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Research Centre**



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**Monitoring and simulating threats to aquatic biodiversity in the
Okavango Delta:
field and laboratory methods**

Methods report to Darwin Initiative 162/14/029

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ECRC Research Report 129**

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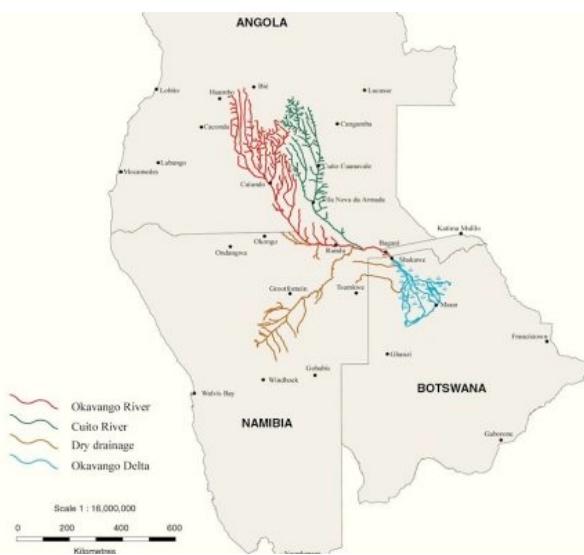
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1. INTRODUCTION

1.1 Project Background

The Okavango Delta, situated in northwest Botswana between E22.0°-E24.0° and S18.0°-S20.5°, is the world's second largest inland wetland region. The Delta is actually an alluvial fan and is fed mainly by the Okavango River whose catchment lies largely in the highlands of central Angola (Fig 1). The river flows south-east through the Caprivi Strip in eastern Namibia, before entering into Botswana as a large river, some 200 m in width. The size of the Delta changes significantly throughout the year - during the dry season, the Delta is approximately 7,000 km², and more than doubles in size to over 15,000 km² during the wet season (Ramberg et al. 2006).

Figure 1: Catchment area of the Okavango River flowing into the Okavango Delta, Botswana



The Okavango Delta is a flood-pulsed wetland – rain falling in the Angola catchment during November pulses down the Okavango River, reaching the northern parts of the Delta by early March. The annual flooding in the Delta is out of phase with the local wet season so that uniquely it provides a water resource in the dry season. It is one of

the WWFs top 200 eco-regions of global significance and the world's largest Ramsar site. The Okavango river system is considered by many to be the last near pristine river in Africa. However, the system is under threat from potential development initiatives in the basin and from climate change. This project aims to build capacity in key institutions involved in conservation of biodiversity in the Delta, to assist in implementation of the Convention on Biological Diversity (CBD). This will involve an integrated, multi-disciplinary programme of: (a) scientific research to develop baseline aquatic biodiversity characterisations (macroinvertebrates, aquatic macrophytes and diatom assemblages) and their relationship with hydrological drivers such as hydroperiod and water quality characteristics; (b) training in methods of aquatic biological data collection, analysis and system modelling. This will enable for simulation of aquatic diversity responses to scenarios of future changes to basin climate and hydrology.

1.2 Previous work

In this project we specifically set out to provide improved baseline information on aquatic organisms, as these are the least well characterised for the Delta (Ramberg et al. 2006). In 2000, *Conservation International* collaborated with the Okavango AquaRAP Team and undertook an extensive survey of fish, invertebrates, plants and birds from 37 geo-referenced sites across the Delta (Alonso & Nordin 2003). They also measured some water chemistry parameters including pH, conductivity, dissolved oxygen and water clarity. This survey was undertaken during a period of high water level throughout most of the Delta. This study was followed up in 2003 by a second survey (AquaRAP II) of 29 geo-referenced sites during low water levels (e.g. Dallas & Mosopele 2007; Murray-Hudson & Heinl in press). These surveys therefore provide useful baselines for the current Darwin programme – indeed, many of the sites selected in these two AquaRAP surveys were revisited to assess longer-term dynamics in biological diversity.

Biological sampling in this Darwin programme:

- (i) significantly builds on previous work by increasing the number of locations sampled throughout the Delta to over 230 geo-referenced sites
- (ii) significantly builds on previous work by increasing the range of biological organisms being investigated. For this current Darwin programme diatoms have been identified, counted and analysed. Samples have also been taken for other algae and zooplankton (see Section 2.2.4)
- (iii) significantly extends environmental gradients found in the Delta and for the first time quantitatively relates these to aquatic diversity
- (iv) takes into account important seasonal differences related to water levels in the Delta

1.3 Project Partnerships

The two main project partners are:

- Department of Geography, University College London
 - <http://www.geog.ucl.ac.uk/>
- Harry Oppenheimer Okavango Research Centre, University of Botswana
 - <http://www.orc.ub.bw>

Other departments and organisations are also involved in the project:

- Department of Biology, University of Botswana: Dr Hillary Masundire is co-supervising the Darwin postgraduate student Richard Mazebedi
- Biokavango: a major GEF funded programme charged with building local capacity for the conservation and sustainable use in biodiversity in the Okavango Delta (www.orc.ub.bw/biokavango)
- IUCN: are co-ordinating a EU FP6 programme on freshwater biodiversity in southern Africa: *Integration of freshwater biodiversity in the development process throughout Africa: mobilising information and site demonstrations. Okavango case study* (<http://www.iucn.org>)

- University of Cape Town (South Africa): Dr Helen Dallas is helping to develop an IBI based on macroinvertebrate data

2. METHODOLOGIES

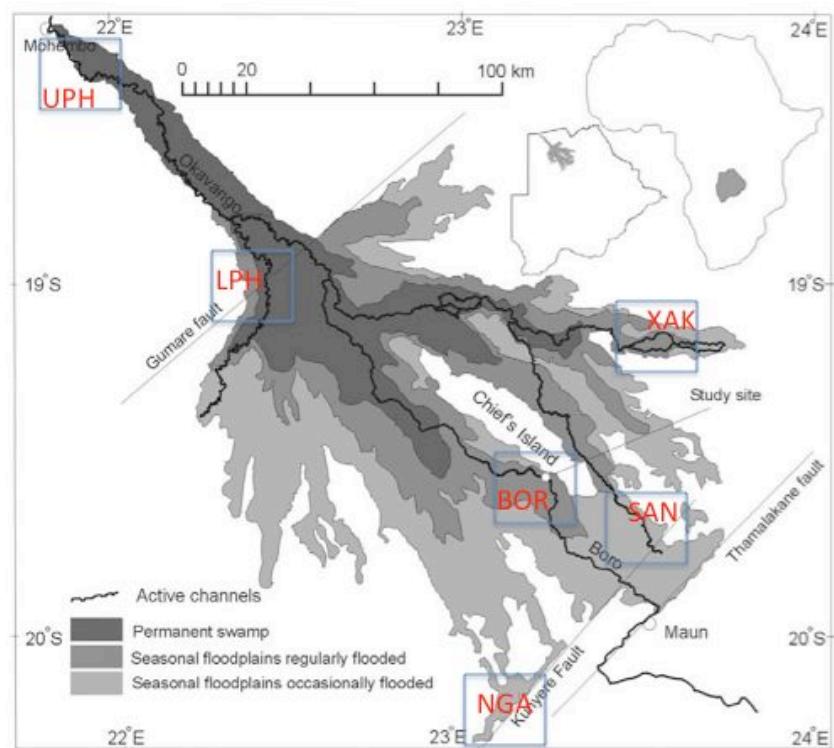
2.1 Site Selection

The main criteria for the selection of sites in our programme was to maximise the diversity of organisms collected with respect to flooding frequency and hydroperiod across the Okavango Delta. The majority of the sites contained a mosaic of vegetation habitats, which are known to influence the communities of other organisms such as macroinvertebrates and diatoms. To incorporate this variation, where relevant 2-3 sites were sampled at key locations. Site selection was based on a number of criteria which had to remain flexible enough to take account of unexpected events in the field. From previous work already carried out on the Delta four regions were identified for sampling according to their hydrological characteristics (e.g. Alonso & Nordin 2003, Dallas & Mosopele 2007):

- The most northerly region (Fig 2) is in the Upper Panhandle (UPH), a region dominated by fluvial input from the Okavango River. There are very few islands or lagoons in the UPH, and the region is considered to have rather low habitat diversity in comparison to the rest of the Delta. Erosional and depositional processes determine vegetation distribution across the UPH. The main channels in the UPH are dominated by *Cyperus papyrus*, *Phragmites mauritianus* and *Vossia cuspidata*. Where flow was reduced in side channels, *Nymphaea nouchali* was abundant.
- Downstream from the UPH is the region known as the Lower Panhandle (LPH), where the Okavango River meanders across the floodplain. The main channel is still fringed by dense permanent swamp, although seasonal swamp becomes more common with increasing distance from the main channel. Several ox-bow lagoons exist where old meanders have become isolated, including Guma Lagoon, one of the largest in the region (Fig 2).
- South of the LPH, the waters of the Okavango River spread out into numerous narrower and shallower channels, which get progressively smaller with distance.

The differentiation of relatively separate distributaries into the characteristic fan-shape of the Delta is due, in the main, to the presence of many islands (Wolski & Murray-Hudson 2005). The main distributary is an extension of the Okavango River and flows to the east, just to the north of the largest island in the Delta, Chief's Island (CI). Principal channels here include Nqoga, Maunachira and Khwai river systems. This region forms part of the extensive Moremi Game Reserve (MGR), one of Botswana's protected National Parks. The Nqoga distributary with its associated channels and lagoons (e.g. Xakanaka Lagoon) and the many isolated seasonally-flooded pools and seasonally rain-fed pools ensures a diverse range of habitats in this region and correspondingly high levels of diversity (Ramberg et al. 2006).

Fig 2: Permanent swamp and seasonal flood extent in the Okavango Delta.



- Several secondary distributaries branch off from the Okavango River, including the Boro, to the west of Chief's Island (Fig 2). This region is also part of the Moremi Game Reserve, and due to the HOORC field station situated on the island, some environmental monitoring has been on-going here since the mid-

1990s. In the channels, *Papyrus* and *Phragmites* are still common, although submergent and emergent species dominate especially in the many lagoons and flooded grasslands. Many of the shallow water lagoons also contain extensive coverings of *Nymphaea* species.

In addition to these four regions, we sampled two regions that are infrequently flooded, as these locations represent extremes of the hydroperiod gradient. As these regions are infrequently flooded, they were only visited once during a period of vry high flood in the south of the Delta in September 2006:

- Lake Ngami sits at the end of the Xudum distributary, and in recent decades very little water has flowed into the lake. In 2004 however, floodwaters from the Xudum entered the lake for the first time since 1998. During our visit in 2006, the lake was also flooded quite extensively.
- Santantadibe is another secondary distributary and it branches off from the Nqoga east of Chief's Island (Fig 2). Although quite a heavily populated region, since the 1990s, flooding extent has declined significantly. In 2006, the area flooded extensively, and locations were visited along the inundated floodplain.

Previous work investigating e.g. macroinvertebrates and aquatic macrophytes in the Delta were carried out to provide a ‘snap-shot’ of conditions, and so fieldwork was carried out in a relatively short time period:

- AquaRap I (high water survey): June 5-22 2000 (18 days)
- AquaRap II (low water survey): Jan 31st – Feb 21st 2003 (22 days)

The Darwin programme spent a total over 60 days in the field, including fieldwork undertaken during both high and low water periods, and times in between.

- Trip 1: 1st – 9th Sept 2006 (9 days)
- Trip 2: 28th Nov – 10th Dec 2006 (13 days)
- Trip 3: 23th Apr – 6th May 2007 (14 days)
- Trip 4: 24th Jul – 7th Aug 2007 (15 days)
- Trip 5: 2nd Oct – 12th Oct 2007 (11 days)

Over the course of the Darwin programme, over 120 locations were visited during the five fieldtrips and samples collected from over 230 sites. Sites visited in the UPH are prefixed with SHA because of Shakawe, the main settlement in the region. Sites visited in LPH are prefixed with GUM because of the main lagoon in the region, Guma Lagoon. Sites visited in the MGR are prefixed with XAK, because of the large Xakanaka Lagoon. Sites visited around the Boro and on Chiefs Island are prefixed with BOR. Sites visited around Santantadibe are prefixed SAN, and those visited at Lake Ngami, NGA. Every site visited was geo-referenced using a Garmin eTrex summit GPS navigator (Appendix 1).

2.2 Collection of biological datasets

2.2.1 Macroinvertebrates

At every site, macroinvertebrates were sampled (“scooped”) using a 30 x 30 cm net for exactly 2 minutes (Fig 3). After removal of leaves and other large bits of vegetation from the net, a preliminary assessment of the main families was made on board the boat. The sample was then placed into a labelled plastic container and ethanol added to fix organisms. Each sample was then kept in the dark and transported back to HOORC laboratory for analyses.

2.2.2 Epiphytic diatoms:

Epiphyte diatom sampling protocol was similar to that used in other monitoring studies (e.g. the UK Acid Waters Monitoring Network). Epiphytic diatoms form a brown/green slime on submerged stems of aquatic macrophytes (Fig 4). 5 submerged stems of the dominant macrophyte species characterising each location were selected. 5 cm sections were cut from the desired plant c. 20 cm below the water surface, and placed in a labelled tube. Both dead stems and new shoots were avoided where possible. Plant selection was partly determined by the habitat being sampled. For example, in habitats with marginal vegetation, only edge plants from emergent species were selected. In open water habitats, stems from floating vegetation were sampled. The sampled stems were stored in a dark, cold icebox until they could be fixed with c. 5 mls of ethanol. Samples were subsequently transported back to UCL for diatom slide preparation and identification (see section 2.5).

Figure 3: Richard Mazebedi collecting macroinvertebrates from Santantidibe, Okavango Delta.



Figure 4: Diatoms present on submerged stems of *Papyrus*.

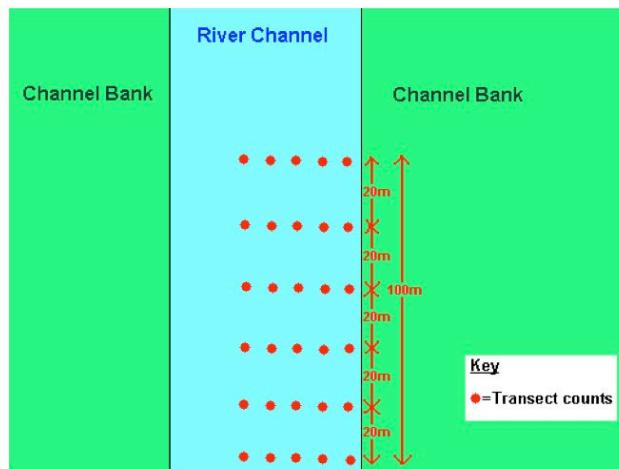


2.2.3 Macrophytes

Aquatic macrophytes were investigated mainly during Trip 3 in the UPH, and at sites around the MGR (Xakanaka and Boro regions). Sites in the LPH could not be sampled due to the very high water levels preventing access to Guma Lagoon. At each sample site, aquatic macrophytes were measured using a number of transects (Fig 5). Initially, a 100 m long transect was established running along the riparian zone. At 20 m intervals emergent, floating and riparian plant species were recorded through observation, and abundance scores were given, ranging from 1-5 (1 being rare and 5 being dominant). At each 20 m interval another transect was extended perpendicularly towards the main channel. Four separate counts were made along each perpendicular

transect increasing in distance from the channel bank until the last recording point was in the middle of the channel. Distances between the 4 intervals were proportionate to the width of the channel at each specific sample site.

Figure 5. Diagram showing sampling protocol for aquatic macrophytes.



In order to accurately record submerged aquatic macrophytes a bathoscope was used (Fig 6). However, in turbid waters when an observation could not be made, a rake sample was also taken. Macrophytes were identified to species level when possibly according to Ellery (1997) and Cook (2004). Samples of unidentified species were pressed, and later identified at both HOORC and UCL laboratories. Water depth (measured to the nearest 10 cm) and total plant biomass (estimated using a 0-5 scale) were recorded at each count in each transect.

Figure 6: Tom Davidson identifying submerged macrophytes in the Boro region of the Okavango Delta, Botswana.



2.2.4 Sampling for other aquatic organisms

While undertaking sampling of sites, we also took the opportunity to sample for other aquatic organisms which were not originally built into the Darwin proposal, but which we knew we could archive for later studies, should the opportunity arise. This extra sampling was done at no extra cost to the Programme.

Phytoplankton

Phytoplankton samples were collected in 1.5 litre bottles at the same sites as water chemistry (see Section 2.3.4 below). Samples were stored in a dark cool-box whilst out in the field. Each night, the sample bottles were left upright so that algal material would settle overnight. In the morning, excess water was carefully poured out until c. 20 mls remained. This small sample containing phytoplankton was then stored into a labelled tube and a few drops of Lugol's iodine added for preservation. These samples were then transported back to UCL and are currently archived.

Zooplankton

Samples were collected using a 2 m tube of 7 cm diameter to take an integrated sample of the water column in deeper water (Fig 7). In the shallow water of flood plains the tube was placed at an angle into the water to collect the entire water column but to maximise the volume of water collected. Care was taken to avoid the debris and vegetation. A number of samples were taken per location (up to 6) to account for spatial heterogeneity common in zooplankton communities, these samples were pooled by pouring them through a 50 micron net. The pooled samples were washed into a sample bottle and preserved to >50% IMS. These samples were then transported back to UCL and are currently archived.

2.3 Collection of explanatory variable datasets

Three types of explanatory variables were collated in our study:

- (i) location and habitat characteristics of aquatic organisms
- (ii) hydrological characteristics of said sites
- (iii) water chemistry characteristics of main locations

Figure 7. Florah Joshua and Rebaone Moshongo sampling the Okavango River for zooplankton



2.3.1 Location and habitat variables

Every site visited was geo-referenced and coded according to the delta region, e.g. UPH, XAK etc (Appendix 1). Water depth (cm) was measured using a *Plastimo Echotest II* handheld depth sounder. In locations with dense submerged vegetation water depths were instead measured using a simple graduated metal pole. Velocity of water flow (m/s) at each site was 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. This instrument was kindly loaned to the Darwin project by Wolski.

The dominant habitat of each location was also recorded. The vegetation and hydrology in the delta are inextricably linked, so that permanently wet regions and seasonally inundated regions are botanically distinct, and therefore have a major influence on e.g. macroinvertebrate and diatom communities. Previous investigations on the Delta have recognised several distinct habitat types within the Delta associated with episodic to permanent flooding (Aquarap1 and 2; Dallas & Mosepele 2007). Relevant habitats characterised in this study include:

- Marginal vegetation in channels (MV-IC)
- Marginal vegetation in lagoons (MV-L)
- Marginal vegetation in backwaters (MV-B)
- Inundated floodplains (IF)
- Floating vegetation (FV)

- Instream / submerged vegetation (IV)
- Marginal vegetation in lakes (MV-L)
- Isolated, seasonally flooded pools (SP)
- Isolated temporary rain-filled pools (TP)

2.3.2 Hydrological variables

The second group of explanatory variables relate to hydrological characteristics of each location with respect to flood frequency, hydroperiod and amplitude of annual water level fluctuations. Inundation in the Okavango Delta displays strong variability at seasonal (annual flood event) and inter-annual (year to year variation in size of flood; sequences of low or high flood years) scales. Additionally, there is a strong spatial variability in hydrological responses resulting from e.g. its flat topography and vegetation-controlled channel-floodplain interactions. For example, the amplitude of channel water level fluctuations changes by 3 m in the upstream locations, 0.15 m in mid Delta, 0.8 m in its distal parts, and by 2.5 m in the Thamalakane River draining the system to the south. Consequently, extrapolation of hydrological responses from a limited network of hydrometric stations (there are only 35 within the 12000 km² of the Delta) is not possible without additional data. A set of inundation maps was therefore used to aid determination of hydroperiod conditions at sampling sites.

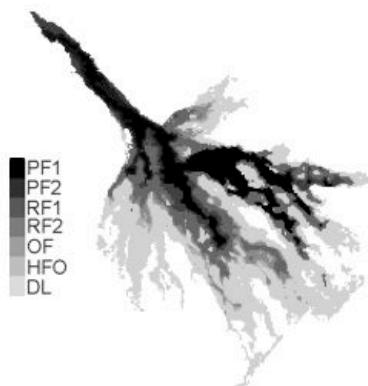
The set comprises 16 inundation maps depicting maximum annual inundation extent during years 1989-2007 (excluding 1991 and 2003). The maps were obtained from Landsat (5 and ETM) images, using a classification procedure involving spectral clustering and contextual classification (Wolski & Murray-Hudson, 2005). To depict the Okavango Delta, four Landsat scenes had to be merged for each coverage. Maps had spatial resolution of 30 by 30 m, and 97% accuracy of determination of inundation extent.

Number of years inundation occurred was calculated for the entire Delta on a pixel-by-pixel basis by stacking up all individual maps. Inundation frequency was then obtained by dividing number of years with inundation by the total number of flood maps. Inundation frequency for the project's sampling sites was obtained by averaging inundation frequency from the nine pixels corresponding to the surrounding

of the sampling point. This was done to reduce possible influence of errors resulting from image misclassification and misregistration.

Figure 8. Flood classes calculated across the Okavango Delta.

Floodplain class	Sub-class	flood frequency	flood duration (months/year)
Permanent floodplain	proper	PF1	1
	fringe	PF2	1 8-12
Regularly flooded seasonal floodplain	RF1	1	4 - 8
	RF2	0.5-1	
Occasionally flooded seasonal	OF	0.1-0.5	1-4
	HFO	<0.1	<2
Dryland	DL	0	0



Inundation frequency in the Okavango Delta can be considered as a proxy for mean duration of inundation: i.e. hydroperiod. However, a relationship between the amplitude of annual water level fluctuations and flood frequency is different for different parts of the system. Available hydrometric data were not enough to formalize these relationships in strict mathematical form. Instead, a set of hydroperiod/amplitude classes was defined, and a class was attached to each of the project sampling sites. The classification was based on flood frequency maps, hydrometric data from hydrometric stations in the vicinity of sampling points, and on expert assessment of hydrological conditions at the sampling points (Fig 8).

2.3.3 Water chemistry

The third group of explanatory variables consists of a variety of chemical variables measured both in the field and at the HOORC laboratory from water samples

collected from each location. Logistically (laboratory resources and time), not every site could be analysed for water chemistry, so one site from each location was chosen for analyses. At each site two water samples were collected. One 2 litre sample bottle was rinsed thoroughly three times before water samples were collected and stored in a cool-box with ice whilst on board boat. A second water sample was collected in an acid-washed 500 ml bottle and stored in the cool box.

Chemical variables measured in situ at each location were pH, conductivity ($\mu\text{S}/\text{s}$) and dissolved oxygen (DO) (mg/l). pH and conductivity were measured using a portable *Fisher Scientific accumet* AP85 portable waterproof pH/conductivity meter, bought specifically for HOORC with Darwin funds. A *YSI 550* dissolved oxygen instrument was used to measure DO and water temperature ($^{\circ}\text{C}$) simultaneously. This instrument was also bought specifically for HOORC using Darwin funds.

Total Dissolved Solids (TDS)

Before setting off for fieldwork, GF/C 47mm filter papers were dried at 105°C for an hour, weighed using a *Mettler Toledo* (model AG204) four-digit balance, and weight of the filter paper recorded on the filter itself. At field camp each evening, 1 litre of water from each 2l bottle sampled for water chemistry was filtered through one of the pre-weighed filter papers. The filter papers were then placed into separately labelled Whirlpak bags and stored in a cool-box before being transported back to the HOORC laboratory for analyses.

Chlorophyll A (ChlA)

At field camp each evening, 1 litre of water was filtered through a GF/C 47mm filter paper. Filter papers were wrapped in aluminium foil and labelled well before being put into separately labelled Whirlpak bags. These samples were stored in a cool-box with ice-blocks before being transported to HOORC where they were kept refrigerated prior to analyses. Importantly, the filtrate was kept for further analyses – see below.

Anions (CO_3 , HCO_3 , Cl , NO_3 , SO_4 , PO_4 , DOC)

250ml of the water already filtered through a GF/C 47mm filter paper for ChlA was collected into rinsed (de-ionised), acid-washed 250 ml bottles. Bottles were labelled

and stored in a cool-box and transported to HOORC where they were refrigerated prior to analyses.

Cations (Na, K, Mg, Ca, Fe)

After collection of water samples from sampling sites in the field, 250ml of water samples was filtered using cellulose nitrate filter papers and the filtered samples were stored in acid washed plastic sample bottles. Immediately after filtration the samples were acidified with three drops of concentrated HNO₃. The water samples were labelled and stored in a cool box until reaching the lab where the samples were stored in a refrigerator until analysis.

Silica

After collection of water samples from sampling sites in the field, 250ml of water samples was filtered using cellulose nitrate filter papers and the filtered samples were stored in acid washed plastic sample bottles.

2.4 Laboratory analyses of water chemistry variables

Cations (Na, K, Mg, Ca, Fe)

Determination of Mg, Ca and Fe was undertaken by flame atomic absorption spectrometry using a *Varion Spectra 220* instrument. The gas used was a combination of nitrous oxide or air and acetylene. PERADE-120 (lot number 106) made from South African supplier: Industrial Analytical (Pty) LTD was used as a reference material. Lanthanum was used as a releasing agent to reduce interferences during calcium analysis. Analytical procedures were followed as described in part 3111 of Standard Methods by Eaton *et al.* (1995). Sodium (Na) and potassium (K) were determined by flame photometry using a *Sherwood Flame Photometer 410* instrument. Propane gas was used in this analysis.

Anions (CO₃, HCO₃, Cl, NO₃, SO₄, PO₄)

The anions were analysed by ion chromatography using a *DX-120* ion chromatograph. The instrument's pump pressure and total conductivity was set at 1728 psi and 19.878 µs respectively. The eluent concentration was 3.5mM NaCO₃ plus 1.0 mM NaHCO₃.

and the flow rate was set at 1.20mL/min. A series of guard and analytical columns were used, guard columns protect the analytical columns from humics and the analytical columns transport the analyte through the analyser. Guard column (AG14 guard column) and analytical column (AS14 analytical column) were 4 x 50mm and 4 x 250mm in length respectively. The sample loop size was 1000 μ l. The reference material used was QCI-051 anions from South African supplier: Industrial Analytical (Pty) LTD. The procedure followed in this analysis is same as described in part 4000 (Eaton et al. 1995).

DOC

An approximation of DOC was obtained by measuring the absorbance of the water samples at 280 nm using a *Perkin Elmer Lambda 20* UV/Vis spectro-photometer. Some organic compounds found in water such as lignin, tannin, humic substances and some aromatic compounds absorb ultraviolet radiation. The UV absorption is a useful approximate measure of DOC in the water of the Okavango Delta and absorbance at 280 nm in surface water has been found to give the best correlation ($r = 0.97$; Mladenov et al., 2005) for water samples from the Okavango Delta.

Determination of total phosphates (TP) and total nitrogen (TN)

Total phosphates (TP) and total nitrates (TN) were analysed by ion chromatography using a *BRAN + LUEBBE Auto analyser 3* (AA3). For determination of TP AA3 method number G.-179-96B of the BRAN + LUEBBE analyser was followed. Prior to AA3 analyses the following reagents were prepared and loaded in the instrument: a digestion mixture was made by adding 25 ml of concentrated sulphuric acid into 500ml of de-ionised (DI) water and then dissolving 35 g and 1 g of ascorbic acid and sodium dodecyl sulphate (SDS) respectively into the solution. Molybdate was prepared by adding 200ml of concentrated sulphuric acid to 25 ml of stock antimony potassium tartrate. The mixture was then diluted with 500 ml DI water and stored in a dark place. When all reagents were prepared and loaded into the AA3 instrument reagent lines were placed into their respective containers.

Before analyzing water samples, five standard solutions, 5ml each, were put in 5ml cuvettes and loaded in the AA3 instrument and used to calibrate the instrument. The instrument was set to suck from each cuvette for 90 seconds, which corresponds to

1ml. After calibration, water samples were put in 5 ml cuvettes and loaded into the instrument and analysed. A reference material was loaded into the instrument after analysis of every ten water samples. The reference material used was QCI-028-2 lot 011314 from South African supplier: Industrial Analytical (Pty) LTD.

To analyse for TN method number G-218-98 Rev.1 (Multitest MT23) of the *BRAN + LUEBBE analyzer* was employed. The procedure for TN analysis and the instrument set-up is similar to that of TP analysis except for different reagents used. Before any analyses were undertaken levels of reagents in the instrument were checked and where necessary the reagents were prepared and refilled into the instrument. The reagents used in TN analysis are as follows: The wetting reagent was prepared by adding 30 ml of 30% Brij-35 solution to 200 ml of DI water and mixing thoroughly. The digestion mixture was made by dissolving 1.2 g of sodium hydroxide, 0.6 g of boric acid and 2.0 g of potassium persulphate in 175 ml DI water. The solution was then diluted to 250 ml and used within two weeks. Colour reagent was prepared by adding 30 ml concentrated hydrochloric acid and 2 g of sulfanilamide to 140ml DI water and dissolving completely. 0.1g of N-1-Naphthylenediamine dihydrochloride was then added to the solution and dissolved before diluting the solution to 200ml with DI water. Ammonium chloride solution was prepared by dissolving 10g ammonium chloride in 180 ml of DI water. The pH was adjusted to 8.5 with ammonia solution before diluting the solution to 200 ml with DI water. 0.2 ml Brij-35 solution was added and the solution was mixed thoroughly.

For some chemical variables analysed, there is not a complete dataset for each of the 5 fieldtrips. Relationships between species and selected chemistry are therefore assessed on a season-by-season basis, on the basis of data available (Table 1).

Table 1: chemical variables successfully analysed during each of the five field trips

Sep 06:	carbonates, alkalinity, TOC, Na, K, Fe, ChlA, SiO ₂
Dec 06:	carbonates, alkalinity, DOC, Na, K, Mg, Ca, Cl, NO ₃ , SO ₄ , PO ₄ , TN, TP, Fe, ChlA, SiO ₂
May 07:	carbonates, alkalinity, DOC, TDS, Na, K, Mg, Ca, Cl, NO ₃ , SO ₄ , PO ₄ , TN, TP, Fe, ChlA, SiO ₂
Jul 07:	carbonates, alkalinity, DOC, TDS, Na, K, Mg, Ca, Cl, NO ₃ , SO ₄ , PO ₄ , TN, TP, Fe, ChlA, SiO ₂
Oct 07:	carbonates, alkalinity, DOC, TDS, Na, K, Mg, Ca, F, Cl, NO ₃ , SO ₄ , PO ₄ , TN, TP, Fe, ChlA, SiO ₂

2.5 Laboratory diatom preparations

At UCL, sub-samples of plant stems (c. 1.5 cm) were put into a test tube. 5 mls of an oxidising agent (30% H₂O₂) was added and then put into a heated water-bath at 60 °C for c. 2-4 hours. Samples were then left overnight and fresh H₂O₂ with further heating if necessary. Much of the stem material by this time (after a day or two) was visibly bleached. A few drops of HCl (50%) were then added to remove any excess H₂O₂ and carbonates. After washing in distilled water, samples were centrifuged at 1200 rpm for 4 minutes and the supernatant decanted off. Washing was repeated three more times (so four times in total). Permanent diatom slides were made up by allowing the diatom suspension to settle out on a cover slip overnight, as described below. This produces an even spread of diatoms over the cover slip but it can take up to two days.

Initially, cleaned diatom suspensions were diluted to a suitable concentration. The suspension should look neither totally clear or milky. Fine particles in suspension should be just visible when the suspension is held up to the light. Metal settling out trays with cover slips were placed in a position where they will not be disturbed, away from dust sources and air currents. Using the 1 ml pipette, 0.5ml of well-mixed diatom suspension was placed on each cover slip and left to dry. The tray should be covered to keep off dust. Drying may take up to two days. Once dry, 1 drop of *Naphrax* (refractive index 1.73) was placed onto a heated glass slide (heated on a hot-plate at 130 °C) and the cover slip with the dried diatoms inverted over the drop. The slides were then heated on the hotplate at c. 100 °C for 15 minutes to drive off the

toluene in the *Naphrax*. Slides were allowed to cool. To check that the cover slips were fixed properly, they were pushed with a fingernail. If the coverslip moved, then they were heated for a little longer.

All diatoms were counted using oil immersion phase contrast light microscopy at x1000 magnification. At least 350-400 valves were counted for each sample, with taxa identified according to published papers and books (e.g. Camburn & Charles 2000; Cholnoky 1966; Gasse 1986; Krammer 2000; Krammer and Lange-Bertalot, 1991a; Krammer and Lange-Bertalot, 1991b; Krammer and Lange-Bertalot, 1999a; Krammer, K., Lange-Bertalot, H., 1999b; Lange-Bertalot 2001; Sonneman et al. 1996). Only one of these (Cholnoky 1966) has looked at diatoms specifically from the Okavango Delta.

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APPENDIX 1: list of geo-referenced sites visited during this programme. Sites arranged along a N-S gradient

site	location	Coordinates	coordinates
DSHA6-3	Shakawe (UPH)	S18.274880	E021.780190
DSHA1B-1	Shakawe (UPH)	S18.339083	E021.837138
DSHA1B-2	Shakawe (UPH)	S18.339083	E021.837138
DSHA1B-3	Shakawe (UPH)	S18.339083	E021.837138
DSHA1B-4	Shakawe (UPH)	S18.339083	E021.837138
DSHA1C-1	Shakawe (UPH)	S18.340445	E021.836097
DSHA1C-2	Shakawe (UPH)	S18.340445	E021.836097
DSHA1C-3	Shakawe (UPH)	S18.340445	E021.836097
DSHA1C-4	Shakawe (UPH)	S18.340445	E021.836097
DSHA2-1	Shakawe (UPH)	S18.405812	E021.885823
DSHA3B-1	Shakawe (UPH)	S18.405818	E021.885450
DSHA3B-3	Shakawe (UPH)	S18.405818	E021.885450
DSHA3A-1	Shakawe (UPH)	S18.409695	E021.886945
DSHA3A-3	Shakawe (UPH)	S18.409695	E021.886945
DSHA4A-1	Shakawe (UPH)	S18.410927	E021.881083
DSHA4A-2	Shakawe (UPH)	S18.410927	E021.881083
DSHA4A-3	Shakawe (UPH)	S18.410927	E021.881083
DSHA4A-4	Shakawe (UPH)	S18.410927	E021.881083
DSHA4B-1	Shakawe (UPH)	S18.411170	E021.880840
DSHA4B-3	Shakawe (UPH)	S18.411170	E021.880840
DSHA8-3	Shakawe (UPH)	S18.411833	E021.889112
DSHA8-4	Shakawe (UPH)	S18.411833	E021.889112
DROTSK	Drotski's Cabins	S18.414063	E021.885477
DSHA7B-3	Shakawe (UPH)	S18.426806	E021.971444
DSHA7B-4	Shakawe (UPH)	S18.426806	E021.971444
DSHA7A-3	Shakawe (UPH)	S18.426972	E021.971389
DSHA7A-4	Shakawe (UPH)	S18.426972	E021.971389
DSHA7C-3	Shakawe (UPH)	S18.427134	E021.970752
DSHA7C-4	Shakawe (UPH)	S18.427134	E021.970752
DSHA5B-1	Shakawe (UPH)	S18.427472	E021.981195
DSHA5B-2	Shakawe (UPH)	S18.427472	E021.981195
DSHA5B-3	Shakawe (UPH)	S18.427472	E021.981195
DSHA5B-4	Shakawe (UPH)	S18.427472	E021.981195
DSHA5A-1	Shakawe (UPH)	S18.427485	E021.981188
DSHA5A-2	Shakawe (UPH)	S18.427485	E021.981188
DSHA5A-3	Shakawe (UPH)	S18.427485	E021.981188
DSHA5A-4	Shakawe (UPH)	S18.427485	E021.981188
DSHA5C-1	Shakawe (UPH)	S18.427740	E021.980670
DSHA5C-2	Shakawe (UPH)	S18.427740	E021.980670
DSHA5C-3	Shakawe (UPH)	S18.427740	E021.980670
DSHA5C-4	Shakawe (UPH)	S18.427740	E021.980670
DSHA9B-3	Shakawe (UPH)	S18.428200	E021.919230
DSHA9A-3	Shakawe (UPH)	S18.428403	E021.919382
DSHA9C-3	Shakawe (UPH)	S18.429220	E021.918910

DGUM1	Guma (LPH)	S18.842305	E022.403612
DGUM1C-1	Guma (LPH)	S18.842512	E022.404298
DGUM1C-2	Guma (LPH)	S18.842512	E022.404298
DGUM1B-1	Guma (LPH)	S18.842858	E022.404603
DGUM1B-2	Guma (LPH)	S18.842858	E022.404603
DGUM1A-1	Guma (LPH)	S18.843205	E022.404515
DGUM1A-2	Guma (LPH)	S18.843205	E022.404515
DGUM8B-4	Guma (LPH)	S18.865278	E022.418812
DGUM8A-4	Guma (LPH)	S18.865341	E022.419814
DGUM3B-1	Guma (LPH)	S18.878745	E022.391268
DGUM3B-2	Guma (LPH)	S18.878745	E022.391268
DGUM3B-4	Guma (LPH)	S18.878745	E022.391268
DGUM3B-5	Guma (LPH)	S18.878745	E022.391268
DGUM3-1	Guma (LPH)	S18.879028	E022.391055
DGUM3A-1	Guma (LPH)	S18.879032	E022.391048
DGUM3A-2	Guma (LPH)	S18.879032	E022.391048
DGUM3A-4	Guma (LPH)	S18.879032	E022.391048
DGUM3A-5	Guma (LPH)	S18.879032	E022.391048
DGUM2-1	Guma (LPH)	S18.879112	E022.391195
DGUM2-2	Guma (LPH)	S18.879112	E022.391195
DGUM2-4	Guma (LPH)	S18.879112	E022.391195
DGUM2-5	Guma (LPH)	S18.879112	E022.391195
DGUM6-1	Guma (LPH)	S18.917593	E022.409447
DGUM6-4	Guma (LPH)	S18.917593	E022.409447
DGUM6-5	Guma (LPH)	S18.917593	E022.409447
DGUM10-5	Guma (LPH)	S18.935230	E022.408940
DGUM4C-1	Guma (LPH)	S18.955450	E022.376333
DGUM4C-2	Guma (LPH)	S18.955450	E022.376333
DGUM4C-4	Guma (LPH)	S18.955450	E022.376333
DGUM4C-5	Guma (LPH)	S18.955450	E022.376333
DGUM4A-1	Guma (LPH)	S18.956098	E022.377158
DGUM4A-2	Guma (LPH)	S18.956098	E022.377158
DGUM4A-4	Guma (LPH)	S18.956098	E022.377158
DGUM4A-5	Guma (LPH)	S18.956098	E022.377158
DGUM4B-1	Guma (LPH)	S18.956482	E022.376987
DGUM4B-2	Guma (LPH)	S18.956482	E022.376987
DGUM4B-4	Guma (LPH)	S18.956482	E022.376987
DGUM4B-5	Guma (LPH)	S18.956482	E022.376987
DGUM4	Guma (LPH)	S18.956695	E022.377250
DGUM5B-1	Guma (LPH)	S18.959685	E022.383172
DGUM5B-2	Guma (LPH)	S18.959685	E022.383172
DGUM5B-4	Guma (LPH)	S18.959685	E022.383172
DGUM5B-5	Guma (LPH)	S18.959685	E022.383172
DGUM5A-1	Guma (LPH)	S18.960190	E022.382820
DGUM5A-2	Guma (LPH)	S18.960190	E022.382820
DGUM5A-4	Guma (LPH)	S18.960190	E022.382820
DGUM5A-5	Guma (LPH)	S18.960190	E022.382820
DGUM5C-1	Guma (LPH)	S18.960588	E022.382635
DGUM5C-2	Guma (LPH)	S18.960588	E022.382635
DGUM5C-4	Guma (LPH)	S18.960588	E022.382635
DGUM5C-5	Guma (LPH)	S18.960588	E022.382635

DGUM9A-4	Guma (LPH)	S18.961050	E022.406857
DGUM9B-4	Guma (LPH)	S18.961050	E022.406857
DGUMLOD-1	Guma (LPH)	S18.962600	E022.373920
DXAK10A-2	Xakanaxa (Moremi G.R)	S19.125260	E023.379063
DXAK10B-2	Xakanaxa (Moremi G.R)	S19.125692	E023.379853
DXAK2A-2	Xakanaxa (Moremi G.R)	S19.145432	E023.383543
DXAK2C-2	Xakanaxa (Moremi G.R)	S19.146020	E023.383210
DXAK2B-2	Xakanaxa (Moremi G.R)	S19.146037	E023.383598
DXAK16-3	Xakanaxa (Moremi G.R)	S19.172583	E023.440806
DXAK4A-2	Xakanaxa (Moremi G.R)	S19.175258	E023.419887
DXAK4A-3	Xakanaxa (Moremi G.R)	S19.175258	E023.419887
DXAK4A-5	Xakanaxa (Moremi G.R)	S19.175258	E023.419887
DXAK4B-2	Xakanaxa (Moremi G.R)	S19.175332	E023.419895
DXAK4B-3	Xakanaxa (Moremi G.R)	S19.175332	E023.419895
DXAK4B-5	Xakanaxa (Moremi G.R)	S19.175332	E023.419895
DXAK15-3	Xakanaxa (Moremi G.R)	S19.177250	E023.438028
DXAK1B-2	Xakanaxa (Moremi G.R)	S19.182738	E023.398312
DXAK1B-3	Xakanaxa (Moremi G.R)	S19.182738	E023.398312
DXAK1C-2	Xakanaxa (Moremi G.R)	S19.182997	E023.397405
DXAK1C-3	Xakanaxa (Moremi G.R)	S19.182997	E023.397405
DXAK14-3	Xakanaxa (Moremi G.R)	S19.183000	E023.440861
DXAK1A-2	Xakanaxa (Moremi G.R)	S19.183048	E023.397720
DXAK1A-3	Xakanaxa (Moremi G.R)	S19.183048	E023.397720
DXAK3B-2	Xakanaxa (Moremi G.R)	S19.186472	E023.396075
DXAK3A-2	Xakanaxa (Moremi G.R)	S19.186622	E023.396618
DXAK5B-2	Xakanaxa (Moremi G.R)	S19.187408	E023.434062
DXAK5A-2	Xakanaxa (Moremi G.R)	S19.187812	E023.433718
DXAK5A-4	Xakanaxa (Moremi G.R)	S19.187812	E023.433718
DXAK5A-5	Xakanaxa (Moremi G.R)	S19.187812	E023.433718
DXAK7-2	Xakanaxa (Moremi G.R)	S19.188850	E023.452418
DXAK7-3	Xakanaxa (Moremi G.R)	S19.188850	E023.452418
DXAK7-4	Xakanaxa (Moremi G.R)	S19.188850	E023.452418
DXAK13-3	Xakanaxa (Moremi G.R)	S19.191445	E023.451972
DXAK13-4	Xakanaxa (Moremi G.R)	S19.191445	E023.451972
DXAK13-5	Xakanaxa (Moremi G.R)	S19.191445	E023.451972
DXAK6A-2	Xakanaxa (Moremi G.R)	S19.191927	E023.432120
DXAK6B-2	Xakanaxa (Moremi G.R)	S19.192233	E023.433820
DXAK6-5	Xakanaxa (Moremi G.R)	S19.192253	E023.432630
DXAK18-4	Xakanaxa (Moremi G.R)	S19.195720	E023.441300
DXAK9-2	Xakanaxa (Moremi G.R)	S19.201925	E023.460653
DXAK9-4	Xakanaxa (Moremi G.R)	S19.201925	E023.460653
DXAK8-2	Xakanaxa (Moremi G.R)	S19.202530	E023.460793
DXAK11-2	Xakanaxa (Moremi G.R)	S19.213987	E023.385062
DXAK12-2	Xakanaxa (Moremi G.R)	S19.240080	E023.356937
DXAK12-3	Xakanaxa (Moremi G.R)	S19.240080	E023.356937
DXAK12-4	Xakanaxa (Moremi G.R)	S19.240080	E023.356937
DXAK12-5	Xakanaxa (Moremi G.R)	S19.240080	E023.356937
DBOR14A-4	Nxaraga (boro)	S19.526833	E023.150667
DBOR14A-3	Nxaraga (boro)	S19.526901	E023.150616
DBOR14A-5	Nxaraga (boro)	S19.526901	E023.150616
DBOR14B-3	Nxaraga (boro)	S19.527056	E023.150710

DBOR14B-5	Nxaraga (boro)	S19.527056	E023.150710
DBOR11-2	Nxaraga (boro)	S19.528937	E023.182492
DBOR10B-3	Nxaraga (boro)	S19.533310	E023.182860
DBOR10A-2	Nxaraga (boro)	S19.533420	E023.183143
DBOR10A-3	Nxaraga (boro)	S19.533420	E023.183143
DBOR10A-4	Nxaraga (boro)	S19.533420	E023.183143
DBOR10A-5	Nxaraga (boro)	S19.533420	E023.183143
DBOR12-2	Nxaraga (boro)	S19.533640	E023.183285
DBOR5-2	Nxaraga (boro)	S19.534358	E023.194837
DBOR15-3	Nxaraga (boro)	S19.538028	E023.184139
DBOR8A-2	Nxaraga (boro)	S19.538495	E023.088402
DBOR8A-3	Nxaraga (boro)	S19.538495	E023.088402
DBOR8A-4	Nxaraga (boro)	S19.538495	E023.088402
DBOR13C-3	Nxaraga (boro)	S19.538520	E023.118130
DBOR13C-5	Nxaraga (boro)	S19.538520	E023.118130
DBOR17A-4	Nxaraga (boro)	S19.538711	E023.114553
DBOR17A-5	Nxaraga (boro)	S19.538711	E023.114553
DBOR17B-4	Nxaraga (boro)	S19.538971	E023.113325
DBOR17B-5	Nxaraga (boro)	S19.538971	E023.113325
DBOR6B-2	Nxaraga (boro)	S19.539210	E023.047585
DBOR13B-3	Nxaraga (boro)	S19.539360	E023.118610
DBOR13B-5	Nxaraga (boro)	S19.539360	E023.118610
DBOR6C-2	Nxaraga (boro)	S19.539410	E023.047362
DBOR13A-3	Nxaraga (boro)	S19.539528	E023.118667
DBOR13A-5	Nxaraga (boro)	S19.539528	E023.118667
DBOR8B-2	Nxaraga (boro)	S19.539652	E023.089635
DBOR8B-3	Nxaraga (boro)	S19.539652	E023.089635
DBOR8B-4	Nxaraga (boro)	S19.539652	E023.089635
DBOR6A-2	Nxaraga (boro)	S19.540635	E023.048277
DBOR7B-2	Nxaraga (boro)	S19.543710	E023.047797
DBOR20A-5	Nxaraga (boro)	S19.545420	E023.184670
DBOR20B-5	Nxaraga (boro)	S19.545420	E023.184670
DBOR19-4	Nxaraga (boro)	S19.546492	E023.186723
DBOR19-5	Nxaraga (boro)	S19.546492	E023.186723
DBOR7A-2	Nxaraga (boro)	S19.546840	E023.044735
DBOR9A-2	Nxaraga (boro)	S19.549053	E023.177108
DBOR9A-3	Nxaraga (boro)	S19.549053	E023.177108
DBOR9A-4	Nxaraga (boro)	S19.549053	E023.177108
DBOR9A-5	Nxaraga (boro)	S19.549053	E023.177108
DBOR9B-2	Nxaraga (boro)	S19.549433	E023.177520
DBOR9B-3	Nxaraga (boro)	S19.549433	E023.177520
DBOR9B-4	Nxaraga (boro)	S19.549433	E023.177520
DBOR9B-5	Nxaraga (boro)	S19.549433	E023.177520
DBOR9C-5	Nxaraga (boro)	S19.549433	E023.177520
DBOR4A-2	Nxaraga (boro)	S19.549895	E023.179033
DBOR4C-2	Nxaraga (boro)	S19.550012	E023.179417
DBOR4B-2	Nxaraga (boro)	S19.550232	E023.179460
DBOR3-2	Nxaraga (boro)	S19.555828	E023.200225
DBOR2C-2	Nxaraga (boro)	S19.565955	E023.203215
DBOR2C-3	Nxaraga (boro)	S19.565955	E023.203215
DBOR2C-4	Nxaraga (boro)	S19.565955	E023.203215

DBOR2C-5	Nxaraga (boro)	S19.565955	E023.203215
DBOR2B-2	Nxaraga (boro)	S19.566605	E023.204318
DBOR2B-3	Nxaraga (boro)	S19.566605	E023.204318
DBOR2B-4	Nxaraga (boro)	S19.566605	E023.204318
DBOR2B-5	Nxaraga (boro)	S19.566605	E023.204318
DBOR2A-2	Nxaraga (boro)	S19.567102	E023.204587
DBOR2A-3	Nxaraga (boro)	S19.567102	E023.204587
DBOR2A-4	Nxaraga (boro)	S19.567102	E023.204587
DBOR2A-5	Nxaraga (boro)	S19.567102	E023.204587
DBOR18-4	Nxaraga (boro)	S19.570031	E023.204447
DBOR18A	Nxaraga (boro)	S19.570424	E023.204428
DBOR16-3	Nxaraga (boro)	S19.575889	E023.201583
DBOR1C-2	Nxaraga (boro)	S19.611612	E023.218820
DBOR1C-2	Nxaraga (boro)	S19.611612	E023.218820
DBOR1B-1	Nxaraga (boro)	S19.611627	E023.218565
DBOR1B-2	Nxaraga (boro)	S19.611627	E023.218565
DBOR1A-1	Nxaraga (boro)	S19.612012	E023.218685
DBOR1A-2	Nxaraga (boro)	S19.612012	E023.218685
DSAN4-1	Santantadibe	S19.622978	E023.379247
DSAN2-1	Santantadibe	S19.627482	E023.421058
DSAN5-1	Santantadibe	S19.633340	E023.403977
DSAN1-1	Santantadibe	S19.637170	E023.420307
DSAN6-1	Santantadibe	S19.638080	E023.414852
DSAN3-1	Santantadibe	S19.641405	E023.424000
DSAN01	Santantadibe	S19.651743	E023.429798
DSAN7-3	Santantadibe	S19.658150	E023.346040
DTHA1-4	Thalamakane	S19.943250	E023.488610
DTHA2-4	Thalamakane	S19.991220	E023.428060
DTHA3-4	Thalamakane	S20.006890	E023.475810
DTHA4-4	Thalamakane	S20.047690	E023.377420
DNGA1-1	L. Ngami	S20.389597	E022.934810
DNGA2-1	L. Ngami	S20.436227	E022.830828

Site: Date: Sample No:	Habitat Description: General comments:				Water chemistry		
		Temp (°C)		DO (mg/l)			
		pH		Cond (us/m)			
TAXON	Pres	Abun	Species?	TAXON	Pres	Abun	Species?
PORIFERA				TRICHOPTERA			
COELENTERATA				Ecnomidae			
TURBELLARIA				Hydropsychidae			
ANNELIDA				Philopotamidae			
Oligochaeta				Hydroptilidae			
Hirudinea				Leptoceridae			
CRUSTACEA				COLEOPTERA			
Anostraca				(Hydrophiloidae)			
Cladocera				Dyticidae			
Conchostraca				Elmidae/Dryopidae			
Ostracoda				Gyrinidae			
Amphipoda				Haliplidae			
Potamonautidae				Helodidae/Solrtidae			
Palaemonidae				Limnichidae			
Atyidae				Noteridae			
HYDRACARINA				Spercheidae			
PLECOPTERA				DIPTERA			
Perlidae				Ceratopogonidae			
EPHEMEROPTERA				Chironomidae			
Baetidae				Culicidae			
Caenidae				Dixidae			
Heptageniidae				Empididae			
Leptophlebiidae				Ephydriidae			
Oligoneuridae				Muscidae			
Polymetaciidae				Psychodidae			
Trycorithidae				Simulidae			
ODONATA				Syrphidae			
Chlorocyphidae				Tabanidae			
Coenagrionidae				Tipulidae			
Lestidae				GASTROPODA			
Aeshnidae				Ancylidae			
Cordulidae				Ampullaridae			
Gomphidae				Bithniidae			
Libellulidae				Lymnaeidae			
LEPIDOPTERA				Planorbidae			
Crambidae (Perlidae)				Physidae			
HEMIPTERA				Thiaridae			
Belostomatidae				Viviparidae			
Corixidae				PELECYPODA			
Gerridae				Corbiculidae			
Hebridae				Sphaeridae			
Hydrometridae				Mutelidae			
Mesovillidae				Unionidae			
Naucoridae							
Nepidae				OTHER			
Notonectidae							
Pleidae							
Villidae							

Appendix 2: Macroinvertebrate record sheet