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# Diatom sensitivity to hydrological and nutrient variability in a subtropical, flood-pulse wetland

**Short title:**

Diatoms of the Okavango Delta

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## ABSTRACT

The principal aim of this study was to disentangle hydrochemical influences on primary producers in a pristine, flood-pulse ecosystem. This was undertaken by analyzing diatoms from 100 sample points from hydrologically distinct regions in the Okavango Delta, Botswana. Cluster analysis was undertaken using two-way indicator species analysis (TWINSPAN), and groups used to classify sample points in a principal components analysis (PCA) biplot. Linear discriminant analysis (LDA) was performed using hydrological data and diatom guilds as explanatory variables. A series of ordinations using redundancy analysis (RDA) was undertaken to assess which variables significantly accounted for diatom variation across the Delta. Species response curves for major taxa were generated using generalized additive models (GAMs). Cluster analysis revealed six distinct groups. Groups 5 and 6 consisted mainly of seasonally inundated floodplain sites, which lay at one end of a significant gradient revealed by PCA. Floodplain diatoms were characteristically N-heterotrophs, requiring elevated concentrations of key resources such as total nitrogen (TN) and $SiO_2$. Using forward selection, constrained RDA reveals five variables were significant in explaining diatom distributions across the Delta: hydroperiod class, flood frequency, flow velocity, and nutrients $SiO_2$ and TN. Species response curves show that motile diatoms were most abundant in seasonally inundated floodplains. Species diversity was significantly higher in the upper Panhandle region of the Delta, which may be related to moderate levels of disturbance and increased resource limitation. Species diversity was significantly lower during the period of maximum flood extent, which may in turn be related to fewer limiting resources.

**Keywords**

Diatoms, biodiversity, wetlands, multivariate techniques, water level regime, diversity indices, Okavango Delta, Botswana

## Introduction

Tropical and subtropical wetlands are of profound importance for humankind, providing vital ecosystem services through the provision of freshwater, food and biodiversity (Millennium Ecosystem Assessment, 2005). Many of these wetlands experience periodic, regular flooding (flood-pulse concept; Junk et al., 1989), which in turn controls nutrient recycling and biological growth in floodplain habitats. Where flood pulses are regular, organisms develop adaptations to exploit periodic wetting; hydrological regimes have been shown to be a major influence on primary productivity and on the composition of primary producers, especially diatom microalgae (Class Bacillariophyceae) (e.g. Gell et al., 2002; Weilhoefer et al., 2008). Microalgae cause changes in water chemistry (e.g. pH) through photosynthesis and respiration. For example, diurnal fluctuations in oxygen concentrations can affect temporal and spatial distributions of invertebrates and fish (Suthers and Gee, 1986). Microalgae also contribute to nutrient cycling and wetland biogeochemistry. Diatoms in particular are very important because they have siliceous frustules which contribute to both carbon and silicon regulation (Struyf and Conley, 2009). Diatoms are also extensively used to monitor wetland ecosystems and human impact (Lane and Brown, 2007) including nutrient enrichment (e.g. McCormick and Stevenson, 1998) and salinisation (e.g. Tibby et al., 2007). However, in regions that experience strong hydrological variability, bioassessments using biological proxies such as diatoms are prone to significant uncertainty associated with the changing flood pulse (Wilcox et al., 2002; Weilhoefer et al., 2008).

In Africa, water availability is distributed very unevenly, and in semi-arid regions availability is especially sensitive to rainfall. Wetland degradation in Africa is highly significant; for example in recent decades over 80% of freshwater wetlands in Niger have disappeared (UNEP, 2000). However, across the continent as a whole, scientific research on wetlands has been rather inconsistent, such that estimates of area covered range between 220,000 and 1,250,000 km$^2$ (Bullock et al. in Schuyt 2005). The Okavango Delta (hereafter referred to as the Delta) is one of Africa's few remaining pristine wetlands, and therefore modelling impacts of changing hydrology on primary producers can be undertaken with minimal influence from anthropogenic stressors.

The Delta is part of the Okavango River Basin (ORB), which straddles Angola, Namibia and Botswana (Fig 1). It lies in the lowest region of the Kalahari Basin, and its resources are fundamental to the health and livelihood of hundreds of thousands people, the majority of whom live below the poverty line (Kgathi et al., 2006). Angola has only recently emerged from decades of civil war, and economic development is relatively weak. However, there is great potential for water to be used for irrigation and hydropower generation (Pinheiro et al., 2003). In northeast Namibia, the Okavango River transverses through the semi-arid, highly populated Kavango region, a major centre of food production that is strongly reliant on the Okavango River for irrigation water (Kgathi et al., 2006). There have been several plans to build hydro-electric dams in Namibia, although these have yet to be realised (Mendelsohn et al., 2010). In Botswana, the Delta is a major source of income through tourism, but because the majority of water enters from neighbouring countries, the Delta is potentially at risk from upstream activities and economic development (Ashton, 2002). For example, modelling studies have suggested that water abstraction and dam building may lead to an increase in dryland regions (Murray-Hudson et al., 2006), while deforestation of the riparian zones may lead to an increase in permanently flooded regions (Andersson et al., 2006).

The Delta is a flood-pulse ecosystem and receives water mainly from the Okavango River (c. $9 \times 10^9$ $m^3$ $yr^{-1}$) and from local summer rainfall (c. $6 \times 10^9$ $m^3$ $yr^{-1}$) (McCarthy et al., 2003). The Delta consists of a complex mosaic of wetland and dryland habitats (linked by its variable, annual flood regime), and is a Ramsar Wetland of International Importance. The Delta supports very high levels of local biological productivity and mammalian biomass (estimated to reach 12 t $km^{-2}$; Junk et al., 2006), because its spatial extent is greatest during winter when rainfall is at its lowest. The Delta is therefore a significant biodiversity reservoir, representing the southernmost limit of many Zambezian species, and the largest breeding populations of various Red Data species globally (Ramberg et al., 2006).

Seasonal inundated floodplains are of profound importance in the Delta; they link terrestrial and aquatic habitats, and are the regions where nutrients are mobilized on wetting, resulting in new cycles of biological production (Mendelsohn et al., 2010). Only limited work in the Delta has been done investigating primary producers, and microalgae research, especially diatoms, has been particularly neglected. Many fish species spawn in the Delta floodplains, but the interactions between microalgae, fish and protected consumers (e.g. the Wattled crane) are poorly understood. Previously, Cholnoky (1966) undertook a qualitative investigation of diatoms in the ORB, although only a few sites were from the Okavango Delta and neighbouring regions. Therefore, as far as we are aware, this is the first, and certainly largest quantitative, systematic study of microalgae in the Delta, let alone Botswana. Given that the nature of the flood pulse drives ecosystem functioning of this globally important wetland, understanding relationships between hydrological drivers on primary producers and diversity patterns is of the utmost importance.

## Methods

### Site Selection

Sample points were selected with a view to maximising potential diatom responses to hydrological variation (Fig 1). The upper Panhandle (UPH; n=23) is dominated by fluvial input from the Okavango River, and is characterised by permanently flooded habitats. Further downstream, the Okavango River meanders across the floodplain into the lower Panhandle region (LPH; n=27). As distance from the main channel increases, seasonal swamps become more common, while old meanders result in the formation and isolation of several large lagoons. South of the LPH, the presence of numerous islands causes the Okavango River to split into separate distributaries (Wolski and Murray-Hudson, 2006a). Just to the north of the Delta's largest island (Chief's Island) the main distributary flows to the east forming the Maunachira / Khwai river system, with many large, flow-through lagoons such as Xakanaxa (XAK; n=25) (Fig 1). Secondary distributaries include the Jao / Boro river system (BOR; n=14) and Santantadibe (SAN; n=7) (Fig 1). In 2006, Santantadibe flooded extensively for the first time since the 1990s. Lake Ngami (NGA; n=2) sits at the end of the Xudum distributary, and in recent decades very little water has flowed into the lake. In 2004 however, floodwaters entered the lake for the first time since 1998. Finally, two sample points were sampled from the Thamalakane river (THA; n=2), which flows along the terminal edge of the Delta (Fig 1).

### Diatom sampling

Diatom samples were collected during the following dates: 1st – 9th Sep 2006 (flood recession; n=30); 28th Nov – 10th Dec 2006 (low flood; n=30); 23th Apr – 6th May 2007 (flood expansion; n=31); 24th Jul – 7th Aug 2007 (high flood; n=9). Habitats investigated include 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, while IF and SP experience wet-dry cycles. At each sample point, 5-cm sections from characteristic dominant macrophyte species were cut from desired plants c. 20 cm below the water surface (avoiding dead stems and new shoots) and fixed with ethanol. The dominant habitat of each sample point was recorded and geo-referenced using a Garmin GPS.

Diatoms were prepared for counting using standard techniques (Battarbee et al., 2001). Sub-samples of plant stems were oxidised using 30% $H_2O_2$ and carbonates removed using 50% HCl. Permanent diatom slides were made using *Naphrax* and duplicates archived both at UCL and University of Botswana. Diatoms were counted using oil immersion phase contrast light microscopy at x1000 magnification. At least 350-400 valves were counted for each sample, and taxa identified with the assistance of several flora (e.g. Cholnoky, 1966; Foged, 1966; Carter and Denny, 1982; Gasse, 1986; Krammer and Lange-Bertalot, 1999a; Krammer and Lange-Bertalot, 1999b; Lange-Bertalot, 2001).

Diatom species adopt different growth forms (guilds) with which they are able to respond to resource availability (e.g. nutrients, light) and disturbance (e.g. river flow, grazing) (Biggs et al., 1998). Every diatom species was assigned to one of three ecological guilds: low profile (guild 1), high profile (guild 2) or motile guild (guild 3) (after Passy, 2007). Guild 1 diatoms attach themselves to substrates in prostrate, adnate and erect forms (e.g. species in the genus *Achnanthidium*), but we also include solitary centric species which do not form chains (e.g. species in the genus *Cyclotella*), and slow moving species (e.g. species in the genus *Amphora*) which have more in common with non-moving taxa than species that are able to move quickly (Hudon and Legendre, 1987). Guild 2 diatoms include those that are tall in stature, or form filamentous chains, or are composed of colonial centrics, e.g. species in the genera *Aulacoseira*, *Eunotia*, *Gomphonema*. Guild 3 diatoms are relatively fast moving, low adhesive species (Passy, 2007), e.g. species in the genera *Craticula*, *Navicula*, *Nitzschia*.

Measures of diversity (Shannon and N2) were undertaken to determine if statistical differences existed between (i) different regions of the Delta, (ii) different stages of the flood cycle, and (iii) permanently wet and seasonally inundated sample points. Shannon index takes into account the number of species present and their evenness, while N2 diversity index relates to the effective number of species in a sample (ter Braak and Šmilaeur 2002). Shannon diversity analysis was undertaken using Past Version 2.08 (Hammer 2011). N2 diversity analysis was undertaken using C2 Data Analysis Version 1.5.1 (Juggins 2007).

Collation of hydrological variables

The influence of four hydrological variables (water depth, flow velocity, flood frequency and hydroperiod class) on diatom communities was initially explored for every sample point analysed for diatoms. Water depth was measured using a *Plastimo Echotest II* handheld depth sounder. Velocity of water flow was measured 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 points 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; only around 30 hydrometric stations exist in the Delta, approximately 1 per 400 km$^2$. Instead, a set of seven hydroperiod/amplitude classes was defined, and a hydroperiod class was attached to each of the sample points. The hydroperiod class gradient spans sample points selected from permanently flooded regions with low amplitude (under 0.3 m; Class 7) to samples points selected from newly inundated drylands (Class 1). Classes 6 and 5 were also permanently flooded regions, but flood amplitude varied between 0.3-1.0m and 1.0-3.0m respectively. Sampling points allocated to class 4 and 3 have, since 2004, been flooded every year, but over the longer term (20 years) were once only seasonally flooded, with flood duration in class 4 longer than class 3. Class 2 sample points were only seasonally flooded for under 6 months of the year. The classification was based on flood frequency maps and hydrometric data from hydrometric stations in the vicinity of sampling point.

## Collation of hydrochemical variables

Surface water chemistry variables are only available for selected diatom sample points during Dec06, May07 and Jul07 trips (n=53). pH and conductivity (μS cm$^{-1}$)

were measured in situ using a portable *Fisher Scientific Accumet* AP85 portable waterproof pH/conductivity meter. 250ml of Delta water were filtered through a GF/C filter into acid-washed bottles for non-metal ($HCO_3$, Cl, $SO_4$, dissolved organic carbon (DOC)) analyses. 250ml of water were also filtered using cellulose nitrate papers for cation analyses (Na, K, Mg) and immediately acidified with three drops of concentrated $HNO_3$. A further 250ml of water was filtered using cellulose nitrate filter papers for $SiO_2$ analysis. All filtered samples were stored in acid-washed plastic sample bottles in cool-boxes, with bags of ice, then transported to the Okavango Research Institute. $HCO_3$ 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. Determination of Mg (mg $L^{-1}$) was undertaken 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 $L^{-1}$) and K (mg $L^{-1}$) were determined by flame photometry using a *Sherwood Flame Photometer 410* instrument. $HCO_3$ (mg $L^{-1}$) was analysed using an auto-titrator (Mettler Toledo model DL 50). Cl (mg $L^{-1}$) and $SO_4$ (mg $L^{-1}$) were analysed by ion chromatography using a *DX-120* ion chromatograph (Eaton et al. 1995). An approximation of DOC (mg $L^{-1}$) 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 $L^{-1}$)) and total nitrogen (TN (mg $L^{-1}$)) were analysed by an air segmented flow analyser (*Bran + Luebbe* AA3) after persulphate digestion. $SiO_2$ (mg $L^{-1}$) was measured using the heteropoly blue method at 815nm.

Statistical Analyses

Cluster analysis using two-way indicator species analysis (TWINSPAN) was undertaken to determine broad patterns in diatom community composition (Hill, 1979). Species occurring in relative abundances < 1%, or < 3 sites were excluded from analyses, because they had little effect on derived groupings. Patterns in diatom community composition were initially analysed using the indirect, ordination technique of detrended correspondence analysis in order to determine whether species responses were mainly linear or unimodal. The axis 1 gradient length was 2.218

standard deviation units, indicating that linear ordination methods were most appropriate for our datasets (Lepš and Šmilaeur, 2003). Derived clusters were then used to classify sites analysed using principal components analysis (PCA), with symmetric scaling of the ordination scores to optimise scaling for both samples and species. Species data were log (x+1) transformed and both species and samples were centred to give a log-linear contrast PCA, appropriate for closed, relative abundance data (Lotter et al. 1993). A broken stick model was sued to test the significance of the PCA axes (Joliffer 1986) using BSTICK v.1.0 (Line and Birks 1996). A 'crisp' classification (where sites belonging to a particular TWINSPAN group are coded 1 or 0) was determined in order that Fisher's linear discriminant analysis (LDA) could be undertaken to assess the influence of (i) hydrological variables and (ii) guild structure on the classes defined by TWINSPAN (Lepš and Šmilaeur, 2003). LDA was undertaken using canonical correspondence analysis (focusing on species distances and using Hill's scaling) in Canoco 4.5 (Lepš and Šmilaeur, 2003).

Hydrological influences on diatom species composition across 100 sample points was determined using redundancy analysis (RDA). 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. Exactly one-fifth of the sample points (n=20) were visited on more than one occasion. Consequently, a partial RDA with season as covariable was undertaken, together with Monte Carlo permutation tests (p = 0.002; n = 499). A major feature of spatial datasets such as ours is the influence of sample point proximity (Legendre, 1993). Here we explore this influence by partialling out sample point location co-ordinates using RDA. In order to identify a minimum subset of variables that significantly explain variation in the diatom data, redundant variables were removed through a form of step-wise regression (forward selection) together with Monte Carlo permutation tests (p = 0.002; n = 499) and associated Bonferroni corrections (ter Braak and Šmilaeur, 2002). Species response curves for selected taxa to hydroperiod class were explored using generalized additive models (GAMs). Poisson distributions were selected and binomial total settings used maximum values. Significance of diatom responses to hydroperiod class were assessed using Akaike Information Criterion (AIC) to determine the parsimony of response models constructed. Prior to all analyses explanatory variables were tested for normality and were subsequently log(x+1) transformed (except pH). Ordination analyses were undertaken using Canoco v. 4.5 (Lepš and Šmilaeur, 2003). GAMs were undertaken using Canodraw for Windows 4.1 (Lepš and Šmilaeur, 2003).

Differences in diversity measures were analysed using SPSS 17.0. Levene's tests for equality of variances were undertaken to ensure that appropriate parametric and non-parametric tests were used. Regional diversity indices had equal variance and were analysed using analysis of variance (ANOVA), and differences between the regions were further investigated using post-hoc tests with Bonferroni corrections. Flood-stage diversity indices had unequal variance, and were analysed using Kruskal-Wallis (KW) tests. KW has no equivalent post-hoc test, so where the KW test did reveal significant variation, pairwise Mann-Whitney U tests were undertaken to determine significant differences between stages of the flood cycle. Differences between sites that were permanently or seasonally flooded, were analysed using a 2-tailed t-test.

## Results

Full names, taxonomic authorities, Hill's N2 diversity and maximum abundance of each of the 167 species are detailed in Appendix S1 in Supplementary Information. Two-way indicator species analysis revealed six distinct groups (Table 1). Group 1 consists only of sample points visited in the upper Panhandle during May 2007, which LDA reveals to be associated with deep-water sites with high flow velocities and is dominated by species with guild 1 (low) growth form (Fig 2). Group 2 consists of sample points from each of the four main regions with highest hydroperiod class and diatoms with guild 2 (high) growth form. Sample points in group 3 were sampled during flood recession and low flood (except for Thamalakane) in waters with low flow velocity (Fig 2a), while sample points in group 4 were mainly sampled during flood expansion and peak flood. Diatom guilds are distinctly different in these two groups, with guild 2 diatoms dominating group 4 (Fig 2b). Group 5 consists of sample points not sampled during peak flow, while group 6 consists only of sample points in the Boro and Xakanaka regions during flood expansion (Table 1). Both groups are best characterised by shallow, low hydroperiod class sample points, and diatoms dominated by motile species in guild 3 (Fig 2a,b).

The first two PCA axes account for 15.2% and 12.1%% of variation in the species data respectively. Broken stick reveals both these values to be significant (Table 2). Samples in the PCA biplot (axes 1 and 2) have been classified according to their TWINSPAN groupings (Fig 3). Axis 1 represents an environmental gradient with groups 5 and 6 at one end, characterised by seasonally inundated floodplains and pools (IF+SP). The other end of the gradient is characterised by permanently inundated sample points (e.g. in group 2) with highest hydroperiod class (MV+FV). The four hydrological variables significantly explain 10% of total diatom variation that cannot be explained by site proximity (Table 3). However, unique variation explained by each variable is also significant, as is variation when season is partialled out as a co-variable (Table 4). In each of these analyses, hydroperiod class remains the variable that significantly explains most variation in the diatom data. In the reduced dataset with both hydrology and hydrochemistry as explanatory variables (Table 5), $K^+$ and $Mg^{2+}$ displayed high variance inflation factors, indicating a substantial amount of collinearity (ter Braak and Šmilaeur, 2002) and were excluded from analyses. Constrained ordination using forward selection determined five variables significantly accounted for almost 30% of the variation in the diatom dataset (Table 6). Axis 1 is controlled mainly by the gradient between diatoms associated with high hydroperiod class and flood frequency, and diatoms associated with nutrients, TN and $SiO_2$ (Fig 4). Species highly correlated along this axis are similar in composition to PCA determined above. Species composition along axis 2 is different however, and is now primarily characterised by species associated along a flow velocity gradient (Fig 4).

Selected diatom response curves for hydroperiod class have been grouped together according to their guilds (Fig 5). Guild 1 diatoms in general show skewed unimodal responses (Fig 5a). Guild 2 diatoms show a heterogeneous set of responses (Fig 5b, c). Several species however did not exhibit any significant response curves: *Synedra acus* v. *acus* and *Staurosirella pinnata* have rather flat null responses, whereas *Eunotia pectinalis* v. *minor* shows an increasing trend which does not reach a maximum value. Guild 3 species generally display declining monotonic responses, although the response for *Nitzschia bacata* is rather more complex, while that for *Navicula cryptotenella* shows no significant trend. (Fig 5d).

Between regions, only Shannon index shows significant variation (p=0.002), with sample points in the UPH region having higher values than either LPH (p=0.001) or BOR (p=0.012). Both Shannon and N2 indices exhibited significant variation in relation to different stages of the flood cycle: lowest values were recorded during high flood, and these values were statistically lower than during each of the other stages. Neither Shannon (p=0.623) nor N2 (p=0.815) indices showed any significant difference between permanently flooded and seasonally inundated sample points.

## Discussion

### Spatial trends in diatom communities in the Okavango Delta

Hydrology and surface water chemistry are influential in determining ecological guilds and species community composition in the Okavango Delta. The most important pattern is the significant gradient between species that inhabit seasonally inundated floodplains and pools, and species which show greater preference for permanently inundated sites. Species with high positive PCA axis 1 correlations (found mainly in groups 5 and 6; Fig 3) exhibit several adaptations that allow them to colonise seasonally inundated habitats. *Craticula buderi*, *Sellaphora pupula* and *Navicula cryptotenella* are highly motile and therefore competitive with regard to both disturbance pressures and limited resources (Passy, 2007). Species such as *Nitzschia bacata* and *Nitzschia communis* are adapted to growing in seasonally wet habitats (Gasse, 1986) and are obligate N-heterotrophs (van Dam et al., 1994) that need continuously elevated concentrations of organically bound nitrogen to thrive. Other species grow best in slow moving waters e.g. *S. acus* v. *tenera* (Patrick and Reimer, 1966). *Craticula buderi* and *Cyclotella meneghiniana* can tolerate brackish waters while other species thrive when soil surfaces are only periodically inundated (e.g. *Pinnularia gibba*) (van Dam et al., 1994). *Hantzschia amphioxis* is an aerophilous species that can tolerate drying, and in our study, this species was only found in the recently flooded regions of Santantadibe and Lake Ngami. Motile diatoms such as those found in groups 5 and 6 are classed as epipelon, and inhabit the soft surface sediments of the floodplains. Epipelon help to mediate efflux rates of nutrients from sediment interstitial water (Goldsborough and Robinson, 1996), and therefore influence nutrient status of surface waters.

Species with high negative PCA axis 1 scores occur in permanently inundated sites. These species are commonly N-autotrophs and grow best in well-aerated, oligotrophic environments, e.g. *Achnanthidium minutissimum, Eunotia naegelli* and *Eunotia rhomboidea*. *Achnanthidium minutissimum* is sensitive to organic pollution and nutrient enrichment, and so axis 1 also likely represents a nutrient gradient, linked to frequency of inundation. The gradient along PCA axis 2 largely represents a split between TWINSPAN group 3 and groups 1, 2 and 4. *Nitzschia amphibia* is a facultative N-heterotroph, and so periodically needs elevated concentrations of organically bound nitrogen (van Dam et al., 1994). In contrast, *Cymbella leptoceros* is an N-autotroph, while *Rhopalodia gibba* is able to fix its own nitrogen via spheroidal bodies which likely have cyanobacterial origin (Prechtl et al., 2004). *Eunotia pectinalis* v. *minor* and *Eunotia flexuosa* species are also N-autotrophs and grow best in circumneutral, low alkalinity environments (Patrick and Reimer, 1966). Axis 2 therefore is unlikely to represent a simple nutrient gradient.

### Factors driving observed spatial trends

The flood-pulse nature of the Delta results in surface water chemistry being associated with hydrological drivers. For example, RDA reveals that sites of low hydroperiod class, i.e. those that are seasonally inundated, have elevated concentrations of key nutrients such as TN and $SiO_2$ (Table 2; Fig 4). Cations and anions are between c. 2-3 times greater in the distal regions (e.g. Boro river system) than the upper Panhandle (Cronberg et al., 1996; Mackay et al., in review). These differences are mainly caused by the greater area of seasonally inundated floodplains (Krah et al., 2006) and by evapotranspiration processes (Dinçer et al., 1978). Generally, motile species exhibit monotonic (declining) responses to increasing hydroperiod class (Fig 5). Floodplain nutrients come from a number of sources including river flow (Cronberg et al., 1996), windblown dust (Krah et al., 2006), animal faeces (Lindholm et al., 2007) and from within the wetland sediments themselves which have accumulated nutrients over thousands of years (Mendelesohn et al., 2010).

Nitrogen and phosphorous can limit plant growth, which makes them the two most important wetland macronutrients. Perhaps surprisingly, our analyses indicate that TP has no significant impact on diatom community composition (Table 6). TN however does significantly influence diatom variation across the Delta (Table 6). Highest TN concentrations are associated with seasonally inundated floodplains in the Boro and Xakanaka regions sampled during the period of flood expansion. The high concentration of TN present in the floodplains allows for the proliferation obligate and facultative N-heterotrophs such as *Nitzschia bacata* and *Nitzschia amphibia* respectively (Fig 4). Species which are very sensitive to excess nitrogen enrichment, such as *Achnanthidium minutissimum*, conversely are most abundant in sites with very low concentrations of TN (Fig 4), while the species response curve for *Achnanthidium minutissmum* shows that it grows optimally in sites with high hydroperiod class (Fig 5). The susceptibility of aquatic ecosystems to algal blooms can be assessed by determining TN/TP ratios. Small ratios (e.g. under 10) can suggest elevated supply of phosphorus, whereas pristine environments (where P is likely to be limiting) are more likely to have ratios > 15 (Hecky and Kilham, 1988). TN/TP ratio for TWINSPAN groups 5 and 6 together is 38, while for TWINSPAN groups 1-4 together, the ratio is much lower at 14. The high ratio in groups 5 and 6 is above the minimum for limitation for P (Cronberg et al., 1996). However, the relatively low value for the remaining sites is unlikely to be due to elevated concentrations of TP (which we have established are low throughout the Delta), but most likely due to a number of processes including intensive denitrification (a major factor for N loss from wetland soils (Reddy and Patrick, 1984; Mitsch and Gosselink, 2000)), the coupling of P with sediment loads (Cronberg et al., 1996) and uptake by aquatic macrophytes.

$SiO_2$ is an essential component of diatom metabolism, and highest concentrations are found in the seasonally inundated floodplains and pools. Increasing $SiO_2$ concentrations are most highly correlated with increasing abundances in several diatom species, including *Aulacoseira granulata*, *Pinnularia gibba*, *Synedra acus* v. *tenera*, *Craticula buderi*. Species such as *Aulacoseira granulata* require high concentrations of $SiO_2$ for valve development. For other species however, it is not clear whether they are growing well due to high concentrations of $SiO_2$, or whether they are responding more to other factors such as elevated TN, or even simply periodic wetting of the habitat. Species which grow best in very slow moving, nutrient rich waters include *Gomphonema parvulum, Aulacoseira distans* v. *africana* and *Aulacoseira ambigua*. All three species have high profile growth forms and so are able to tolerate the low flow velocities impacting those sites (Fig 4). *Gomphonema parvulum*, is a facultative N-heterotroph, and although it has a broad tolerance to nutrients it grows best when nutrients are elevated (van Dam et al., 1994). Like *Aulacoseira granulata*, *Aulacoseira distans* v. *africana* and *Aulacoseira ambigua* require high concentrations of $SiO_2$. In East African lakes, Gasse (1986) found that *Aulacoseira ambigua* and *Aulacoseira distans* v. *africana* grew best in shallow waters dominated by Ca-Mg carbonates, colonized by swampy vegetation (habitats very like the distal regions of the Delta).

Diatom species associated with high hydroperiod class include *Achnanthidium minutissimum*, *Eunotia pectinalis* v. *minor* and *Navicula radiosa*, each of which represents a different guild (low, high and motile respectively). This is perhaps not surprising as the vectors for hydroperiod class and velocity are orthogonal to each other (Fig 4), so the diatoms associated with increasing hydroperiod class will not necessarily show any trend with increasing velocity (i.e. growth form). In the Panhandle region there are many lagoons, which although deep also have very little flow. Both *Navicula radiosa* and *Achnanthidium minutissimum* require well aerated, highly oxygenated waters (Cholnoky, 1966; Gasse, 1986; van Dam et al., 1994), which ties in well with their occurrence in waters which are permanently inundated, but whose environments do not become shallow or stagnant. *Eunotia naegelli*, *Eunotia rhomboidea*, *Synedra amphicephela* and *Gomphonema gracile* exhibit high profile growth forms, and are able to flourish when disturbance pressures such as high velocities are low. *Gomphonema gracile* for example also shows high affinity with rather stagnant waters in the East African great lakes (Gasse, 1986), but is intolerant of elevated nitrogen concentrations (van Dam et al., 1994). Species in the *Eunotia* genus however exhibit highest abundances when hydroperiod classes are high (Fig 5b). *Eunotia* species are all N autotrophs, and therefore not dependent on high nitrogen concentrations. The exception here is *Eunotia okawangoi*. It grows best in seasonally inundated floodplains with low hydroperiod class (Fig 5b). Further work is needed to better characterise this species ecological requirements.

Diatom species associated with high flow velocities include group 1 indicator taxa *Frustulia crassivervia* and *Brachysira brebissoni* (Table 1), *Eunotia pectinalis* v. *undulata*, *Synedra acus* v. *acus*, *Encyonema mesiana*, *Encyonema minuta* and *Encyonema gracilis* (Fig 4). Group 1 sites are generally taken from deep channel margins along the southern most reaches of the Okavango River. Many diatom species here exhibit low profile growth forms (guild 1). For example, *Encyonema* species grow in mucilaginous communities and are able to tolerate relatively high flow velocities (Biggs et al., 1998) such as those in the UPH.

The Shannon index is a function of both species richness and evenness, i.e. the distribution of individuals among species. Significantly high values of Shannon index in the UPH suggests that diatom communities in this region contain many taxa which have few individuals, especially in comparison to communities in LPH and BOR regions. High diversity values may be interpreted in the context of the intermediate disturbance hypothesis (IDH) (Connell 1978), such that moderate levels of disturbance can prevent competitive exclusion. Diversity can also be interpreted within the context of resource-competition theory. For example, high algal diversity can be associated with conditions where many resources are limiting (e.g. Interlandi and Kilham 2001). The fast flowing waters in the UPH therefore may result in sufficient disturbance to maintain high diatom diversity. These relationships are apparent in both Table 1 and axis 2 in RDA (Fig 4). The RDA biplot also reveals a negative association between high velocity and TN and $SiO_2$, i.e. such that key nutrients at these sample points were also lowest, which conforms to findings by Interlandi and Kilman 2001). Significantly lower diversity values during peak flood suggest that fewer taxa dominate communities, such as *Achnanthidium minutissimum*, which is abundant in regions with high hydroperiod class (Fig 5). Our data may suggest therefore that at this stage in the flood cycle, few resources are limiting, and levels of disturbance low. In a recent comprehensive study of surface water chemistry across the Delta, Mackay et al. (in review) showed that TN concentrations were significantly highest during peak flood, which fits well with the hypothesis that key nutrients at this time were not limiting (Interlandi and Kilman 2001).

It was also hypothesised that seasonally inundated sample points would contain higher levels of diatom diversity than permanently inundated sample points due to the intermediate disturbance hypothesis (Connell 1978). However, no statistical difference in diatom diversity was found between these two groups of sample points. This may be because the rate of inundation is very slow, and diatom communities are adapted to exploiting available niches quickly, or that there is no difference in resource limitation between either permanently or seasonally flooded habitats. Our data certainly show different communities living in the two groups of sample points, but the gradual inundation has not resulted in shifts between e.g. stress tolerant and stress sensitive species (Stevenson 1997). However, other factors may also play a role, such as grazing by zooplankton and macroinvertebrates (Jones and Sayer 2003), because consumer diversity at this time is highest (unpublished data). Future work will explore these concepts in more detail.

Modelled impacts of changing hydrology from increasing development in the ORB are relatively small in comparison to observed variability in river flow in recent decades and modelled variability forced by predicted future climate change (Murray-Hudson et al., 2006). 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). However, over the next 100 years, climate change scenarios in southwest Africa are still highly uncertain (Hughes et al. 2010). A key question therefore remains as to how an increase in volume of water in the flood pulse will influence diatom communities in the Delta. From our analyses, changes in the extent of inundated floodplains will have a significant impact on the composition of diatom communities related to, e.g. nutrient availability, although determining the impacts on diatom diversity needs further work.

## Conclusions

Hydrological variability may be a deciding factor in biogeochemical cycling in flood-pulse wetlands (Struyf and Conley, 2009), and it certainly influences surface water chemistry and habitat availability for many organisms. This is the first study to model diatom responses to hydrological and hydrochemical variability in the Okavango Delta. As such, this study forms an important baseline for future work into biogeochemical cycling in the Delta and food-web interactions between species highly adapted to seasonally inundated floodplain environments and species recognised for their international conservation value. We have also demonstrated that diatoms in the Delta are sensitive to surface water chemistry, including nutrients such as TN and $SiO_2$. Diatoms therefore have the potential to make excellent sensors of environmental change for current and future biomonitoring programmes as long as hydrological variability is also considered. Diatoms would, for example, be amongst the first organisms to respond to any future deterioration in water quality. Ecosystem health of the Delta is also under threat from future climate variability and hydro-technological development, which would attenuate the nature of the flood pulse. However, not only would this alter the availability of substratum for microalgae to grow, but any decline in flow would also have a detrimental effect on growth and primary production (Goldsborough and Robinson, 1996). Such changes could have severe negative consequences for the ecosystem functioning of this globally important wetland.

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**Author contributions**

A.W.M. and M.T. conceived the ideas; A.W.M., T.D., P.W., S.W., R.M. collected the data; P.W. undertook analyses of remotely sensed data; W.R.L.M. supervised the analyses of the hydrochemistry data; A.W.M. and T.D. analysed the data; A.W.M. led the writing; ALL contributed to the writing of the manuscript.

Table 1: Sites grouped according to their TWINSPAN classification. Most abundant species in each group are given in the bottom panel, while indicator taxa are highlighted in bold. Refer to Appendix S1 for species codes.

| **Group 1 (9)** | **Group 2 (30)** | **Group 3 (33)** | **Group 4 (11)** | **Group 5 (7)** | **Group 6 (10)** |
|---|---|---|---|---|---|
| UPH1B-3 | BOR12-2 | LPH1B-1 | BOR13B-3 | BOR16-3 | BOR10A-3 |
| UPH3B-3 | LPH1A-1 | LPH1B-2 | BOR17A-4 | BOR8A-3 | BOR10B-3 |
| UPH4B-3 | LPH1A-2 | LPH1C-1 | BOR18-4 | NGA2-1 | BOR14A-3 |
| UPH5B-3 | LPH2-1 | LPH1C-2 | BOR19-4 | SAN4-1 | BOR14B-3 |
| UPH6-3 | LPH2-2 | LPH4B-2 | LPH4C-2 | XAK7-2 | BOR15-3 |
| UPH7B-3 | LPH3A-1 | LPH5A-2 | SAN7-3 | XAK7-3 | BOR2B-3 |
| UPH8A-3 | LPH3A-2 | LPH5C-2 | XAK13A-3 | XAK9-2 | BOR9A-3 |
| UPH8B-3 | LPH3B-1 | NGA1-1 | XAK14-3 | | XAK13B-3 |
| UPH9C-3 | LPH3B-2 | SAN1-1 | XAK15-3 | | XAK13C-3 |
| | LPH4A-1 | SAN2-1 | XAK16A-3 | | XAK1A-3 |
| | LPH4A-2 | SAN3-1 | XAK16B-3 | | |
| | LPH4B-1 | SAN5-1 | | | |
| | LPH4C-1 | SAN6-1 | | | |
| | LPH5A-1 | THA2-4 | | | |
| | LPH5B-1 | THA3-4 | | | |
| | LPH5C-1 | UPH1B-2 | | | |
| | LPH6-1 | UPH1B-1 | | | |
| | LPH8A-4 | UPH1C-1 | | | |
| | LPH9A-4 | UPH3A-1 | | | |
| | LPH9B-4 | UPH3B-1 | | | |
| | UPH4A-1 | UPH4A-2 | | | |
| | UPH4B-1 | UPH5A-2 | | | |
| | UPH5A-1 | UPH5B-1 | | | |
| | XAK10B-2 | UPH5B-2 | | | |
| | XAK12-3 | UPH5C-1 | | | |
| | XAK19A-4 | UPH5C-2 | | | |
| | XAK3B-2 | XAK1-2 | | | |
| | XAK4B-3 | XAK11-2 | | | |
| | XAK5A-2 | XAK2A-2 | | | |
| | XAK5B-2 | XAK2B-2 | | | |
| | | XAK6A-2 | | | |
| | | XAK6B-2 | | | |
| | | XAK8-2 | | | |
| EunPectM | **GompParv** | GompParv | GompParv | GompParv | PinnGibb |
| EunPectU | GompGrac | **GompGrac** | EunPectM | GompGrac | NitzBaca |
| **BracBreb** | EunPectM | **EunPectM** | **EunPectU** | EunPectM | GompParv |
| **FrusCras** | EunRhomb | AchMinut | EunNaege | NitzBaca | CratBude |
| | EunNaege | **EunNaege** | | CratBude | **AulGranu** |
| | AchMinut | | | **AulAmbig** | |

Table 2: Principal components analysis (PCA) of diatom dataset.

| | PCA Axes 1 | 2 | 3 | 4 | Total Variance |
|---|---|---|---|---|---|
| Eigenvalues | 0.152 | 0.121 | 0.095 | 0.056 | 1 |
| Cum. % var. spp .data | 15.2 | 27.3 | 36.8 | 42.4 | |
| Broken stick variances | 0.048 | 0.039 | 0.034 | 0.031 | |

Table 3: Redundancy analysis (RDA) of species data with four hydrological variables as explanatory variables, and latitude – longitude coordinates partialled out as co-variables

| | RDA Axes | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | Total Variance |
| Eigenvalues | 0.052 | 0.023 | 0.016 | 0.009 | 1 |
| Cum. % var spp. data | 5.7 | 8.3 | 10.1 | 11 | |
| Sum of all eigenvalues | | | | | 0.903 |
| Sum of all canonical eigenvalues; p=0.002 | | | | | 0.1 |

Table 4: % contributions to diatom variation of each of the four hydrological variables as determined using RDA of 100 sample points. Unique contributions have also been calculated, with the remaining 3 variables as co-variables. Contributions to diatom variation with season and site proximity as co-variables are also shown. All values are significant to at least p = 0.008, except * (p>0.05).

| | Single variable | Other hydrological variables partialled out | Season partialled out | Proximity partialled out |
|---|---|---|---|---|
| Depth | 3.7 | 1.8 | 3.3 | 1* |
| Velocity | 2.2 | 2.2 | 2.1 | 1.8 |
| Flood frequency | 4.1 | 2.3 | 4.2 | 2.5 |
| Hydroperiod class | 5.5 | 4 | 4.6 | 2.5 |

Table 5: Summary statistical analyses for hydrological and hydrochemical data measured for the reduced dataset of 53 sample points. Summary statistical data for the 4 hydrological variables are also given for the full dataset of 100 sample points for comparison.

| **Explanatory variable** | N | Minimum | Maximum | Mean | S.D. |
|---|---|---|---|---|---|
| Depth (m) | 100 | 0.0 | 4.4 | 1.5 | 1.1 |
| Velocity (m/s) | 100 | 0.0 | 1.6 | 0.1 | 0.3 |
| Flood frequency | 100 | 1.0 | 15.0 | 13.0 | 3.9 |
| Hydroperiod class | 100 | 1.0 | 7.0 | 4.9 | 1.9 |
| | | | | | |
| Depth (m) | 53 | 0.0 | 4.3 | 1.6 | 1.2 |
| Velocity (m s$^{-1}$) | 53 | 0.0 | 1.6 | 0.2 | 0.4 |
| Flood frequency | 53 | 1.0 | 15.0 | 13.9 | 2.9 |
| Hydroperiod class | 53 | 1.0 | 7.0 | 5.0 | 1.7 |
| pH (pH units) | 53 | 4.7 | 9.1 | 6.7 | 0.7 |
| Conductivity ($\mu$S cm$^{-1}$) | 53 | 20.7 | 236.0 | 64.7 | 35.3 |
| DOC (mg L$^{-1}$) | 53 | 3.3 | 34.4 | 8.8 | 6.2 |
| SiO$_2$ (mg L$^{-1}$) | 53 | 4.1 | 32.3 | 15.7 | 8.6 |
| TP (mg L$^{-1}$) | 53 | 0.0 | 0.1 | 0.0 | 0.0 |
| TN (mg L$^{-1}$) | 53 | 0.1 | 1.9 | 0.6 | 0.4 |
| HCO$_3$ (mg L$^{-1}$) | 53 | 3.5 | 100.0 | 49.2 | 21.6 |
| Cl (ug L$^{-1}$) | 53 | 0.2 | 14.5 | 1.2 | 2.4 |
| SO$_4$ (mg L$^{-1}$) | 53 | 0.0 | 12.6 | 0.7 | 1.8 |
| K (mg L$^{-1}$) | 53 | 0.5 | 17.1 | 2.6 | 2.4 |
| Na (mg L$^{-1}$) | 53 | 0.5 | 3.0 | 1.3 | 0.6 |
| Mg (mg L$^{-1}$) | 53 | 0.1 | 2.6 | 0.7 | 0.5 |

Table 6: RDA of species data (86 taxa) in 53 sample points, with five forward selected variables as explanatory data.

| | RDA Axes | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | Total Variance |
| Eigenvalue | 0.133 | 0.079 | 0.036 | 0.021 | 1 |
| Cum. % var spp. data | 13.3 | 21.3 | 24.9 | 27.0 | |
| Sum of all canonical EVs; p = 0.002 | | | | | 0.286 |

## Figure Legends

Figure 1: Map of the Okavango Delta and its catchment basin straddling three countries Angola, Namibia and Botswana. Principal regions studied are indicated: UPH (upper Panhandle); LPH (lower Panhandle); XAK (Xakanaxa region) BOR (Jao /Boro river system), SAN (Santantadibe), THA (Thamalakane), NGA (Lake Ngami).

Figure 2: Biplots of linear discriminant analysis of diatom communities classified according to their TWINSPAN grouping; (a) hydrological variables and (b) diatom guilds as predictor variables. Both biplots show Axis 1 plotted against Axis 2.

Figure 3: Principal components analysis (PCA) biplot (axes 1 and 2) of diatom species. Sample points (n=100) have been coded according to their TWINSPAN groupings.

Figure 4: Redundancy (RDA) triplot (axes 1 and 2) with sample points (n=53) coded according to their TWINSPAN grouping, and forward selected explanatory variables shown.

Figure 5: Species response curves (SRC) for major taxa fitted with a generalized additive model (GAM). SRCs are grouped according to (a) guild 1 (low); (b, c) guild 2 (high); (d) guild 3 (motile).

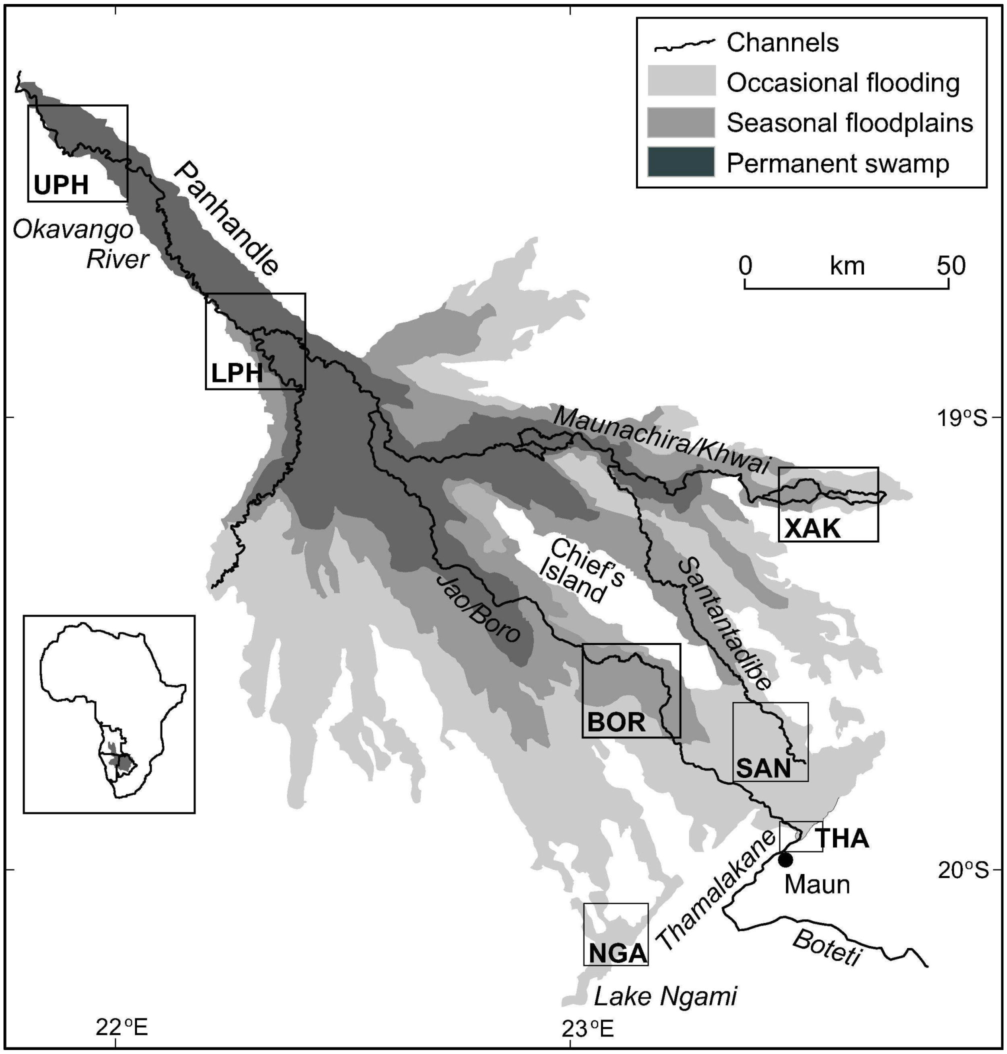

Figure 1: Map of the Okavango Delta and its catchment basin straddling three countries Angola, Namibia and Botswana. Principal regions studied are indicated: UPH (upper Panhandle); LPH (lower Panhandle); XAK (Xakanaxa region) BOR (Jao /Boro river system), SAN (Santantadibe), THA (Thamalakane), NGA (Lake Ngami).
105x110mm (600 x 600 DPI)

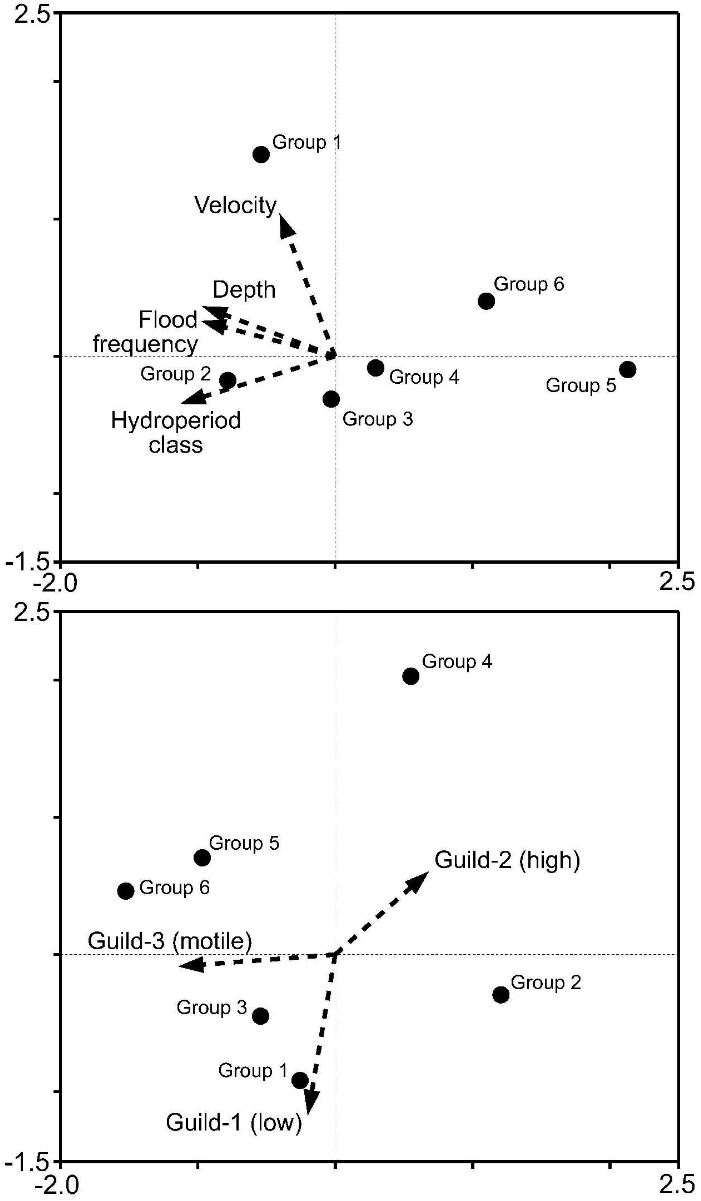

Figure 2: Biplots of linear discriminant analysis of diatom communities classified according to their TWINSPAN grouping; (a) hydrological variables and (b) diatom guilds as predictor variables. Both biplots show Axis 1 plotted against Axis 2
106x182mm (600 x 600 DPI)

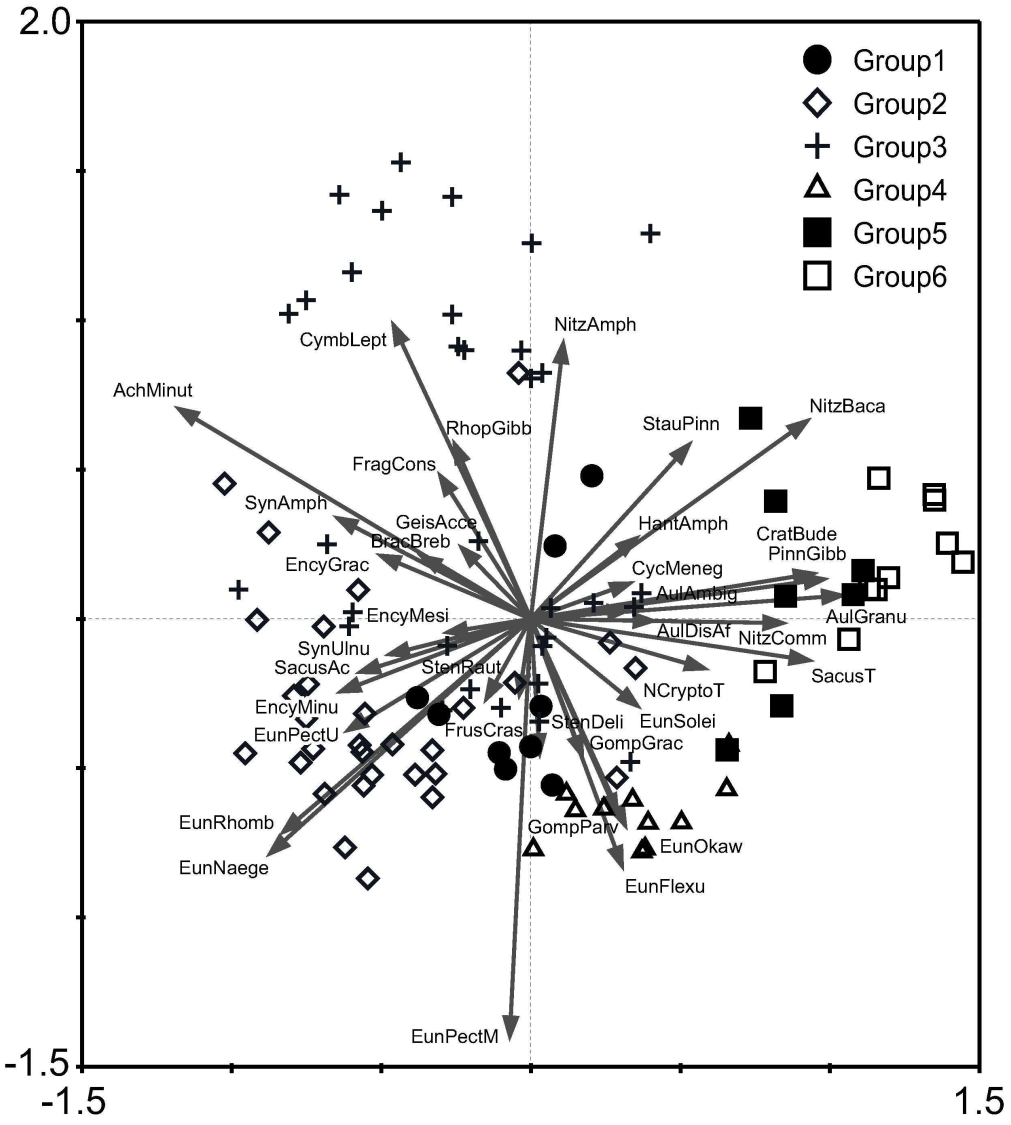

Figure 3: Principal components analysis (PCA) biplot (axes 1 and 2) of diatom species. Sample points (n=100) have been coded according to their TWINSPAN groupings.
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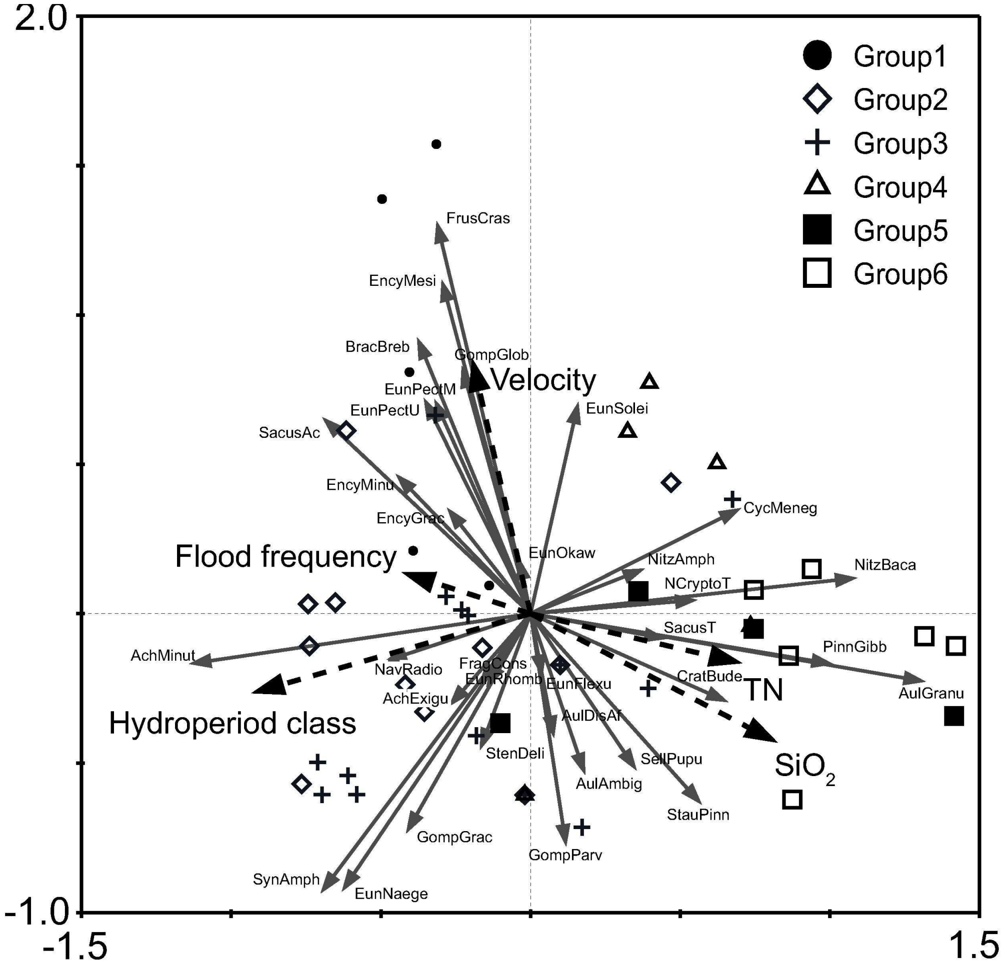

Figure 4: Redundancy (RDA) triplot (axes 1 and 2) with sample points (n=53) coded according to their TWINSPAN grouping, and forward selected explanatory variables shown.
106x102mm (600 x 600 DPI)

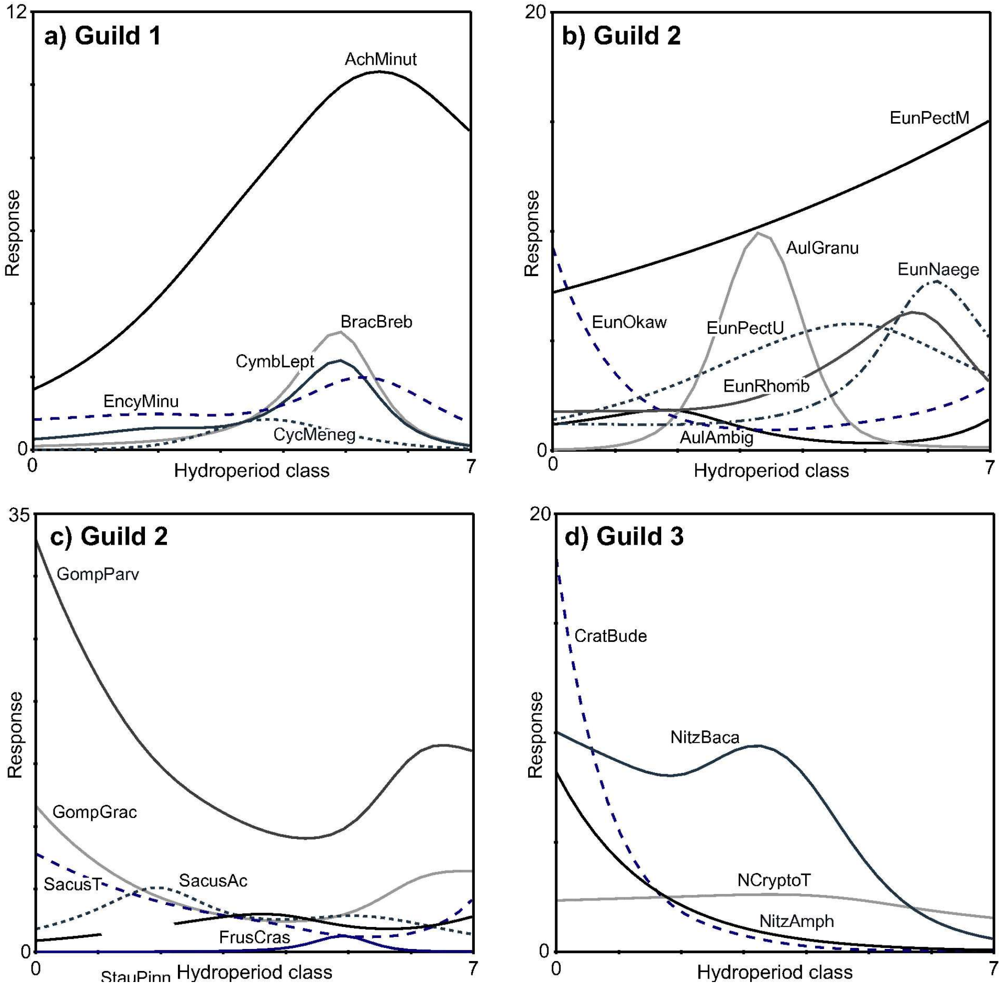

Figure 5: Species response curves (SRC) for major taxa fitted with a generalized additive model (GAM). SRCs are grouped according to (a) guild 1 (low); (b, c) guild 2 (high); (d) guild 3 (motile).
161x158mm (600 x 600 DPI)