conductivity, DOC, K, SiO_2, Mg, HCO_3, Na, and NO_3 (Table 1; Fig. 4). Floodplains contain greater concentrations of chemical constituents because flooding acts to mobilise ions and nutrients from previously dry soil surfaces into solution (Cronberg et al. 1996b). Nutrients in the floodplains are derived from a number of sources, including river flow (Cronberg et al. 1996b), in situ from accumulated sedimentary stores over many thousands of years (Mendelesohn et al. 2010), from wind-blown dust (Garstang et al. 1998; Krah et al. 2006), and animal faeces (Lindholm et al. 2007). Lindholm et al. (2007) measured highly elevated nutrient concentrations during a period of low flood extent in a BOR floodplain, which they attributed to low dilution of stored nutrients. Further work needs to be done, however, to determine if such observations are more widely applicable, or are particular to that floodplain. Nitrogen and phosphorus are two of the most important macronutrients in wetlands, dependent on a number of factors, including oxygen content, redox potential, temperature, pH, and microbiological processes (Mitsch and Gosselink 2000). Our measurements of macronutrients are rather incomplete; nitrate was not measured during low flood, and there were technical problems with analyses of phosphate, nitrite, and ammonium, and so those are not presented here. However, TN and TP have been measured in every region during each stage of the flood cycle, and these still serve as useful macronutrient indicators. Inorganic NO_3 concentrations did not vary significantly with seasonal changes in hydrology (Table 1), perhaps confirming observations by Krah et al. (2006) who also did not detect any nitrate “boost” as the flood arrived and expanded. Garstang et al. (1998) suggests that this may be because most of the nitrogen has been taken up by plant growth at the end of the previous flood period or has undergone denitrification. However, TN concentrations are significantly higher during the period of high flood, perhaps because the floodwaters contain slightly higher nitrogen concentrations than wetland soils (Cronberg et al. 1996a). Mean TP concentrations are low (<0.04 mg/L), similar to levels determined by Krah et al. (2006) for a Boro floodplain, but lower than values obtained by Lindholm et al. (2007). Statistically, however, TP shows no significant regional variation (Table 1). TP concentrations do vary significantly with stages of the flood cycle (Table 1, Fig. 3), especially during flood recession, when concentrations are highest. These findings are in contrast to previous studies, which reported the highest concentrations of TP occurred during the initial flood stage at the Boro floodplain (e.g., Krah et al. 2006; Lindholm et al. 2007), and as the flood expanded, concentrations declined. Lindholm et al. (2007) undertook a comparison of two years of nutrient data with different flood sizes, and concluded that local differences are overwhelmed when flood sizes are large because connectivity between river channels and floodplains is increased. It may be that our TP data are a reflection of overall flood size; however, more work needs to be done on the regional and long-term changes of this important macronutrient. TN/TP ratios are useful in determining whether P and/or N is limiting growth in freshwater ecosystems, and how susceptible that ecosystem is to developing algal blooms. For example, high levels of TP might suggest impacted ecosystems through elevated phosphorus supplies (and will give a smaller ratio, e.g., less than 10), while ratios above 15 suggest potential P-limitation (Abell et al. 2010). The TN/TP ratio for BOR was 20.4, a figure very similar to that determined by Cronberg et al. (1996b), which suggests potential P limitation in this region. Ratios for the other regions were lower: 13.6 (UPH), 11.8 (LPH), 15.7 (XAK), and it is likely that the availability of N and P in these regions is close to that needed for balanced growth of primary producers, i.e., these nutrients are co-limiting (Abell et al. 2010). Threats of nutrient enrichment from increasing economic activity (e.g., tourism and aquaculture) pose serious 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.

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

Cronberg et al. (1996a) concluded that spatial heterogeneity and seasonal flow was responsible for most of the variation of water chemistry in the Jao/Boro river system. Here we provide the first quantitative estimates that seasonal flow and spatial heterogeneity significantly account for 19% and 17% variation in Delta chemistry, respectively (Table 3). The influence of biogeochemical processes that take place in seasonally inundated floodplains on surface water chemistry is very important (e.g., Cronberg et al. 1996a,b; Mladenov et al. 2005; Krah et al. 2006). By grouping the sites as seasonally or permanently flooded, we show that this habitat distinction accounts for 8% significant variation in the chemistry data (Table 3iii). This lower value may be a reflection of larger flood sizes experienced at the time of the study, diluting nutrients, and other chemical constituents in the floodplains (Lindholm et al. 2007), and the balance between the 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 dataset will be subject to spatial autocorrelation, especially given the proximity of many sample points to each other (Fig. 1; Legendre 1993). This can lead to over-estimation of the interactions between the chemical variables and hydrological predictors. Variation captured by each hydrological variable taking into account proximity, declines by approximately two-thirds (Table 4v). It is notable, however, that depth and hydroperiod class variables are still highly significant.

The size of the flood pulse in the Delta has increased over the last five years, and is likely to continue to increase into the near future due to a quasi, 80-year, climatic oscillation (Gumbricht et al. 2004). A key question therefore remains as to how an increase in volume of water in the flood pulse will influence surface water chemistry. Here we use t-value biplots to further explore significant relationships between hydroperiod class and water depth with chemical variables. Variables that fall within the Van Dobben circles in the t-value biplot are significantly correlated either positively (solid circle) or negatively (dashed circle) with the variable under investigation (Fig. 7). Hydroperiod class has a significant, positive relationship with pH values in the Delta, but a significant, negative relationship with conductivity, HCO_3, Mg, and DOC. Therefore, an increasing area of sites with higher hydroperiod class may result in a decline in DOC, conductivity, HCO_3, and certain cations such as Mg, through dissolution of precipitated salts (Cronberg et al. 1996a). An increase in water depth would not have a positive effect on any of the variables that we measured, but would result in a significant decline in concentration of many variables (conductivity, HCO_3, DOC, Mg, K, and SiO_2). Water depth likely represents a proxy for volume of water, and so in shallow water regions, concentrations of solutes are greater. Therefore, in sites that currently experience flooding, an increase in flood size is likely to result in an overall dilution of chemical constituents (Cronberg et al. 1996a). Other factors not measured here are also likely to have a strong influence on water chemistry in the Delta, especially history of the water prior to sampling, and vegetation composition at any one location. Moreover, an increase in flood size will also result in new, dryland regions becoming inundated for the first time in many decades, resulting in new, productive floodplains. This is a large and important uncertainty and requires much more intensive spatial and temporal monitoring in key regions.

Conclusions

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

Acknowledgments

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Figure Legends

Figure 1: Map of the Okavango Delta with site locations indicated on aerial photographs from each of the four study regions

Figure 2: Boxplots for hydrochemical variables that show significant regional variation (see text for details). The central line is the median value, while the bottom and top of the box indicate 25th and 75th percentiles. T-bars extend to 1.5 times the height of the box. Outliers are given as points, whereas extreme outliers (i.e. values that are more than 3x the height of the box) are given as asterisks.

Figure 3: Boxplots for hydrochemical variables, that show significant variation among different stages of the flood cycle.

Figure 4: Boxplots for hydrochemical variables that show significant variation between sites that are either permanently or seasonally flooded.

Figure 5: PCA biplot of surface water chemistry in the Okavango Delta. Sample points have been coded according to their location in each of one of the four principal regions. Broken stick reveals that that first axis significantly explained 30% variation.

Figure 6: RDA triplot showing sample points (coded according to their location), surface water chemistry variables, and 4 hydrological explanatory variables (depth, velocity, flood frequency, and hydroperiod class).

Online Resource 1: PDF consisting of a number of worksheets: (i) sample point co-ordinates; (ii) PPMC analyses of chemistry data; (iii–v) statistical analyses of data showing main results from Levene’stests for equality, ANOVA, 2-tailed t-tests, Kruskal-Wallis tests, pairwise Mann-Whitney tests for (iii) regions; (iv) different stages of the flood cycle; (v) between permanently and seasonally inundated sites; and (vi) decompiled mean data for regions at different stages of the flood cycle.

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
pH	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*
TP	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*

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

Axes	**1**	**2**	**3**	**4**	**Total 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	

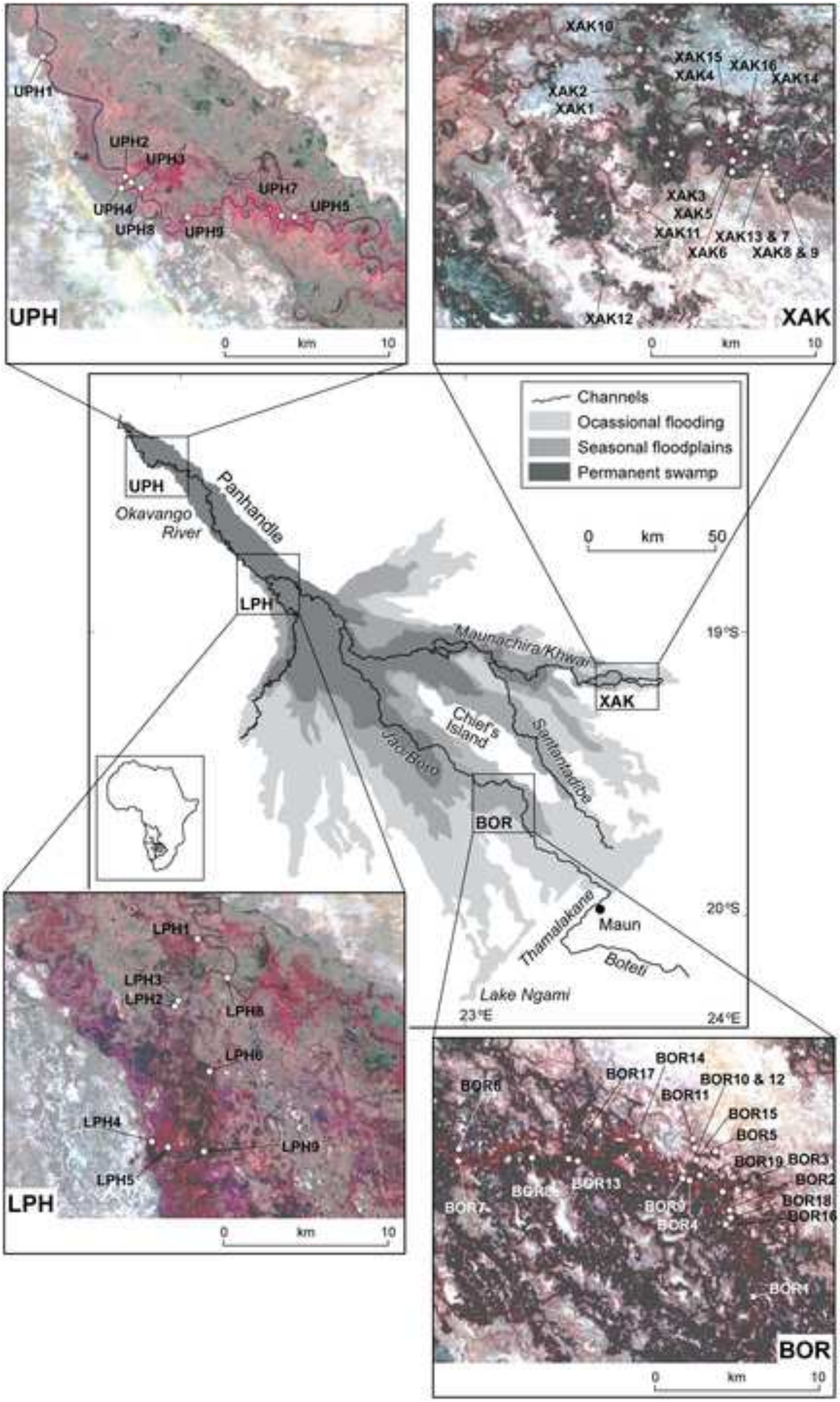
Table 3: Redundancy analysis (RDA) of surface water chemistry with (i) four hydrological variables; (ii) flood extent; (iii) habitat type; (iv) site locations as explanatory variables.

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	
∑ 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	
∑ 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	
∑ 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	
∑ 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. $p \leq 0.05$; n=499 permutations).

Hydrological Variables	**(i)**		**(ii)**		**(iii)**		**(iv)**		**(v)**	
	% var	p	% var	p	% var	p	% var	p	% var	p
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 1



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XAK11
XAK13 & 7
XAK6
XAK8 & 9
XAK12
XAK
0 km 10
Channels
Ocassional flooding
Seasonal floodplains
Permanent swamp
UPH
Okavango River
Panhandle
0 km 50
LPH
19°S
Maunachira/Khwai
XAK
Chief's Island
Santantadibe
Jao/Boro
BOR
20°S
Maun
Thamalakane
Boteti
Lake Ngami
23°E
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Fig 2

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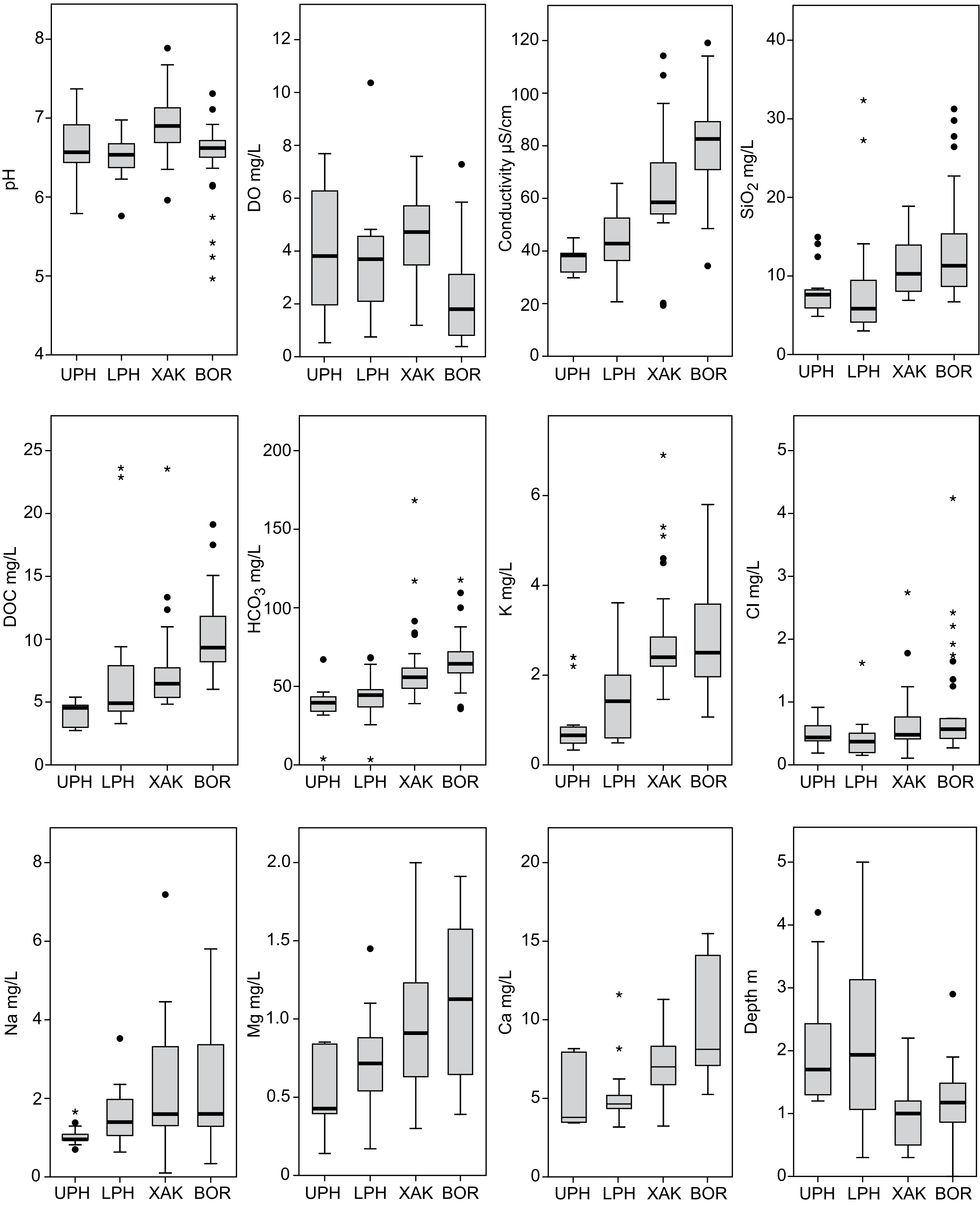

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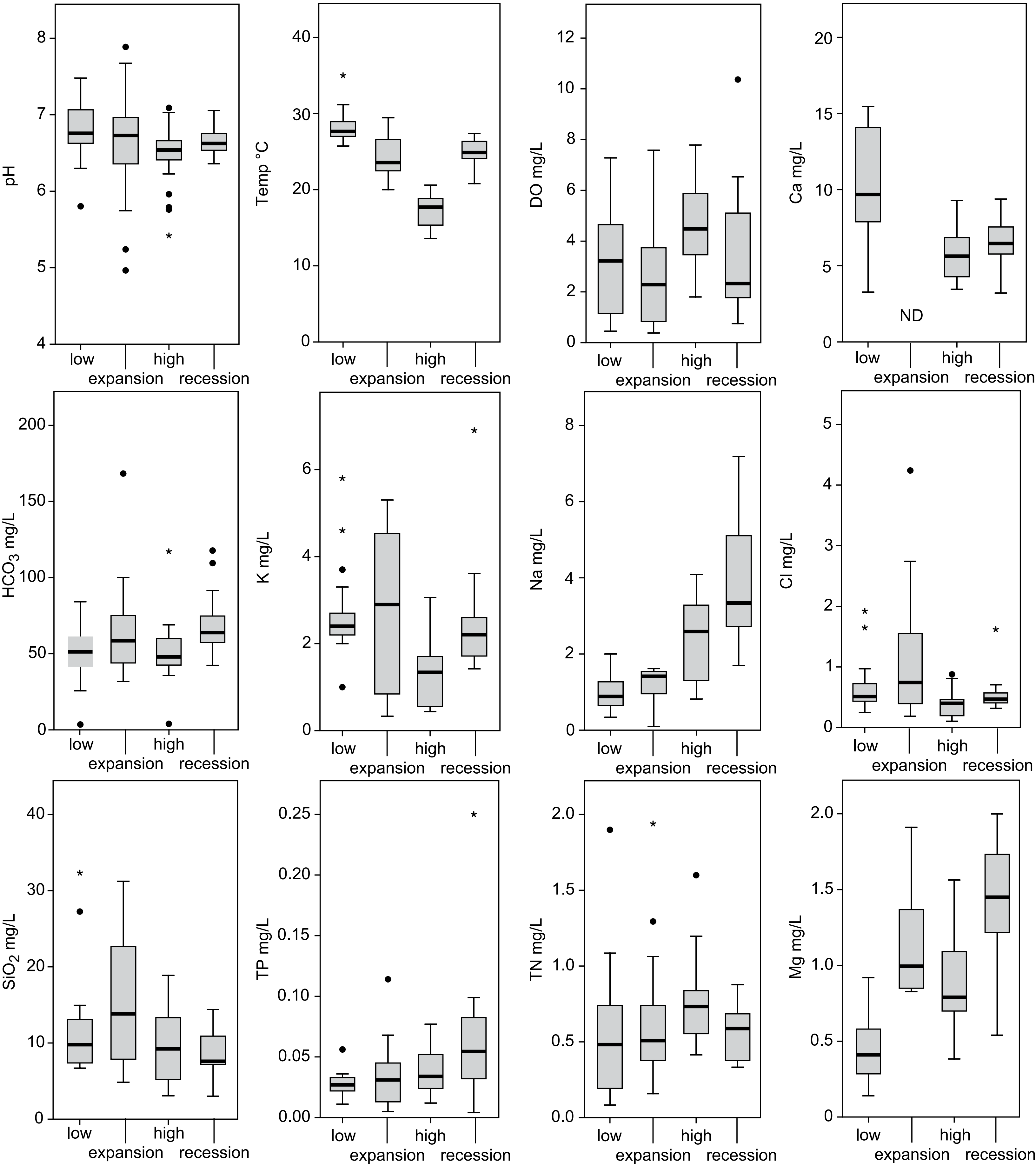
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K mg/L
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Na mg/L
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SiO$_2$ mg/L
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Fig 4

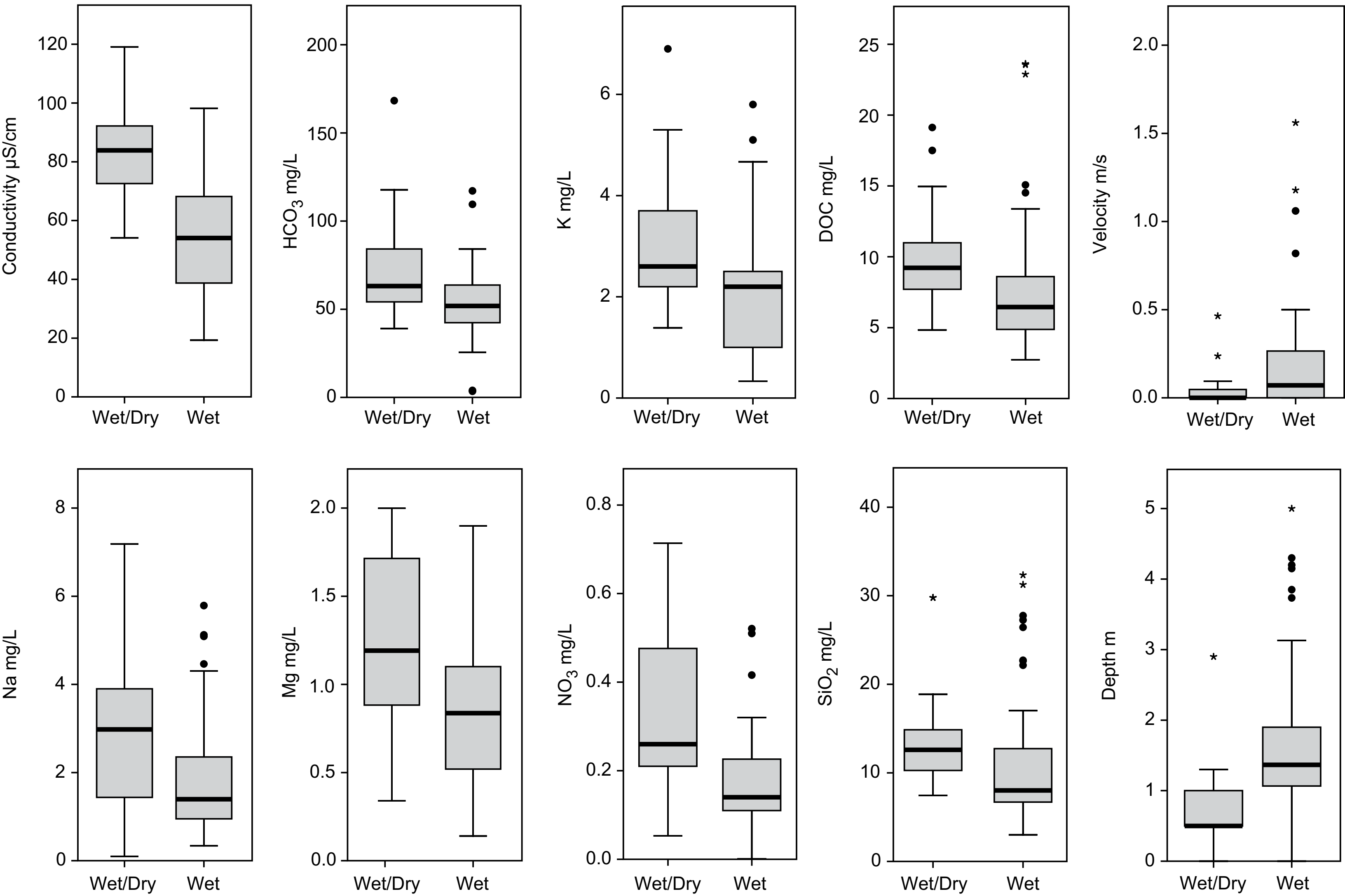
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Fig 5

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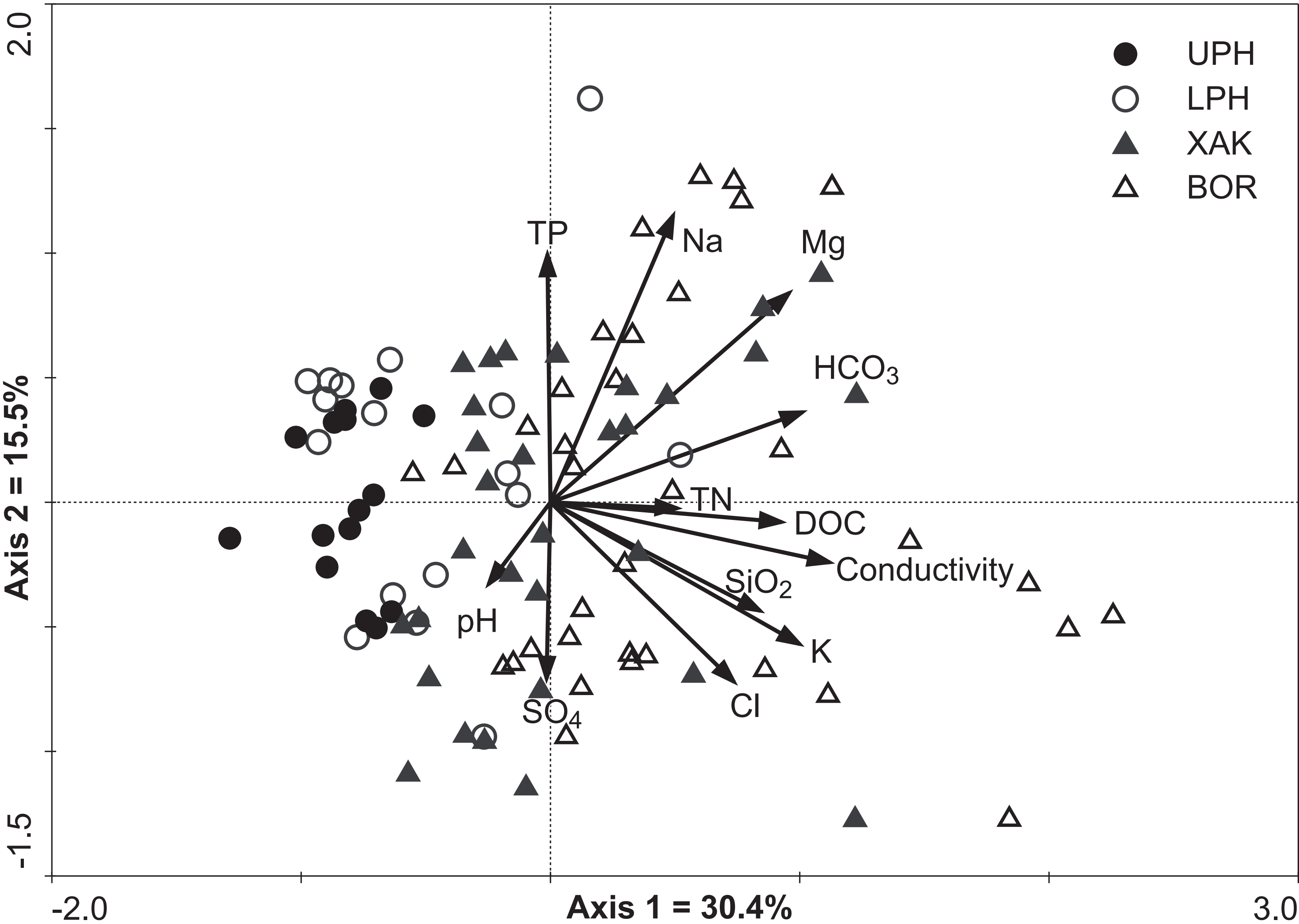

Fig 6

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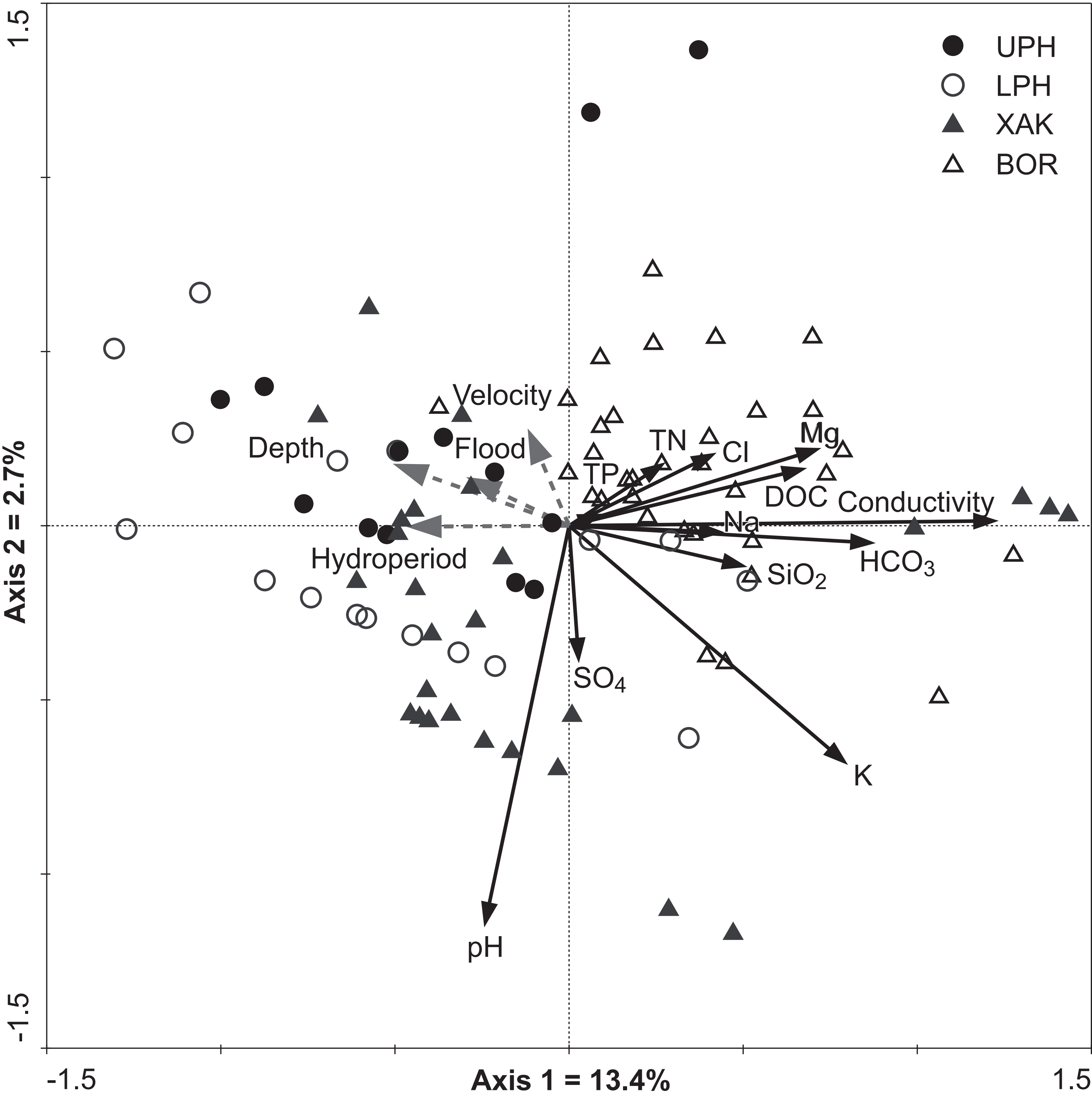
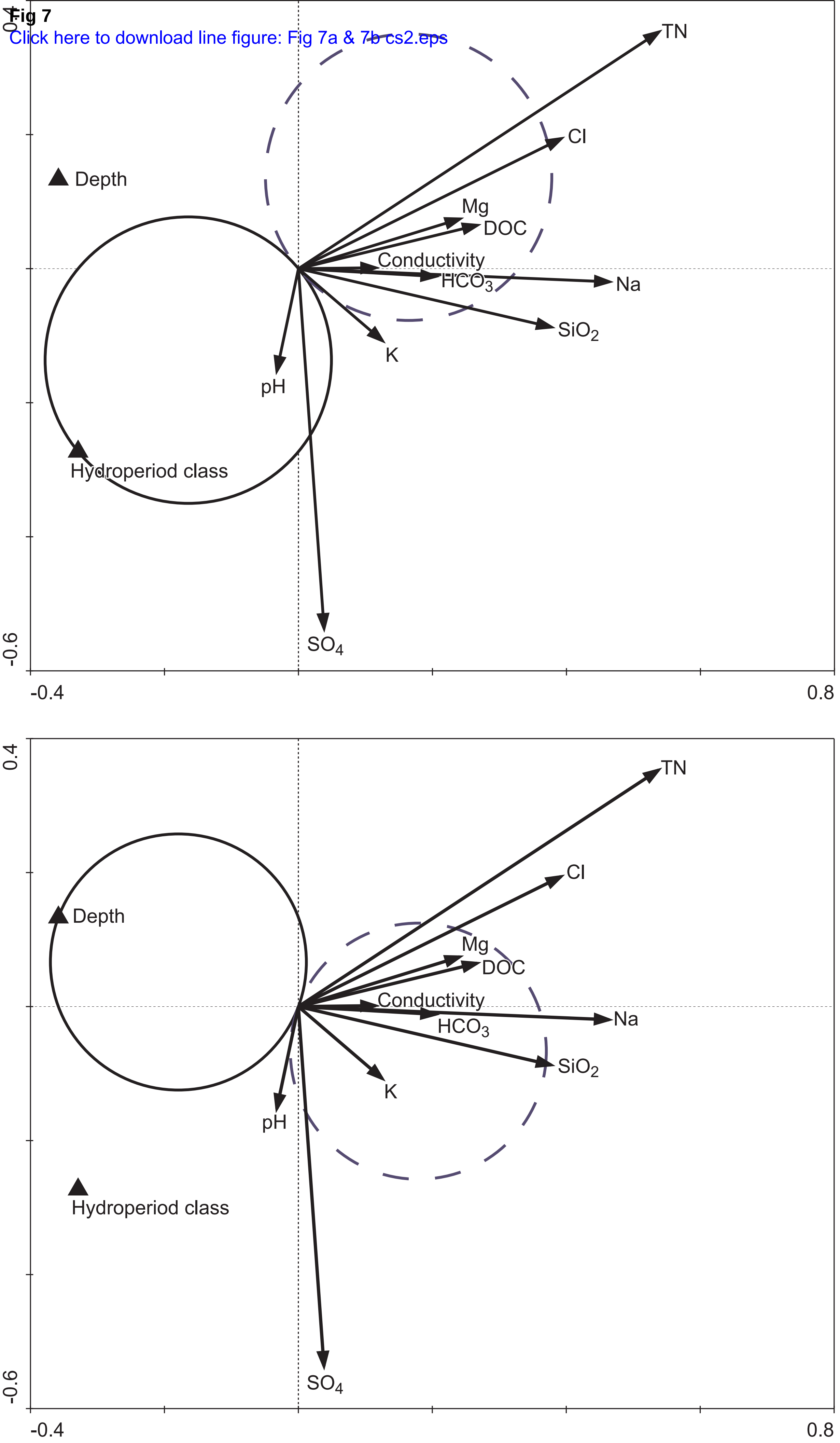


Fig 7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HCO_3
SiO_2
K
pH
Hydroperiod class
SO_4

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

Journal: ***Wetlands***

Anson W. Mackay[1]
Thomas Davidson[1,2]
Piotr Wolski[3]
Richard Mazebedi[3]
Wellington R.L. Masamba[3]
Philippa Huntsman-Mapila[3]
Martin Todd[1,4]

1ECRC, Department of Geography, UCL, Gower Street, London WC1E 6BT
2 Institute of Bioscience, NERI, Aarhus University, Vejlsøvej 25, DK-8600 Silkeborg, Denmark
3 ORI, University of Botswana, Private Bag 285, Maun, Botswana
4 Department of Geography, University of Sussex, Brighton, BN1 9RH UK

Corresponding author:
Anson W. Mackay
a.mackay@ucl.ac.uk
p: +44 (0)20 7679 0558; f: +44 (0)20 7679 0565

Online Resource 1(i)

ESM-1(i): locational co-ordinates; 2=low flood; 3=flood expansion; 4=high flood; 5=flood recession

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).

	pH	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**					
TP								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)

Test of Homogeneity of Variances for Regions

	Levene Statistic	df1	df2	Sig.
pH	.804	3	94	.495
Temp	4.907	3	94	.003
DO	1.620	3	94	.190
Conductivity	4.511	3	94	.005
DOC	3.658	3	94	.015
HCO3	1.167	3	94	.326
K	1.503	3	94	.219
Na	14.136	3	94	.000
SiO2	5.926	3	94	.001
Cl	4.238	3	94	.007
TP	1.267	3	94	.290
TN	2.096	3	94	.106
NO3	4.894	3	57	.004
TSS	6.329	3	83	.001
SO4	21.348	3	94	.000
Mg	2.234	3	94	.089
Ca	18.685	3	71	.000
Depth	13.611	3	94	.000
Velocity	5.749	3	94	.001

ANOVA of variables with equal variances

	Sum of Squares	df	Mean Square	F	Sig.
pH	**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**
K	**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**

Post Hoc Tests

Multiple Comparisons with

Dependent Variable	(I) REGION	(J) REGION	Mean Diff (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
pH	BOR	LPH	-0.029	0.125	1.000	-0.365	0.307
		UPH	-0.109	0.130	1.000	-0.460	0.242
		XAK	-0.411	0.104	0.001	-0.691	-0.131
	LPH	BOR	0.029	0.125	1.000	-0.307	0.365
		UPH	-0.079	0.149	1.000	-0.482	0.323
		XAK	-0.382	0.127	0.021	-0.725	-0.039
	UPH	BOR	0.109	0.130	1.000	-0.242	0.460
		LPH	0.079	0.149	1.000	-0.323	0.482
		XAK	-0.302	0.133	0.150	-0.660	0.055
	XAK	BOR	0.411	0.104	0.001	0.131	0.691
		LPH	0.382	0.127	0.021	0.039	0.725
		UPH	0.302	0.133	0.150	-0.055	0.660
DO	BOR	LPH	-1.553	0.556	0.038	-3.053	-0.054
		UPH	-2.043	0.581	0.004	-3.608	-0.477
		XAK	-2.401	0.464	0.000	-3.652	-1.150
	LPH	BOR	1.553	0.556	0.038	0.054	3.053
		UPH	-0.489	0.667	1.000	-2.286	1.308
		XAK	-0.848	0.568	0.833	-2.379	0.683
	UPH	BOR	2.043	0.581	0.004	0.477	3.608
		LPH	0.489	0.667	1.000	-1.308	2.286
		XAK	-0.359	0.592	1.000	-1.954	1.237
	XAK	BOR	2.401	0.464	0.000	1.150	3.652
		LPH	0.848	0.568	0.833	-0.683	2.379
		UPH	0.359	0.592	1.000	-1.237	1.954
HCO3	BOR	LPH	23.537	5.852	0.001	7.765	39.309
		UPH	28.761	6.109	0.000	12.296	45.226
		XAK	5.300	4.882	1.000	-7.858	18.459
	LPH	BOR	-23.537	5.852	0.001	-39.309	-7.765
		UPH	5.224	7.012	1.000	-13.675	24.124
		XAK	-18.236	5.974	0.018	-34.338	-2.135
	UPH	BOR	-28.761	6.109	0.000	-45.226	-12.296
		LPH	-5.224	7.012	1.000	-24.124	13.675
		XAK	-23.461	6.226	0.002	-40.241	-6.680
	XAK	BOR	-5.300	4.882	1.000	-18.459	7.858
		LPH	18.236	5.974	0.018	2.135	34.338
		UPH	23.461	6.226	0.002	6.680	40.241
Mg	BOR	LPH	0.439	0.126	0.005	0.099	0.780
		UPH	0.579	0.132	0.000	0.224	0.935
		XAK	0.189	0.105	0.456	-0.095	0.473
	LPH	BOR	-0.439	0.126	0.005	-0.780	-0.099
		UPH	0.140	0.151	1.000	-0.268	0.548
		XAK	-0.250	0.129	0.332	-0.598	0.097
	UPH	BOR	-0.579	0.132	0.000	-0.935	-0.224
		LPH	-0.140	0.151	1.000	-0.548	0.268
		XAK	-0.390	0.134	0.028	-0.753	-0.028
	XAK	BOR	-0.189	0.105	0.456	-0.473	0.095
		LPH	0.250	0.129	0.332	-0.097	0.598
		UPH	0.390	0.134	0.028	0.028	0.753

Kruskal Wallis Test

	Temp	Conduct	DOC	Na	SiO2	Cl	NO3	TSS	SO4	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	Cl	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	Cl	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	Cl	Ca	Depth
Mann-Whitney U	9	0	131	122	192	38	94
Sig. (2-tailed)	.000	.000	.005	.003	.136	.006	.000

LPH / XAK	Conduct	DOC	Na	SiO2	Cl	Ca	Depth
Mann-Whitney U	99	184	193	121	161	61	118
Sig. (2-tailed)	.000	.087	.129	.002	.027	.000	.002

LPH / UPH	Conduct	DOC	Na	SiO2	Cl	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	Cl	Ca	Depth
Mann-Whitney U	45	14	80	110	190	53	44
Sig. (2-tailed)	.000	.000	.000	.004	.319	.078	.000

Online Resource 1(iv)

Test of Homogeneity of Variances for Flood extent

	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

ANOVA of variables with equal 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 hoc tests: multiple comparisons with Bonferroni

Dependent Variable	(I) flood extent	(J) flood extent	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence	Upper Bound
Temp	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	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	-9.386
		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
DO	low	expand	0.360	0.570	1.000	-1.177	1.897
		high	-1.536	0.547	0.036	-3.009	-0.063
		recede	-0.270	0.593	1.000	-1.869	1.329
	expand	low	-0.360	0.570	1.000	-1.897	1.177
		high	-1.896	0.575	0.008	-3.446	-0.346
		recede	-0.631	0.620	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
HCO3	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	-29.329	3.590
		recede	-17.532	6.349	0.042	-34.645	-0.419
	recede	low	18.007	6.301	0.032	1.025	34.990
		expand	4.663	6.580	1.000	-13.072	22.398
		high	17.532	6.349	0.042	0.419	34.645
Mg	low	expand	-0.688	0.086	0.000	-0.921	-0.456
		high	-0.409	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	0.921
		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	K	Na	SiO2	Cl	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	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	K	Na	SiO2	Cl	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	Cl	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	Cl	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

expand/high	pH	K	Na	SiO2	Cl	TP	TN	TSS
Mann-Whitney U	260	143	162	175	150	239	182	245
Sig. (2-tailed)	.326	.001	.004	.008	.002	.161	.012	.199

expand/recede	pH	K	Na	SiO2	Cl	TP	TN	TSS
Mann-Whitney U	229	176	0	119	160	144	224	117
Sig. (2-tailed)	.971	.184	.000	.007	.088	.035	.884	.465

high/recede	pH	K	Na	SiO2	Cl	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(iv)

Test of Homogeneity of Variances for Flood extent

	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

ANOVA of variables with equal 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 hoc tests: multiple comparisons with Bonferroni

Dependent Variable	(I) flood extent	(J) flood extent	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence	Upper Bound
Temp	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	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	-9.386
		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
DO	low	expand	0.360	0.570	1.000	-1.177	1.897
		high	-1.536	0.547	0.036	-3.009	-0.063
		recede	-0.270	0.593	1.000	-1.869	1.329
	expand	low	-0.360	0.570	1.000	-1.897	1.177
		high	-1.896	0.575	0.008	-3.446	-0.346
		recede	-0.631	0.620	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
HCO3	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	-29.329	3.590
		recede	-17.532	6.349	0.042	-34.645	-0.419
	recede	low	18.007	6.301	0.032	1.025	34.990
		expand	4.663	6.580	1.000	-13.072	22.398
		high	17.532	6.349	0.042	0.419	34.645
Mg	low	expand	-0.688	0.086	0.000	-0.921	-0.456
		high	-0.409	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	0.921
		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	K	Na	SiO2	Cl	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	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	K	Na	SiO2	Cl	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	Cl	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	Cl	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

expand/high	pH	K	Na	SiO2	Cl	TP	TN	TSS
Mann-Whitney U	260	143	162	175	150	239	182	245
Sig. (2-tailed)	.326	.001	.004	.008	.002	.161	.012	.199

expand/recede	pH	K	Na	SiO2	Cl	TP	TN	TSS
Mann-Whitney U	229	176	0	119	160	144	224	117
Sig. (2-tailed)	.971	.184	.000	.007	.088	.035	.884	.465

high/recede	pH	K	Na	SiO2	Cl	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 for Equality of Variances		t-test for Equality of Means		
	F	Sig.	t	df	Sig. (2-tailed)
pH	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
HCO3	4.888	.029	3.642	96	.000
K	.841	.361	3.221	96	.002
Na	9.613	.003	3.233	96	.002
SiO2	.415	.521	2.069	96	.041
TP	.143	.706	.113	96	.910
TN	.924	.339	1.951	96	.054
NO3	8.771	.004			
TSS	.106	.746	.006	85	.936
Cl	.003	.955	.398	96	.692
SO4	11.355	.001	1.702	96	.092
Mg	2.913	.091	3.747	96	.000
Ca	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)

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

	Depth	Veloc	pH	Temp	DO	Conduct	DOC	HCO3	K	Na	SiO2	Cl	TP	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