

ACTEWAGL DISTRIBUTION MURRUMBIDGEE ECOLOGICAL MONITORING PROGRAM PART 1: ANGLE CROSSING SPRING 2010

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List of abbreviations

ACT – Australian Capital Territory ACTEW – ACTEW Corporation Limited AFDM – Ash Free Dry Mass (periphyton) ALS – Australian Laboratory Services ANOSIM – Analysis of similarities ANOVA – Analysis of Variance (statistics) ANZECC –Australian and New Zealand Environment and Conservation Council APHA – American Public Health Association ARMCANZ – Agriculture and Resource management Council of Australia and New Zealand AUSRIVAS – Australian River Assessment System BACI – Before After Control Impact CMA – Catchment Management Authority CRCFE – Cooperative Research Centre for Freshwater Ecology EC – Electrical Conductivity EIS – Environmental Impact Statement EPA – Environmental Protection Authority EPT – Ephemeroptera, Plecoptera and Trichoptera taxa GL/a – Gigalitres per annum GPS – Global positioning system IBT- Inter-Basin Water Transfer M2G – Murrumbidgee to Googong MEMP – Murrumbidgee Ecological Monitoring Program ML/d – Megalitres per day NATA – National Association of Testing Authorities NMDS – Non-metric Multidimensional Scaling (statistics) NSW – New South Wales NTU – Nephlelometric Turbidity Units PERMANOVA – PERMutational Multiple Analysis Of Variance QA – Quality Assurance QC – Quality Control SIMPER – Similarity Percentages TN – Total Nitrogen TP – Total Phosphorus

Executive Summary

To improve ACT water security for the future, ACTEW Corporation is constructing an additional pumping structure and pipeline to abstract water from the Murrumbidgee River near Angle Crossing (southern border of the ACT).

The proposed pumping system will transfer water from Angle Crossing through an underground pipeline into Burra Creek, and then transfer the water by run of river flows into the Googong Reservoir. The system is being designed to pump up to 100 ML/d, and is expected to be in operation by mid-2012. Abstraction will be dictated by the Googong Reservoir capacity, and by the availability of water in the Murrumbidgee River. The proposal is referred to as Murrumbidgee to Googong project (M2G).

This program aims to determine the baseline river condition prior to the water transfer and then continue monitoring after commencement to determine what changes are taking place that are attributable to abstraction from Angle Crossing.

The key aims of this sampling run were to:

- *Collect current baseline condition macroinvertebrate community data, up- and downstream of Angle Crossing;*
- *Report on water quality up and downstream of Angle Crossing.*
- *Collect periphyton baseline data to help monitor seasonal and temporal change and;*
- *Conduct river health assessments based on AUSRIVAS protocols at key sites potentially affected by the construction and operation of pumping infrastructure at Angle Crossing;*

This report presents the results from biological sampling and monitoring of the Murrumbidgee River upstream and downstream of Angle Crossing in spring 2010. Sampling was completed in November 2010 and was based on the AUSRIVAS sampling protocols, which was extended to include replicated sampling at each site and genus level identifications for selected taxa. The reasons for these variations were to: a) establish estimates of the within-site variability prior to the commencement of pumping; and; b) increase the resolution of the monitoring program to detect subtle changes in the macroinvertebrate community in response to water abstraction impacts. Comparisons between upstream and downstream locations are established in this baseline period, despite M2G being not operational at this stage to recognise any pre-existing location effects that might exist over and above those that might be brought about during the construction or operational phases of the M2G project.

The November 2010 sampling round coincided with a high rainfall period. Five of the six sites were sampled for biological parameters due to high flow condition at MUR 28 (upstream of the Cotter River confluence).

The key results from the spring 2010 sampling of Angle Crossing show that:

- *1) The results from this round of sampling were similar to previous findings for the Angle Crossing study where sampling coincides with high flow periods. For example, communities were dominated by early colonising taxa, sediment-dwelling taxa. Furthermore, there were reduced numbers of high SIGNAL sensitivity rating taxa. This indicates that flows displaced the free living, more sensitive taxa, and the sediment dwellers gain some protection from the shear stress exerted by the increased flows across all monitoring sites. The presence of high numbers of early colonists, suggests that despite the ongoing rainfall and frequent high flow conditions, recolonisation occurs rapidly.*
- *2) Descriptive statistics conducted on the water quality parameters showed no indication of any location effects. The overall trends in the water quality time series data are indicative of responses to changing flow conditions. For example, there are corresponding decreases in electrical conductivity with increasing flows, while the dissolved oxygen daily cycles show less variability during flow peaks. Both water quality stations track fairly constantly through the season, although there are some differences in the diurnal patterns of dissolved oxygen, which are likely to be (over and above the influence of flow) a function of different sensor depths between the two stations, which was observed in previous sampling runs (ALS, 2010).*
- *3) Surface water temperature, displayed an increasing monotonic trend resulting in an average 8°C increase over the spring period (September – November) and mean daily turbidity readings exceeded the recommended guidelines on 58 and 20 days (based on daily mean values) up- and downstream of Angle Crossing respectively*. *pH was slightly above the upper guideline for the first 5 days in October, otherwise all other parameters met the ANZECC and ARMCANZ (2000) guidelines.*
- *4) The in-situ water quality results show that the majority of physico-chemical parameters are within the ANZECC and ARMCANZ (2000) guidelines. The only exception was dissolved oxygen at MUR 15, but given that all the in-situ readings were conducted at different times of day, they are not necessarily comparable between sites. Total Phosphorus (TP) and Total Nitrogen (TN) exceeded the guidelines at all monitoring sites. The TP concentrations represent a two-fold increase from the autumn 2010 results (except MUR 23), and TN concentrations are similar across both seasons.*
- *5) Periphyton production was measured in terms of ash free dry mass (AFDM) and chlorophyll-a concentrations. Mean AFDM and chlorophyll-a concentration did not differ significantly between up and downstream locations. There were higher median chlorophyll-concentrations observed at Point Hut Crossing, which is consistent with previous sampling runs. This continues to imply periodic nutrient enrichment at this site through Point Hut Pond spillages during high flow events.*
- *6) The AUSRIVAS assessment for the riffle habitat showed no change at MUR 16; MUR 18 and MUR 19 since autumn 2010, although direct comparisons on a season by season basis indicates that MUR 15, 16 and MUR 23 have improved riffle habitat assessments than they did in spring 2009, shifting from BAND B to BAND A. By*

comparison, MUR 18 and 19 remained at BAND B, which is the same as they have been since spring 2009.

- *7) Assessment based on edge habitat samples were somewhat biased by the on-going high flow disturbance of edge habitat in this section of the Murrumbidgee River. Such effects were so pronounced at MUR 15 and MUR 18 that no reliable assessment based on edge habitat sampling was possible at these sites. However, edge habitat-based assessments for the other sites revealed a decline in condition at MUR 16 and MUR 19, which were assessed as BAND C and no change in the condition at MUR 23 (Point Hut crossing) compared to spring 2009.*
- *8) Trichoptera were the best represented of the suite of sensitive taxa (EPT) across the sampling sites. Several of the usually common and abundance mayflies (Ephemeroptera) were absent from most sampling sites except for the farthest upstream site, MUR 15, which also had the most stoneflies (Plecoptera). However, for the most part, all of the communities were dominated by Chironomids, Simulids, Oligochaetes and Caddis flies with low to intermediate SIGNAL-2 scores. The edge habitat showed a high degree of variation of taxonomic composition, which is evidenced by the variation seen in the AUSRIVAS results across all sites.*

These results are indicative of communities which have been subjected to a natural disturbance, such as high flow events as the case is here. For example, highly sensitive taxa and usually common, sensitive taxa were missing from all sites with no obvious patterns in their presence or absence. Taxa that are adapted to low velocities, were absent or in very low numbers in the edge samples and although some sensitive taxa were collected in the riffles, they were in much lower abundances and less common than they have been in previous seasons. The overall conclusion based on the water quality and biological information indicates a natural disturbance (i.e. high flows) as the overriding factor influencing the indicators used in this assessment. Based on this evidence we suggest that once flows stabilise, the sites under assessment should improve in their ecological health rating based on AUSRIVAS assessment.

1 Introduction

The Murrumbidgee Ecological Monitoring Program (MEMP) was set up by ACTEW Corporation to evaluate the potential impacts of water abstraction from the Murrumbidgee River. It is being undertaken as part of the ACT water supply security infrastructure upgrade.

The proposed time-line is to undertake sampling in spring and autumn over a three year period that commenced in spring 2008.

There are four component areas being considered:

- Part 1: Angle Crossing
- Part 2: Burra Creek (discharge point for Angle Crossing abstraction)
- Part 3: Murrumbidgee Pump Station
- Part 4: Tantangara to Burrinjuck

This report focuses on Part 1: Angle Crossing

To improve ACT water security for the future, ACTEW Corporation is proposing to construct an additional pumping structure and pipeline to abstract water from the Murrumbidgee River near Angle Crossing (southern border of the ACT). The proposed pumping system will transfer water from Angle Crossing through a 12km underground pipeline into Burra Creek. The water will then be transported a further 13km by run of river flows into the Googong Reservoir.

The system is being designed to pump up to 100 ML/d and is expected to be in operation by mid-2012, with construction due to commence in late 2010. Water abstraction from the Angle Crossing pump station will be dictated by the Googong Reservoir's capacity and by the availability of water in the Murrumbidgee River. This project is referred to as Murrumbidgee to Googong project (M2G).

During periods of low flow (whether climate related or artificially induced), impacts upon aquatic environments can be measured using surrogate indices based on changes to macroinvertebrate communities, such as changes in species richness, abundances and community structure. Such changes can result either directly through invertebrate drift, or indirectly through reductions in habitat diversity or flow conditions which do not suit certain taxa. Dewson et al., (2007) reported that certain macroinvertebrate taxa are especially sensitive to reductions in flow and be useful indicators in flow restoration assessments and can assist in longer term management of flows in regulated river systems. It is expected there will be changes to the aquatic ecosystem within the Murrumbidgee River and Burra Creek as a result of M2G. Some of these effects include, but are not limited to: changes to water chemistry; and changes to channel morphology, velocity and depth. All of these changes have potential knock-on effects to the biota within the river's ecosystem (**APPENDIX A**). This current monitoring program will form the basis of an Ecological Monitoring Program to satisfy EIS requirements.

1.1 Background: The Upper Murrumbidgee River

The Murrumbidgee River flows for 1600 km from its headwaters in the Snowy Mountains to its junction with the Murray River. The catchment area to Angle Crossing is 5096 km^2 . As part of the Snowy Mountains Scheme, the headwaters of the Murrumbidgee River were constrained by the 252 GL Tantangara Dam, which was completed in 1961. The reservoir collects water and diverts it outside the Murrumbidgee catchment to Lake Eucumbene. This has reduced base flows and the frequency and duration of floods in the Murrumbidgee River downstream. The Murrumbidgee River is impounded again at Burrinjuck Dam, after the river passes through the ACT. This region above Burrinjuck Dam is generally known as the Upper Murrumbidgee.

Land-use varies from National Park in the high country to agriculture and farming in the valley regions. Annual rainfall varies from greater than 1400 mm in the mountains, to 620 mm at Canberra, down to 300 mm in the west (B.O.M, 2010).

Drought has had the most significant impact on catchment condition within the upper Murrumbidgee catchments in recent times. More than 80% of catchments have been drought*-*affected since late 2002. Drought-induced land degradation in the upper Murrumbidgee catchments has been significant across all areas and adverse effects include increased stress on surface and groundwater resources, increased soil erosion and a shift from mixed farming and cropping to grazing, and reduced stock numbers. Drought has also led to increased pressure on native vegetation in the catchments, a heightened risk of fire in native forests, and an increase in the abundance of several weed species.

1.2 Project objectives

There are two key phases to this project, which incorporates two sets of objectives, representing long and short term aims, i.e. before and after abstraction (Table 1). Phase 1 of this monitoring program involves the establishment of baseline macroinvertebrate community composition at selected sites up- and downstream of the proposed abstraction point. The focus of Phase 1 will be on the documentation of spatial and seasonal changes in macroinvertebrate and periphyton assemblages as well as monitoring water quality patterns. This will also include monitoring potential effects associated with (either directly or indirectly) the construction of the new pump station at Angle Crossing.

Phase 2, incorporates long term objectives, which aim to delineat e potential ecological effects that are related specifically to the abstraction of water from the Murrumbidgee River at Angle Crossing, outside of what is considered natural, temporal and spatial variation.

The specific aims of this monitoring program are:

1. To determine seasonal and annual variation in the composition and abundance of periphyton at control and test sites before water abstractions commence, and to assist in the monitoring of river ecosystem health once the abstractions begin.

2. To determine baseline macroinvertebrate communities at test and control sites before the water abstractions commence, and to assist in the monitoring of riverine ecosystem health once the abstractions begin.

Table 1. Project objectives and estimated time frames

1.3 **Project scope**

The current ecological health of the sites monitored as part of the Murrumbidgee to Googong (M2G) monitoring program was estimated using AUSRIVAS protocols for macroinvertebrate community data, combined with a suite of commonly used biological metrics and descriptors of community composition. The scope of this report is to convey the results from the spring 2010 sampling. Specifically, as outlined in the MEMP proposal to ACTEW Corporation (Ecowise, 2009) this work includes:

- Water quality, periphyton and macroinvertebrate sampling conducted in spring 2010;
- Macroinvertebrate communities collected from riffle and edge habitats using AUSRIVAS protocols;
- Macroinvertebrate samples counted and identified to the taxonomic level of genus;
- Riffle and edge samples assessed through the appropriate AUSRIVAS model;
- *In-situ* water quality samples analysed for nutrients in ALS's NATA accredited laboratory.

1.4 Rationale for using biological indicators

Macroinvertebrates and periphyton are two of the most commonly used biological indicators in river health assessment. Macroinvertebrates are commonly used to characterise ecosystem health because they represent a continuous record of preceding environmental, chemical and physical conditions at a given site. Macroinvertebrates are also very useful indicators in determining specific stressors on freshwater ecosystems because many taxa have known tolerances to heavy metal contamination, sedimentation, and other physical or chemical changes (Chessman, 2003). Macroinvertebrate community assemblage, and two indices of community condition; the AUSRIVAS index and the proportions of three common taxa (the Ephemeroptera, Plecoptera, and Trichoptera, or EPT index), were used during this survey to assess river health.

Periphyton is the matted floral and microbial community that resides on the river bed. The composition of these communities is dominated by algae but the term "periphyton" also includes fungal and bacterial matter (Biggs and Kilroy, 2000). Periphyton is important to maintaining healthy freshwater ecosystems as it absorbs nutrients from the water, adds oxygen to the ecosystem via photosynthesis, and provides a food for higher order animals. Periphyton communities respond rapidly to changes in water quality, light penetration of the water column and other disturbances, such as floods or low flow, and this makes them a valuables indicator of river health.

Changes in total periphyton biomass and/or the autotrophic component of the periphyton (as determined by chlorophyll*-a*) can vary with changes in flow volume, so these variables are often used as indicators of river condition in relation to monitoring the effects of flow regulation, environmental flow releases or water abstraction impacts (Talsma and Hallam, 1982; Biggs, 1989;; Whitton and Kelly, 1995; Biggs *et al.*, 1999;). Water abstractions from Angle Crossing will not affect the timing or magnitude of higher flows, but it could affect conditions during the seasonal low flow period, such as increasing the nutrient availability through increased residence time, reducing scouring impacts on benthic organism and reducing surface flows over riffle habitats and thus decreasing habitat quality and availability. As changes in flow volume are expected with the proposed changes in the Murrumbidgee River water abstraction regime, periphyton biomass and chlorophyll*-a* are included as biological indices.

2 Materials and Methods

2.1 **Study sites**

Macroinvertebrate community composition, periphyton assemblages and water quality were monitored from replicate sites on the Murrumbidgee River, up- and downstream of Angle Crossing (~2km west of Williamsdale) with the aim of obtaining baseline ecological condition information following the ANZECC guidelines for ecological monitoring (ANZECC & ARMCANZ, 2000).

The upper Murrumbidgee River is impacted by activities in its large catchment, which includes a large array of land-use practices. As such, it was important to select a sufficiently large number of sites to enable the program to provide a reasonable snap-shot of the current status of the macroinvertebrate community in the study area. Sites were chosen based on several criteria, which included:

- Safe access and approval from land owners;
- Sites have representative habitats (i.e. riffle / pool sequences). If both habitats were not present then riffle zones took priority as the they are the most likely to be affected by abstractions;
- Sites which have historical ecological data sets (e.g. Keen, 2001) took precedence over "new sites" –allowing comparisons through time to help assess natural variability through the system. This is especially important in this program because there is less emphasis on the reference condition, and more on comparisons between and among sites of similar characteristics in the ACT and surrounds over time.

Potential sites were identified initially from topographic maps, they were visited prior to sampling and their suitability was subsequently considered based on senior ALS staff and the habitat descriptions in Coysh et al., (2000). Six sites suited the criteria mentioned above (Table 2; Figures 1 and 2). These sites include three sites upstream of Angle Crossing (in NSW) and three sites downstream (all in the ACT).

Table 2. Sampling site locations and details

Figure 1. Angle Crossing sampling locations and gauging stations

MUR 15. Looking upstream (399 ML/d) MUR 15. Looking downstream

MUR 16. "The Willows" near Michelago MUR 16. Looking downstream (399 ML/d)

Mur 18. ~800m Upstream of Angle Crossing Mur 18 looking across to the edge habitat (394 ML/d)

Plate 1. Photographs of sampling sites upstream of Angle Crossing

Mur 19. Downstream of Angle Crossing Mur 19. Looking downstream (475 ML/d)

Mur 23. Point Hut Crossing **Murious Communist Contains Murious Murious Communist C**

Mur 28. Upstream Cotter River confluence highlighting the difficult wading and high flow conditions

Plate 2. Photographs of sampling sites downstream of Angle Crossing

2.2 **Hydrology and rainfall**

River flows and rainfall for the sampling period were recorded at ALS gauging stations located at Lobb's Hole (downstream of Angle Crossing: 410761) and upstream of Angle Crossing (MURWQ09) (see Table 3).

Stations are calibrated monthly and data are downloaded and verified before storage on the database where they are quality coded. Water level data were verified manually by comparing the logger value to the staff gauge value. If there were differences between logger and staff, the logger was adjusted accordingly. Rain gauges were calibrated and adjusted according to ALS work procedures (document number: Fi011). Records were stored on the HYDSTRA[©] database software and downloaded for each sampling period.

Table 3. Location and details of continuous water quality and flow stations

Site Code	Location/Notes	Parameters*	Latitude	Longitude
410761	M'bidgee River @ Lobb's Hole (D/S of Angle Crossing)	WL, Q, pH, EC, DO, Temp, Turb. Rainfall	S 35,5398	E 149.1015
MURWQ09	M'bidgee River U/S Angle Crossing	WL, Q, pH, EC, DO, Temp, Turb, Rainfall	S 35,3533	E 149.0705

 $*$ WL = Water Level; Q = Rated Discharge; EC = Electrical Conductivity; DO = Dissolved Oxygen; Temp = Temperature: Turb = Turbidity: Rainfall = Rainfall $(0.2 \text{ mm}$ increments)

2.3 **Water quality**

Baseline *in-situ* physico-chemical parameters including temperature, pH, electrical conductivity, turbidity and dissolved oxygen were recorded using a multiprobe Hydrolab[®] minisonde 5a at sites indicated in Table 2. The Hydrolab® was calibrated following QA procedures and the manufactures requirements prior to sampling. Additionally, grab samples were taken from each site in accordance with the AUSRIVAS protocols (Coysh *et al.*, 2000b) for Hydrolab verification and nutrient analysis. All samples were placed on ice, returned to the ALS laboratory, and analysed for nitrogen oxides (total NOx), total nitrogen and phosphorus in accordance with the protocols outlined in APHA (2005). Collectively, this information on the water quality parameters will assist in the interpretation of biological data and provide a basis on which to gauge ecosystem changes potentially linked to flow reductions at these key sites following water abstractions.

2.4 **Macroinvertebrate sampling and processing**

At each site, macroinvertebrates were sampled in the riffle and edge habitats where available. Both habitats were sampled to provide a more comprehensive assessment of each site (Coysh *et al.*, 2000a); and allow the program to isolate flow-related impacts from other disturbances. Monitoring both habitats will allow an overall site assessment as the potentially immediate impacts resulting from abstraction may be less consequential given the greater available habitat in the edges and the longer response time to low flow conditions.

Riffle and edge habitats were sampled for macroinvertebrates and analysed in strict accordance with the ACT autumn riffle and edge AUSRIVAS (Australian River Assessment System) protocols (Coysh *et al.*, 2000b) during spring (November $24th$ and $25th$) 2010. At each site, two samples were taken (where possible) from the riffle habitat (flowing broken water over gravel, pebble, cobble or boulder, with a depth greater than 10 cm; (Coysh et al., 2000b) using a framed net (350 mm wide) with 250 μ m mesh size.

Sampling began at the downstream end of each riffle. The net was held perpendicular to the substrate with the opening facing upstream. The stream directly upstream of the net opening was disturbed by vigorously kicking and agitating the stream bed, allowing any dislodged material to be carried into the net. The process continued, working upstream over 10 metres of riffle habitat. The samples were then preserved in the field using 70% ethanol, clearly labelled with site codes and date then stored on ice and refrigerated until laboratory sorting commenced.

The edge habitat was also sampled in strict accordance with the ACT AUSRIVAS protocols. Two samples were taken from the edge habitat. The nets and all other associated equipment were washed thoroughly between sampling events and sites to remove any macroinvertebrates retained on them. Samples were collected by sweeping the collection net along the edge habitat at the sampling site; the operator worked systematically over a ten metre section covering overhanging vegetation, submerged snags, macrophyte beds, overhanging banks and areas with trailing vegetation. Samples were preserved on-site as described for the riffle samples.

Processing of the macroinvertebrate samples followed the ACT AUSRIVAS protocols. Briefly, in the laboratory, the preserved macroinvertebrate samples were placed in a sub-sampler, comprising of 100 (10 X 10) cells (Marchant, 1989). The sub-sampler was then agitated to evenly distribute the sample. The contents of randomly selected cells were removed and the macroinvertebrates within each cell were identified to genus level except for Chironomids (sub-family) and Oligochaeta (class). Specimens that could not be identified to the specified taxonomic level (i.e. immature or damaged taxa) were removed from the data-set prior to analysis. For the AUSRIVAS model, taxa were analysed at family level except for: Chironomidae (sub-family), Oligochaeta (class) and Acarina (order) until 200 animals were identified (identification followed taxonomic keys published by Hawking, (2000)). If 200 animals were identified before a cell had been completely analysed, identification continued until the animals in the entire cell were identified. Data were entered directly into electronic spread sheets to eliminate errors associated with manual data transfer.

2.5 **Periphyton**

Estimates of algal biomass were made using complimentary data from both chlorophyll*-a* (which measures autotrophic biomass) and ash free dry mass (AFDM; which estimates the total organic matter in periphyton samples and includes the biomass of bacteria, fungi, small fauna and detritus in samples) of the periphyton samples (Biggs, 2000).

The six sites shown in Table 2 were sampled for periphyton in autumn in conjunction with the macroinvertebrate sampling. All periphyton - adnate and loose forms of periphyton, as well as organic/inorganic detritus in the periphyton matrix, were collected using the *in-situ* syringe method similar to Loeb (1981), as described in Biggs and Kilroy (2000) (Plate 3). A 1m wide transect was established across riffles at each site. Along each transect, twelve samples were collected at regular intervals, using a syringe sampling device, based on two 60 ml syringes and a scrubbing surface of stiff nylon bristles, covering an area of $~637 \text{ mm}^2$. The samples were then divided randomly into two groups of six samples to be analysed for Ash Free Dry Mass (AFDM $gm⁻²$), and chlorophyll-*a*. Samples for Ash Free Dry Mass (gm-2) and chlorophyll*-a* analysis were filtered onto glass filters and frozen. Sample processing follows the methods outlined in APHA (2005).

Qualitative assessments of the estimated substrate coverage by periphyton and filamentous green algae were also conducted at each site in accordance with the AUSRIVAS habitat assessment protocols (Coysh *et al.*, 2000b) to compliment the quantitative samples.

Plate 3. Diagram of the periphyton sampler (taken from Loeb, 1981)

Plate 4. Periphyton sampler in operation

2.6 **Macroinvertebrate quality control procedures**

A number of Quality Control procedures were undertaken during the identification phase of this program including:

- Organisms that were heavily damaged were not selected during sorting. Attempts were made to obtain more than 200 organisms, to overcome losses associated with damage to intact organisms during vial transfer.
- Identification was performed by qualified and experienced aquatic biologists with more than 100 hours of identification experience.
- When required, taxonomic experts performed confirmations of identification. Reference collections were also used when possible.
- ACT AUSRIVAS QA/QC protocols were followed.
- An additional 10% of samples were re-identified by another senior taxonomist.
- Very small, immature, or damaged animals or pupae that could not be positively identified were not included in the dataset.

All procedures were performed by AUSRIVAS accredited staff.

2.7 **Licences and permits**

All sampling was carried out with current NSW scientific research permits under section 37 of the Fisheries Management Act 1994 (permit number P01/0081(C)).

ALS field staff maintains current ACT and NSW AUSRIVAS accreditation.

2.8 **Data analysis**

2.8.1 Water quality

Water quality parameters were examined for compliance with ANZECC water guidelines for healthy ecosystems in upland streams (ANZECC and ARMCANZ, 2000). Trend analyses of water quality parameters will be conducted at the end of the baseline collection period.

2.8.2 Macroinvertebrate communities

The macroinvertebrate data were examined separately for riffle and edge habitats. Replicates were examined individually (i.e. not averaged) at all sites to examine within-site variation as much as it is to describe patterns among sites. The PERMDSIP routine in PERMANOVA+ was used to test for homogeneity of multivariate dispersions based on Bray-Curtis similarities from the macroinvertebrate similarity matrix. The rationale for conducting this test was based on our previous observations from the Angle Crossing macroinvertebrate data set, which has revealed considerable within-site variation in community assemblages.

Variation in multivariate dispersions can have two potential consequences on both the hypothesis testing component of the community analysis and the interpretation of the ordination plots. Because both the ANOSIM and PERMANOVA tests are sensitive to differences in multivariate dispersions (one of the design assumptions is homogenous dispersions of residuals and random effects), this test serves to test of one of the key assumptions of the macroinvertebrate community modelling. Furthermore, different degrees of variability (multivariate dispersions) among sites or grouping factors can be an important indicator of environmental stress on benthic communities (Warwick and Clarke, 1993); so may be an important component of this monitoring program in its own right (Anderson *et. al*., 2008). All multivariate analyses were performed using PRIMER version 6 (Clarke and Gorley, 2006) and PERMANOVA + (Anderson *et al*., 2008)). Univariate statistics were performed using R version 2.12.1 (R Development Core Team, 2010).

Non-metric multidimensional scaling (NMDS) ordination was performed to reduce dimensionality of the macroinvertebrate data in order to provide a visual representation of the macroinvertebrate relationships between sites and locations. Within the NMDS plot, sites closer together indicate that the macroinvertebrate communities are more similar to one another than sites further apart in the ordination space. In other words, NMDS reduces the dimensionality of the data by describing trends in the joint occurrence of taxa. This procedure was performed on the macroinvertebrate community data following the initial cluster-analysis.

The initial step in this process was to calculate a similarity matrix for all pairs of samples based on the Bray-Curtis similarity coefficient (Clarke and Warwick, 2001). For the macroinvertebrate data collected during this survey, the final number of dimensions was reduced to two. Stress values for each NMDS plot were examined before results were interpreted. The stress level is a measure of the distortion produced by compressing multidimensional data into a reduced set of dimensions and will increase as the number of dimensions is reduced and can be considered a measure of "goodness of fit" to the original data matrix (Kruskal, 1964). Stress values near zero suggest that NMDS patterns are very representative of the multidimensional data, while stress values greater than 0.2 indicate a poor representation and, therefore, the need to interpret NMDS plots with these sorts of Stress values with caution (Clarke and Warwick 2001).

An Analysis of similarities test (ANOSIM) was performed on the macroinvertebrate similarity matrix to test whether macroinvertebrate communities were statistically different upstream and downstream of Angle Crossing. Sites were nested within location for the analysis. The Similarity percentages (SIMPER) routine was carried out on the datasets only if the initial ANOSIM test was significant (i.e. P<0.05), to examine which taxa were responsible for, and explained the most variation among statistically significant groupings (Clarke and Warwick, 2001). This process was also used to determine which taxa characterised particular groups of sites.

In addition to these analyses, variation in the macroinvertebrate data set was modelled against environmental, physical and water quality variables to explore which variable or combination of variable correlate to the macroinvertebrate resemblance matrix. This was performed using the BIO-ENV procedure in PRIMER V6. BIO-ENV which compares the biotic and environmental similarity matrices based on all possible combinations of the environmental variables; resulting in a rank-correlation coefficient (Spearman's Rho was selected) which can take on values between -1 and +1. The extreme Spearman Rho values indicate either complete disagreement or complete agreement respectively, between the two similarity matrices (Clarke and Warwick, 2006). Values around zero indicate no relationship between the biotic and abiotic data sets. Statistical significance of the global test (i.e. between all variables in the abiotic matrix and the macroinvertebrate data set) were obtained by 999 permutations to create a nulldistribution to which our observed value of ρ is compared. The most parsimonious set of variables was selected on the basis of the best fit (i.e. smallest number of variables and highest ρ-value) since there are no formal tests available in this procedure for individual model selection.

2.8.3 AUSRIVAS assessment

In addition to assessing the composition and calculating biometrics from the macroinvertebrate data, riffle and edge samples, river health assessments based on the ACT AUSRIVAS spring riffle and edge models were conducted. AUSRIVAS is a prediction system that uses macroinvertebrate communities to assess the biological health of rivers and streams. Specifically, the model uses site-specific information to predict the macroinvertebrate fauna expected (E) to be present in the absence of environmental stressors. The expected fauna from sites with similar sets of predictor variables (physical and chemical characteristics which cannot be influenced due to human activities, e.g. altitude) are then compared to the observed fauna (O) and the ratio derived is used to indicate the extent of any impact (O/E). The ratio derived from this analysis is compiled into bandwidths (i.e. X, A-D; Table 4) which are used to gauge the overall health of particular site (Coysh *et al.,* 2000). Data is presented using the AUSRIVAS O/E 50 ratio (Observed/Expected score for taxa with a >50% probability of occurrence) and the previously mentioned rating bands (Table 4).

The site assessments are based on the results from both the riffle and edge samples. The overall site assessment was based on the furthest band from reference in a particular habitat at a particular site. For example, a site that had an A assessment in the edge and a B Band in the riffle would be given an overall site assessment of B (Coysh *et al.*, 2000b). In cases where the bands deviate significant between habitat (e.g. $D - A$) then an overall assessment was avoided due to the unreliability of the results.

The use of the O/E 50 scores is standard in AUSRIVAS. However it should be noted that this restricts the inclusion of rare taxa and influences the sensitivity of the model. Taxa that are not predicted to occur more than 50% of the time are not included in the O/E scores produced by the model. This could potentially limit the inclusion of rare and sensitive taxa and might also reduce the ability of the model to detect any changes in macroinvertebrate community composition over time (Cao *et al.*, 2001). However, it should be noted that the presence or absence of rare taxa does vary naturally over time and in some circumstances the inclusion of these taxa in the model might indicate false changes in the site classification because the

presence or absence of these taxa might be a function of sampling effort or the effects of a recent hydrological disturbance rather than truly reflecting ecological change.

2.8.4 SIGNAL-2 (Stream Invertebrate Grade Number – Average Level)

Stream Invertebrate Grade Number – Average Level (SIGNAL) is a biotic index based on pollution sensitivity values (grade numbers) assigned to aquatic macroinvertebrate families that have been derived from published and unpublished information on their tolerance to pollutants, such as sewage and nitrification (Chessman, 2003). Each family in a sample is assigned a grade between 1 (most tolerant) and 10 (most sensitive). Sensitivity grades are also given in the AUSRIVAS output which can then be used as complimentary information to these assigned bandwidths to aid the interpretation of each site assessment.

We conducted linear mixed effect ANOVA models separately for the riffle and edge samples to test for location differences in the univariate metrics: SIGNAL-2 scores and AUSRIVAS OE50 ratios. The factor sites (nested within location) was considered a random effect representing the river condition upstream and downstream of the proposed abstraction point; while location (up- and downstream) was considered a fixed, constant effect. Data transformations were not necessary because the model assumptions were met on all accounts. Models were constructed using lme4 (Bates *et al*., 2011), a statistical package applied in the R environment (R Development Core Team, 2010)). For all analyses, the level of significance (alpha) was set to 5%.

Several metrics in addition to AUSRIVAS and SIGNAL-2 were used. The number of taxa (taxa richness) was counted for each site and other descriptive metrics such as the relative abundances of pollutionsensitive taxa (Ephemeroptera, Plecoptera and Trichoptera- EPT) and, pollution-tolerant taxa, (i.e. Oligochaeta and Chironomids) were examined at family and genus levels. Taxa richness was monitored as a means of assessing macroinvertebrate diversity. In assessing the taxonomic richness of a site, it is important to keep in mind that high taxa richness scores may, though does not always, indicate better ecological condition at a given location. In certain instances high taxa richness may indicate a response to the provision of new habitat or food resources that might not naturally occur as a result of anthropogenic activities.

Table 4. AUSRIVAS band-widths and interpretations for the ACT autumn riffle and edge models

2.8.5 Periphyton

To test whether estimated biomass (AFDM) and live content (chlorophyll*-a*) were different between sites upstream and downstream of Angle Crossing, a mixed effects, analysis of variance was fitted to the Logtransformed data for AFDM and Chlorophyll-a. The site factor was nested within location (upstream or downstream of the abstraction point); consequently, site and location were treated as random and fixed effects, respectively in the ANOVA model. Log-transformation was necessary to meet the assumptions of normality. For the purposes of graphical visualisation, however, raw data are presented.

The relationship between the spring periphyton data and a suite of environmental and physico-chemical water quality parameters was examined using Pearson's product moment coefficients. The Pearson correlation coefficient measures the strength of the relationship between two variables (x and y). The correlation coefficient, denoted as "R", can positive or negative, with the values -1 or +1 indicating that the observations fall along a straight line (either negatively or positively) and 0 indicating no relationship between the variables. Univariate statistics were performed using R version 2.10.1 (R Development Core Team, 2010). Significance testing was not performed on these data because of low sample size (n=6 in all cases).

3 Results

3.1 Summary of sampling and river condition

Sampling for the autumn 2010 sampling run was conducted on the $24th$ and $25th$ of November. At the time of sampling, the mean daily flow recorded at the closest gauging station (MURWQ09: Upstream of Angle Crossing) was 393 ML/d, while flows downstream at Lobb's Hole were 474 ML/d. Sampling was delayed as long as possible in spring due to a series of high flow events that came through the catchment. Following flood events, the ACT AUSRIVAS sampling protocol states that sampling must not occur when a stream is in flood and sampling should resume four weeks after the floods subside (Coysh *et al*., 2000).

Flows remained high through the catchment most of the spring period, which meant a decision was made to go ahead with sampling despite not quite meeting the four week down time between events. This decision was made based on weather patterns at the time (i.e. there was no indication that the rainfall would cease for a long enough period to sample within the AUSRIVAS seasonal guidelines). As a result, the Murrumbidgee River flows - within the domain of the Angle Crossing monitoring program - were still running high, which meant that macroinvertebrates and periphyton at MUR 28 (upstream of the Cotter confluence) was not sampled for in this round of sampling.

During sampling, the ambient temperatures were in the range of 28-33°C and conditions were fine. Despite high river levels, there had been no rain over the previous 8 days. Riffle zones were inundated and flows in the edge habitats were swift (almost three times the current velocity from the previous round of sampling).

3.2 Hydrology and rainfall

As discussed above, high flows were the dominant feature of the Murrumbidgee during the spring period. Analysis of the flow data from Lobb's Hole station (410761) indicate that flows for the three month period in the Murrumbidgee River averaged 889 ML/d, while upstream of Angle Crossing (MURWQ) flows averaged 792 ML/d for the same period (Table 5). The average spring flows (September – November), based on the historical records from Lobb's Hole indicate that these were the highest recorded since 1996, while rainfall gauged at Lobb's Hole (570985) over the same period was the second highest on record (period of record: 1974-present).

Constant rainfall throughout spring triggered several high flow events (Figures $2 \& 3$). The largest of these events occurred in mid-October, where flows peaked at ~16,000 ML/d at Lobb's Hole after 103 mm of rain fell over four days. There was a long recession limb due to the 4-5 days of continual rainfall following the flow peak. Flows remained at approximately 1000 ML/d during the first three weeks of November, corresponding to almost two weeks of continual rainfall (Figures $2 \& 3$). Daily mean flows towards the last week of November dropped back to 300-400 ML/d, which was when sampling took place.

Table 5. Autumn rainfall and flow summaries upstream and downstream of Angle Crossing. Flow values are daily means. Rainfall is total (mm)

Figure 2. Spring hydrograph of the Murrumbidgee River upstream of Angle Crossing (MURWQ09) and downstream of Angle Crossing at Lobb's Hole (410761)

• Note the log scale for discharge on the y-axis

Figure 3. Spring rainfall in the Murrumbidgee River catchment: upstream of Angle Crossing (MURWQ09) and downstream of Angle Crossing at Lobb's Hole (410761).

Water quality

3.2.1 Continuous records

The results of continuous water quality monitoring at the two gauging stations were extracted for the spring period, defined as the period between 1/9/10 and 1/12/10. These results are summarised below.

There are some gaps in the continuous records from both gauging stations (Figures $4 \& 5$). Data are missing for dissolved oxygen on two separate occasions, following high flow events upstream of Angle Crossing. The inaccuracies in the dissolved oxygen sensor were caused by silt deposition on the sensor following the high flow events. Efforts are being made to reduce the reoccurrence of this at this site. The four days of data that were lost in early October from the Lobb's Hole station was a result of lightening damage.

The overall trends in the water quality time series data show are indicative of the responses to changing flow conditions. For example, there were corresponding decreases in electrical conductivity with increasing flows, while the dissolved oxygen daily cycles fell within a shallower range during flow peaks (Figures $4 \& 5$). Both stations tracked fairly constantly through the season, although there were some differences in the diurnal patterns of dissolved oxygen, which were likely to be (over and above the influence of flow) a function of different sensor depths between the two stations. Surface water temperature, displayed an increasing monotonic trend resulting in an average 8°C increase over the spring period (Table 6; Figures $4 \& 5$).

Comparisons of the continuous data records to the ANZECC and ARMCANZ (2000) guidelines show that both stations, upstream and downstream of Angle Crossing, generally fell within the specified guidelines and are highly comparable between both stations (Table 6). The exceptions were mean daily turbidity readings which exceeded the recommended guidelines on 58 and 20 days (based on daily mean values) up- and downstream of Angle Crossing respectively. pH was slightly above the upper guideline for the first 5 days in October upstream of Angle Crossing, otherwise all other parameters met the ANZECC and ARMCANZ (2000) guidelines.

The grab sample results also show that the majority of physico-chemical parameters meet the ANZECC and ARMCANZ (2000) guidelines. The only exception is dissolved oxygen at MUR 15 (Table 6), but given that all the spot readings were conducted at different times of day, they are not necessarily comparable between sites. The strength of the longitudinal gradient for EC and alkalinity was weaker than previously seen during periods of lower, more stable flows; which highlights the homogenising effects of these high flow periods upon water quality. Nutrient data (namely Total Phosphorus and Total Nitrogen) exceeded the guidelines across all monitoring sites. The TP concentrations represent a two-fold increase from the autumn results (except MUR 23), although TN concentrations are similar across both seasons. TP concentrations had a narrow range $(0.4 - 0.5)$, while TN was more variable (range: 0.37-0.51) across sites. The highest TP and TN values were recorded at MUR 16 (Table 6), while the lowest were upstream at MUR 15. Nitrogen oxide values were below detectable limits on this occasion. Ammonia ranged from 0.07 mg/L at MUR 15 to 0.01 mg/L at MUR 28.

Analyte | Temp. C | EC (us/cm) | pH | Turbidity (NTU) Max. in parentheses D.O (% sat.) U/S D/S U/S D/S U/S D/S U/S D/S U/S D/S September 12.4 12.5 47 62 7.09 7.75 62 [1558] 26 [775] 82-98 96-100 October 16.7 17.1 70 72 7.58 7.68 37 [798] 45 [750] 76-98 97-101 November 20.2 20.2 66 82 7.30 7.71 33 [880] 18 [145] 88-102 93-100 Spring 16.5 16.6 61 72 7.30 7.71 44 [1558] 29 [775] 82-99 95-101

Table 6. Monthly water quality statistics from upstream and downstream of Angle Crossing. All values are means, except D.O. % Sat. which is expressed as mean monthly minimums and maximums.

Figure 4. Continuous water quality records from upstream Angle Crossing (MURWQ09) for spring 2010. The shaded area indicates two periods where silt deposition, following rainfall, resulted in inaccurate or no data being recorded for dissolved oxygen.

Figure 5. Continuous water quality records from Lobb's Hole (downstream Angle Crossing: 410761) for spring 2010. The shaded area indicates the four day period when the probe was under repair following lightning damage

Table 7. In-situ water quality results from spring 2010 (ANZECC guidelines are in red). Yellow cells indicate values outside of ANZECC and ARMCANZ (2000) guidelines. *ns = not sampled

EC = Electrical conductivity; TSS = Total suspended solids; D.O = Dissolved oxygen; Alk. mg/L; TP = phosphorus; TN = total nitrogen

3.3 **Periphyton**

The distribution of the chlorophyll-*a* estimates ranged considerably amongst sampling sites, from 220 μ g/m⁻² at MUR 19 to 13900 μ g/m⁻² at MUR 16 (Figure 6a). Average chlorophyll-a concentrations were lower upstream of Angle Crossing (mean= $3868 \mu g/m^2$) compared to downstream (mean= $5492 \mu g/m^2$) however, these differences were not statistical different $(F_{1,3} = 0.01; P > 0.05;$ Table 7). The spatial distribution of chlorophyll-*a* differed to previous sampling runs (ALS, 2009 & 2010) in that we have previously seen a linear increase in median concentrations up to MUR 23, at which point there is a marked spike in chlorophyll-a which is maintained down to MUR 28. While no data are available for MUR 28 for this round of sampling, the patterns in the data show a more or less consistent pattern upstream of MUR 23, although there is still a shift towards higher median values at this site, and an apparent increase in the $25th$ percentile and minimum values (Figure 6a).

Ash free dry mass (AFDM) concentrations displayed similar spatial patterns as chlorophyll-concentrations (*cf*. Figures 6a & 6b). Mean chlorophyll*-a* and mean AFDM were moderately correlated indicating some degree of algal derived chlorophyll-a in the AFDM samples. Like the chlorophyll-a data, AFDM estimates tended to be lower upstream of Angle Crossing (mean= 4519 mg/m^2) than they were downstream (mean =8681 mg/m⁻²) however these differences were not statistically different (F_{1,3} = 0.01; P>0.05; Table 7).

AFDM and chlorophyll-a are correlated $(R=0.72)$ indicating that a proportion of the chlorophyll-a detected in the detrital matter is algal derived. Given the strength of the relationship between these parameters it is not surprising then, that there are similar relationships between these biological parameters and the environmental variables (Table 8).

AFDM and chlorophyll-a concentrations declined with increasing riffle velocity (R=-0.88 and -0.84 respectively) (Table 9). Previous rounds have suggested moderate relationships between periphyton and water quality, but no such relationships are apparent in the current study. Substrate composition appeared to have a minor role in the distribution of periphyton, with weak relationships being detected for AFDM and chlorophyll-a. Bedrock appeared to provide a stable environment for AFDM and chlorophyll-a, whereas AFDM declined as sand increased $(R=0.42)$, and chlorophyll-a declined as pebbles increased in the riffle substrate $(R=0.61)$.

Table 7. Nested analysis of variance results for chlorophyll-a and AFDM concentrations

Table 8. Pearson's correlation coefficients between mean AFDM, mean chlorophyll-a concentrations and the most important environmental parameters (based on the strength of the correlation)

Figure 6. The distribution of a) chlorophyll-a; and b) Ash Free Dry Mass (AFDM) upstream and downstream of Angle Crossing

Strip chart values (in blue) represent the raw data values for each site. See APPENDIX C for an explanation of how to interpret box and whisker plots.

3.4 **Macroinvertebrate communities**

3.4.1 Riffles

The non-metric multidimensional scaling (NMDS) plot of the riffle macroinvertebrate data shows one distinct cluster which contains all of the upstream sites, which represents 70% similarity within this group (Figure 7). Variation of the taxonomic composition between locations (i.e. upstream and downstream) was significantly different, based on the ANOSIM results (R=0.76; P=0.05; APPENDIX C). The high R statistic in this case indicates that the similarities within each level of group "location" are more similar to one another than they are to other samples in the other levels of the group location – in other words, upstream samples are more similar to other upstream samples than they are from samples taken downstream. There are also very different dispersions among groups. Upstream sites are much less dispersed than the downstream sites (Figure 7), which is confirmed by the PERMDISP analysis ($F_{1,28} = 39.51$; P<.0001).

Taxa that contributed to the location differences in this analysis are shown in Table 9. Approximately 70% of the dissimilarity between these two locations was due to differences in the mean abundances of 15 taxa. These taxa ranged in their SIGNAL sensitivity ratings from 2-8, although most were considered moderately tolerant with scores in the 4-6 range.

As in previous sampling runs, taxonomic richness was reasonably consistent across sampling sites (Figure 8). The number of families collected from this assessment ranged from 18 (at MUR 16) to 27 (at MUR 15). The number of genera collected was in a similar range with 24 collected at MUR 19 and the most, 34 collected at MUR 15. The number of these taxa representing the EPT suite of invertebrates ranged from 7-9 families and 11-16 genera. MUR 15 had the most number of EPT families and genera, while MUR 19 (downstream of angle Crossing) had the least.

Patterns in taxa abundances show, that while the distribution EPT taxa was relatively even across all of the sites, there were considerable differences in their relative abundances (Figure 9) and there is an apparent trend of decreasing EPT taxa with longitudinal distance downstream. EPT taxa made up approximately 38% of the community composition at MUR 15, while at MUR 19 on average only 3.8% of the community abundance was comprised of EPT taxa. This pattern is largely driven by decreasing numbers of Hydropsychidae (SIGNAL $=6$) and a reciprocal increase of Simuliidae (SIGNAL $=5$).

Simulids dominated the macroinvertebrate community at MUR 19 and contributed almost 50% to the total abundance estimated for this site. Simulids were also highly abundant at MUR 23, but there was an almost ten–fold increase in the number of Hydropsychidae compared to MUR 19. Despite this, common mayflies remained low in number at MUR 23 (and MUR 19) compared to MUR 15-18. Baetidae (SIGNAL $=$ 5) and Leptophlebiidae (SIGNAL=8) were common and relatively abundant upstream of Angle Crossing, but became sparse and less abundant at MUR 19 and the otherwise ubiquitous Caenidae (SIGNAL=4) was completely absent from MUR 19.

MUR 19 and MUR 23 stand apart from the main group and for different reasons. MUR 19 is dominated by two taxa: Simuliidae and Oligochaeta, which together comprise up to 72% of the total community abundance at this site. MUR 19 also lacks some of the lower level (genus) taxonomic diversity that all the other sites contain, especially within the Caddisfly order (Trichoptera) and perhaps most obvious in the family Hydrobiosidae (SIGNAL= 8) and

Hydroptilidae (SIGNAL $= 4$) (see Table 10 for groups comparisons of mean abundances). MUR 23 on the other hand is split into two groups, the first is contained within the main cluster and is approximately 65% similar to the upstream sites. The second group differs in that the samples are dominated by Simuliidae and is lacking of the common mayflies: Baetidae and Caenidae and, like MUR 19 has lower taxonomic diversity in the Trichoptera group.

The results from the BIO-ENV analysis show a moderate relationship between the observed macroinvertebrate similarity matrix (Bray-Curtis similarity) and riffle current velocity $(\rho=0.49)$; % pebbles (in riffle) $(\rho=0.45)$ and % gravel (in riffle) $(\rho=0.4)$. The combination of the best three variables resulted in an overall spearman's rho correlation coefficient of 0.59, which was significantly different than would be expected by chance based on 999 permutations. The ten best combinations of environmental variables to the macroinvertebrate similarity matrix are given in APPENDIX D.

Figure 7. Non-metric multidimensional scaling of genus data from the spring riffle samples Ellipses represent the 70% similarity groups

Note: Green dots represent upstream sites, blue squares represent downstream sites

Table 9. Results from the SIMPER analysis comparing location effects upstream and downstream of Angle Crossing

Figure 8. Total number of taxa at genus and family levels in the riffle and edge habitats Note that MUR 28 was not sampled in spring 2010

3.4.2 Edges

Taxonomic richness was highest at MUR 15 where 27 families and 35 genera were collected (Figure 8). MUR 19 recorded the low count of taxa at family and genus level. There were no obvious patterns across sites or no obvious location difference, other than the low number at MUR 19, which is immediately downstream of Angle Crossing.

The ordination analysis shows that the multivariate variation (dispersion) between locations is approximately the same for both locations (Figure 10), which is confirmed by the PERMDISP analysis $(F_{1,22} = 0.56; P=0.51)$. ANOSIM results from the edge macroinvertebrate community data indicate no difference between locations (R=0.08; P=0.33), which is consistent with the ordination plot, where it can be seen that sites from both locations form one large cluster containing three sites that are approximately 65% similar (Bray-Curtis distance). The remaining clusters contain sites from specific locations, but MUR 19 (downstream of Angle Crossing) is grouped alone, and some distance from the other sites. Overall, the edge habitat was dominated by tolerant taxa with moderate SIGNAL-2 scores Chironomids and Oligochaetes were the two dominant groups making up to 70% of the total community abundance (i.e. MUR 19). Communities could not be differentiated by ANOSIM analysis because the number of shared taxa and the overall structure between locations was very similar.

The location of MUR 19 in the ordination space (Figure 10) is due to much lower diversity than the other sites, as indicated in Figure 7. Many of the common edge taxa are either completely absent from MUR 19 or poorly represented; these taxa included: Corixidae (SIGNAL=2), Caenidae (SIGNAL=4); Gyrinidae (SIGNAL=4) and Dysticidae (SIGNAL=2). Taxa that characterized the remaining sampling sites tended to have moderate SIGNAL-2 scores. The most dominant taxa across all of the edge communities were Orthocladiinae (SIGNAL $=4$) and Simuliidae (SIGNAL=5), followed by Chironominae (SIGNAL $=3$) and Oligochaeta (SIGNAL=2).

There were fewer individual taxa from the EPT group in the edge samples compared to the riffles and lower diversity at the family and genus level. Most of the usually common EPT taxa, including Leptoceridae (SIGNAL=6); Baetidae and Caenidae, though not absent from these sites were poorly represented. Leptoceridae was absent from MUR 16, 18 and 19. The most prolific of these (EPT) taxa were the Hydroptilidae (SIGNAL =4) at MUR 15 $\&$ 16.

The BIO-ENV results indicate a strong relationship between the observed macroinvertebrate similarity matrix (Bray-Curtis similarity) and a combination of five, best fitting variables, namely: mean current velocity; % boulder, % pebble; % gravel and the per cent composition of shrubs in the riparian zone given a combined Spearman's correlation of $p=0.72$, which was significantly different from zero $(P=0.001;$ APPENDIX D). While this was the best fit the most parsimonious model had three variables and was almost as good as the five-variable model ($p=0.71$; P=0.01). The combined variables in the parsimonious model were: % Sand; mean current velocity and pH. The best selections from the BIO-ENV analysis are given in APPENDIX D.

Figure 10. Non-metric multidimensional scaling of genus level data from spring edge samples

Ellipses represent the 65% similarity groups

Note: Green dots represent upstream sites, blue squares represent downstream sites

3.5 **AUSRIVAS assessment**

The AUSRIVAS assessment for the riffle habitat shows no change at MUR 16, MUR 18 or MUR 19 since autumn, although direct comparisons on a season by season basis indicates that MUR 15, 16 and MUR 23 have improved riffle habitat assessments than they did in spring 2009 (shifting from BAND B to BAND A (Table 12). By comparison, MUR 18 and 19 returned BAND B assessments, which is what they have been since spring 2009. A full list of taxa, predicted to occur from the AUSRIVAS model, but were absent from each habitat are presented in APPENDIX E.

There was no difference between the observed to expected ratios between sites upstream and downstream of Angle Crossing $(F_{1,3} = 1.29; P=0.33; Figure 11)$ despite a slight higher average upstream of Angle Crossing (mean =0.94) compared to downstream (mean=0.86). The effect of location accounted for only 6% of the total variation within the dataset, while site to site variation within location had an estimated variance component of 52%. Similarly, there were no statistical differences detected between locations for the SIGNAL-2 scores (F_{13}) $= 0.025$; P=0.88; Figure 11). The highest SIGNAL- 2 on average were recorded at MUR 18 (mean=5.2), while the lowest was at MUR 15 (mean $=4.79$), however there was a very narrow range across all sampling sites (see Table 12 for detail).

The only obvious pattern relating to missing taxa across sampling sites was the absence of the more sensitive taxa from most sites. For example, Elmidae (SIGNAL=7) was missing from 95% of samples, while where predicted, Psephenidae (SIGNAL=6) was missing from all samples. Other taxa included Gripopterygidae (SIGNAL=8) and Glossomatidae (SIGNAL=9); the later only being collected at MUR 15 in one of the samples. Taxa with low to intermediate SIGNAL-2 scores tended to be absent from selected samples, but were generally not entirely missing from a given site. The exception to this rule was Caenidae $(SIGNAL = 4)$, which was absent from MUR 19.

There were no differences found between locations in either the O/E 50 scores ($F_{1,3} = 0.095$; P=0.78; Figure 12) or the SIGNAL-2 scores ($F_{1,3} = 0.001$; P=0.97; Figure 12) based on the edge AUSRIVAS modelling. Reliable site assessments were problematic for the edge samples, as there was significant within-site variability in O/E50 scores at certain sites, most likely due to the effects of high flow disturbance on edge habit in this section of the Murrumbidgee River. For instance, there are wide confidence intervals for O/E50 scores for edge habitat at sites MUR 15 (OE/50: $0.66 - 1.22$) and MUR 18 (OE/50: 0.55-1.00) (Figure 12). This degree of variability makes determining appropriate AUSRIVAS model bandings less reliable. Hence, no overall AUSRIVAS site assessment is provided as part of this study for MUR 15 and MUR 18. However, the site-based assessments for the remaining sites indicated a decline in condition at MUR 16 and MUR 19, which were assessed as BAND C and no change in the condition at MUR 23 (Point Hut crossing) compared to spring 2009. These comparisons also apply to autumn 2010, when all sites were assessed as BAND B.

Missing taxa from the edge samples spanned a wide range of SIGNAL -2 scores, ranging from the tolerant Corixidae (SIGNAL=2) to the highly sensitive Leptophlebiidae and Gripopterygidae (both SIGNAL=8). There are no clear patterns in the table of missing taxa (APPENDIX E), suggesting perhaps that the absence of these taxa and hence the resulting AUSRIVAS bands are due to stochastic processes rather than a specific cause across the sampling sites.

The poor community structure in the edge habitat is also reflected in the Average SIGNAL-2 scores across sites, which tend to be lower than the riffle samples (Table 12). The lowest, average was recorded at MUR 19 (mean =3.4), whereas the highest was recorded at MUR 23 $mean = 4.4$.

Table 10. AUSRIVAS and SIGNAL scores for spring 2010.

*NRA =No Reliable Assessment

Figure 11. Average AUSRIVAS OE/50 scores (top) and average SIGNAL-2 scores for riffle samples from upstream and downstream of Angle Crossing

Error bars are 95% confidence intervals

Figure 12. Average AUSRIVAS OE50 scores (top) and SIGNAL-2 scores for the edge samples from upstream and downstream of Angle Crossing

Average AUSRIVAS OE50 scores (top) and S

Error bars are 95% confidence intervals

4 Discussion

4.1 **Water Quality**

Sustained periods of high flows through spring resulted in similar values for most of the water quality parameters assessed in this study (Table 7). Turbidity remained above ANZECC and ARMCANZ guidelines for prolonged periods during spring (Figures $4 \& 5$), as a result of ongoing rainfall and catchment runoff. pH exceed the upper threshold of 8.0 during the first week of October and only at the gauging station, upstream of Angle Crossing. The reason for this is unclear, however, despite these values exceeding the water quality guidelines, the pH values are within the range seen throughout this project and indeed in ALS's historical records for this section of the Murrumbidgee River.

The results from the grab samples are comparable to the continuous records, although pH and turbidity levels were within the guidelines at the time of sampling (Table 7), which is due to sampling occurring at the tail of the recession curve on the hydrograph (Figure 2). Total phosphorus (TP) was twice the guideline values across all sampling sites, while Total nitrogen (TN) was a bit more variable across sites.

The total nitrogen values collected in this round of sampling are similar to those collected autumn 2010, which was a period of low, stable flow. While in spring 2009, following a high flow event we found TN levels to be up to five times the concentration of the current study. This demonstrates firstly that despite the current results being collected on the receding limb following extended periods of high flows, the TN levels are within the same range as they are during periods of low, stable flows, suggesting that the background levels within this section of the catchment are high because of the land use history in the catchment.

4.2 **Periphyton**

Although nitrogen and phosphorus are often a limiting factor in plant growth (Allan and Castillo, 2008), there was no relationship between AFDM and chlorophyll-*a* with the nutrient data, or in fact any of the water quality parameters in this study. High flows may have masked any relationship between the periphyton and nutrient data because site to site variation in the nutrient concentrations was less prominent than autumn, when there were strong trends detected between these variables. Current velocity was negatively correlated to both AFDM and chlorophyll-*a*, suggesting that concentrations decrease with increasing velocity (Table 8). On this basis, both AFDM and chlorophyll-*a* should be expected to increase downstream of Angle Crossing relative to upstream reaches under the M2G Project, given that flows will be reduced from current levels downstream of Angle Crossing due to water abstraction.

In this study, there was no evidence of a location effect on chlorophyll-a concentrations or AFDM (Figures 6a and 6b). Although this result is consistent with all other sampling runs to date, comparisons between the current quantitative findings and previous sampling seasons show much higher concentrations of both parameters upstream of Angle Crossing in the current study than in previous studies (Ecowise, 2009; ALS 2010). It is likely that these higher concentrations, particularly at MUR 15 and 16 have increased through periodic nutrient fluxes since the largest flow event that occurred in October. Since then, flows have been relatively stable albeit high, thus perhaps allowing biomass accrual during this period.

The increased AFDM and chlorophyll-*a* concentrations may be an indication that TP is the limiting factor for algal growth (despite the absence of any correlation between the variables as previously indicated) given that TN levels are remarkably similar to previous studies, where AFDM and chlorophyll-*a* concentrations have been markedly lower during much lower base flow conditions. The consistently high concentrations of chlorophyll-*a* and AFDM at MUR 23 (Point Hut Crossing) (Figures 6*a* & 6*b* respectively) are comparable to previous studies (Ecowise 2009; ALS, 2010) showing a very similar spatial pattern. The explanation for these apparent elevated concentrations remain as they have for the

previous studies – that frequent spillages from Point Hut Pond (APPENDIX F) are likely to deliver nutrients to this site at a more constant rate because of the highly urbanised upstream catchment area.

4.3 **Macroinvertebrate communities and AUSRIVAS assessment**

There was some evidence to suggest the upstream riffle communities differed from the downstream communities (Figure 7; APPNDIX C). However, this result should be interpreted with a certain degree of caution because the resulting p-value is on the cusp of the *a-priori* alpha value and therefore provides no firm evidence to neither accept the null hypothesis of no difference nor reject it with a high degree of certainty. Another consideration is the absence of MUR 28 from the analysis, which, based on data from all previous assessments, generally clusters with MUR 16 and 18 at approximately 65% similarity. Finally, this result is complicated by the significantly different multivariate dispersions detected between locations, which can be seen in Figure 7. As previously mentioned, ANOSIM can be sensitive to different among group dispersions (Anderson *et al*., 2008); therefore, the following interpretation of this particular result should take the preceding information into consideration.

The location difference from the riffle data indicates that around 70% of the dissimilarity is based on the cumulative effect of the mean abundance of 15 taxa (Table 9). These taxa had mainly low to intermediate water sensitivity (SIGNAL-2) scores, which indicate that there were no differences in the average abundance of highly sensitive taxa (i.e. sensitive taxa were generally poorly represented in this study irrespective of the location). Generally, there were increased abundances of Simuliidae (SIGNAL =5) and Oligochaeta (SIGNAL =2) downstream of Angle Crossing. Indeed, 72% of the total estimated abundance at MUR 19 consisted of these two taxa (Figure 9) suggestive of two processes. Siltation following the high rainfall, in combination with the following high flows is the likely explanation for the high abundance of Oligochaetes (Hogg and Norris, 1991) because, being sediment dwellers, they are less vulnerable to the impacts of high flow events.

Other contributing taxa to the overall location difference included 8 taxa from the EPT group, including sediment tolerant Caenids (SIGNAL=4) and the usually highly abundant Hydropsychidae (SIGNAL=6). It should be pointed out, that although Table 9 indicates these taxa as discriminating taxa between locations, the main reason for this is their absence and much lower abundance (i.e. Hydropsychidae) at MUR 19. The temporal patterns from the data collected to date show that there is an antagonistic relationship between Simulids and Hydropsycids. Simulids are often outcompeted by Hydropsycids (e.g. Hemphill and Cooper, 1983), but tend to dominate early successional stages of colonisation and become extremely abundant (Radar *et al*., 2008). As more taxa become established, however, they are often outcompeted by Hydropsycids, whose dominance can be enhanced by nutrient enrichment (Hemphill and Cooper, 1983). This pattern is supported in this study, where Hydropsychidae are most abundant at the sites within the highest nutrient readings (MUR 15, 16 and 23), and these sites, conversely, had the lowest Simuliidae abundances.

The ANOSIM results show no evidence of a location difference from the edge samples. The relationships among sampling sites shows that MUR 23 is more similar to MUR 18 (upstream of the Angle Crossing) than MUR 19 which explains the low R –statistic from the ANOSIM output. MUR 19 stands alone in this analysis which represents the low taxonomic diversity (Figure 8) across all sensitivity levels. It should be noted however, that only one edge sample was possible from MUR 19 and as such taxa richness may have been underestimated since it (richness) usually increases as a function of increasing sample size (e.g. Vinson and Hawkins, 1996).

Both highly tolerant and high sensitive taxa were absent from most of the edge samples, which were generally characterised by Orthocladiinae (SIGNAL =4); Chironominae (SIGNAL=3); Simuliidae (SIGNAL=5); Hydroptilidae (SIGNAL=4) and Oligochaetes. These taxa dominated most edge communities, but in varying rank-abundances between sites, which is why ANOSIM detected no difference between the communities. The main feature of the edge communities was the absence of freeliving taxa, namely: Corixidae (SIGNAL = 2); Dysticidae (SIGNAL = 2) and Gyrinidae (SIGNAL = 4) which are not only tolerant to changing water quality, but are usually very common and highly abundant in the edge habitat. The absence of these taxa across all sites in particular strongly suggests flow-related

impacts, such as scouring, sedimentation and high shear stress as the main factor contributing to the observed macroinvertebrate assemblages. Further evidence of flow related disturbance comes from the fact that these taxa were most abundant at MUR 15 and MUR 18, which had the lowest mean velocities at any of the sites. Correlation analysis from the BIO_ENV routine also suggests the patterns in the multivariate structure are related to differences in current velocity for both the riffle and edge habitats (APPENDIX D), but other factors, such as the influence of substrate are also likely to be intrinsically related.

There was no difference in either the AUSRIVAS scores or the SIGNAL-2 scores between upstream and downstream locations for the riffle (Figure 11) and edge (Figure 12) samples. The AUSRIVAS assessment suggests that there was some improvement in the ecological health assessment at MUR 15, 16 and 23 on a season by season basis since spring 2009. However, due to the high within site variation in the edge samples there was no overall sites assessment for MUR 16 or MUR 18.

The "no reliable assessment" attributed to the edge samples is a consequence of the patchy assemblages from the edge samples, which are also due to the effects of high flow related disturbances (Barmuta *et al*.,). Following high flow events, community assemblages commonly contain fewer, and lower abundances of common taxa compared to undisturbed communities (Niemi *et al*., 1990). There appeared to be a lack of structure or pattern in the edge samples compared to previous sampling events. The patchiness in the data set can indicate that the communities are essentially random assemblages of taxa that: happened to persevere during the periods of high flow; are adapted to faster flowing water; or are early colonists following natural disturbances (Lake, 2000; APPENDIX D).

Despite the edge habitat being problematic, the riffle habitat shows signs of improved ecological health at three of the five samples sites (Table 10). MUR 15, 16 and 23 were all assessed as BAND A, or close to reference condition. MUR 18 and 19 remained at BAND B, which is consistent with previous sampling runs. The improved assessment at MUR 15, 16 and 23 resulted from several taxa, including Baetidae (SIGNAL=5), Leptophlebiidae (SIGNAL =8) and Hydropsychidae (SIGNAL=6) being collected in this study, but were absent from these sites in spring 2009. It is likely that despite a period of high flow leading up to this sampling event, the relative stability of the flow regime allowed these taxa to reestablish since the large high flow event in October (Figure 2). The short time since the event in spring 2009 meant that these communities were likely still in a very early post-disturbance phase, meaning that the aforementioned taxa were still absent from the samples.

The high variation (dispersion) in the macroinvertebrate assemblages observed at MUR 19 and MUR 23 (Figure 7) are likely due to their location in this sampling program. Both sites differ from the others in that they are the only ones which have potential point source impacts that have the potential to influence the ecology at each site. MUR 19 is situated immediately downstream of a low level crossing and is flanked by dirt roads on either side; whereas MUR 23 is downstream of the Point Hut spillway. The implications of this is that over and above the direct impacts of high flow events, such as those experience during this study, the impacts of runoff from flanking dirt roads and receiving waters from an urban lake spillway are likely to influence the physical within-site characteristics in different ways such that the macroinvertebrate assemblages become more varied compared to those at sites which are not influenced by additional factors (Hynes, 1970; Lake, 2000). The broader implication of this is that it may, in the end, be difficult to separate the impacts of these types of disturbances from the potential impacts of reduced flows downstream of Angle Crossing under the M2G, or that these disturbances might create significant variability within these sites to mask actual impacts associated with reduced flows.

Once flows in the Murrumbidgee River stabilise, the re-colonisation of edge benthic macroinvertebrates communities should be relatively quick (e.g. Radar et. al., (2008)). However it should be noted that full recovery can take up to four weeks, which is the recommended time to resume sampling following a high flow event under the AUSRIVAS protocols (Coysh et al. 2000a). Once there has been a sufficient recovery period, there should be an increase in sensitive taxa (diversity and abundance), including those from the EPT suite of taxa and the normally ubiquitous free living taxa that are adapted to slow waters, which were missing from this assessment.

5 Conclusions

The results from this study are indicative of the patterns that would be expected following or during periods of high flow and heavy rainfall. The high flows that have affected all of the sampling sites in this study have had a homogenising effect on most water quality parameters so that the values were similar across sampling sites. Nutrient concentrations (TP and TN) were above the recommended upper limit. TN has been above the guidelines since the inception of this sampling program over a range of hydrological conditions, which suggests that background levels within the limits of this program are likely to be high due to the history of land-use practices in the catchment. The exceeded concentrations of TP are related to ongoing rainfall and high flows. During low stable flows TP in these reaches of the Murrumbidgee River, TP levels stay within the guidelines.

It is likely that periphyton responded to high nutrient concentrations over the spring period especially at the upper most sites; but were perhaps keep in check by ongoing high flow events and hence subject to periods of removal through scouring, which is suggested by the correlation analysis and the strong negative relationships with increased velocity.

The AUSRIVAS-based riffle habitat assessment given to three of the five monitoring sites indicate an increase at three of the monitoring sites and no change at MUR 18 and 19 (either side of Angle Crossing). Macroinvertebrate communities from both habitat types, but particularly the edge habitat, were characterised by taxa which are indicative of both high flow disturbance (REF) and early colonisation patterns following disturbance. The riffle communities during this sampling period displayed patterns seen in previous sampling runs during or following high flow periods, which included a dominance of taxa with low to intermediate sensitivity ratings and highly sensitive taxa tended to be sparse and patchy in their distribution. The edge samples lacked many of the common free living taxa that usually inhabit slow moving water. The macroinvertebrate communities in the edge habitat were highly unstructured and patchy, which lead to highly variable AUSRIVAS assessments. The main reason for this is believed to be prolonged seasonal high flows. Periods of stable flow should allow the edge communities to re-establish in future sampling events.

6 Recommendations

The recommendations made in autumn 2010 (ALS, 2010) are again supported for this assessment, and are detailed below.

- 1. The high within-site variation found in this and previous sampling runs suggest that a single replicate is not adequate to capture the variation in composition at a given site. This is consistent with the findings of (Nichols *et al.*, 2006), who recommended taking replicate samples at impaired sites for biological assessments. Taxonomic diversity and abundances differed considerably between replicates and subsamples especially downstream of Angle Crossing, where point source impacts are likely to increase the variation in the physical habitat resulting in considerable variability in the AUSRIVAS bio assessment of a given site. It is recommended that this level of replication be maintained throughout Phase 1 and Phase 2 sampling.
- 2. The additional information gained from the BIO-ENV routine suggests flow and substrate relationships between the community structures as a whole. We recommend maintaining this analysis in the Angle Crossing component of the MEMP project. Additional hydrological metrics should also be considered for incorporation into this project at some stage. This would provide a more detailed assessment of how the communities and even individuals respond to the seasonal flow dynamics that have been observed throughout the course of this project to date. Doing so would enhance ACTEWs ability in making informed decisions regarding flow rules in order to reduce potential environmental impacts related to the M2G project.

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Appendix A –

Potential effects of reduced flow and their knock-on effects on habitat conditions and macroinvertebrate communities

Summary of the effects of reduced flows on various habitat conditions and macroinvertebrate communities from recent literature (Dewson et al. 2007)*. *Reproduced with permission from the authors.

Appendix B –

Interpreting box and whisker plots

Appendix B. Interpreting box and whisker plots.

Box and whisker plots are intended as an exploratory tool to help describe the distribution of the data. The blue points on the inside of the plot area indicate the raw data values that make up the distribution portrayed in the boxplot. The plot below explains how the box and whisker plots should be read.

* The interquartile (IQR) range is the difference between the $25th$ and $75th$ percentile. This value is important when two sets of data are being compared. The closer the values are to the median, the smaller the IQR. Conversely, the more spread out the values are, the larger the IQR.

Appendix C – ANOSIM output for riffle and edge samples

ANOSIM Analysis of Similarities

Two-Way Nested Analysis

RIFFLE

TESTS FOR DIFFERENCES BETWEEN Site GROUPS (across all # Location groups) Global Test Sample statistic (Global R): 0.75 Significance level of sample statistic: 0.1% Number of permutations: 999 (Random sample from a large number) Number of permuted statistics greater than or equal to Global R: 0 TESTS FOR DIFFERENCES BETWEEN # **Location** GROUPS (using Site groups as samples) Global Test Sample statistic (Global R): 0.758 Significance level of sample statistic: 5% Number of permutations: 210 (All possible permutations) Number of permuted statistics greater than or equal to Global R: 1

EDGE

TESTS FOR DIFFERENCES BETWEEN # Site GROUPS (across all # Location groups) Global Test Sample statistic (Global R): 0.963 Significance level of sample statistic: 0.1% Number of permutations: 999 (Random sample from 392392000) Number of permuted statistics greater than or equal to Global R: 0 TESTS FOR DIFFERENCES BETWEEN # **Location** GROUPS (using # Site groups as samples) Global Test Sample statistic (Global R): 0.087 Significance level of sample statistic: 33.9% Number of permutations: 56 (All possible permutations) Number of permuted statistics greater than or equal to Global R: 19

Appendix D – BIO-ENV output for riffle and edge samples

RIFFLE

```
Rank correlation method: Spearman 
Method: BIOENV 
Maximum number of variables: 5 
Resemblance: 
Analyse between: Samples 
Resemblance measure: D1 Euclidean distance 
Variables 
   1 Mode Stream width 
   2 mean riffle depth 
   3 mean velocity 
   4 Sqr(BOULDER) 
   5 PEBBLE 
   6 GRAVEL 
   7 SAND 
   8 Water temp. 
   9 EC 
  10 D.O (% Sat.) 
  11 Turbidity 
  12 Alkalinity 
  13 Ammonia 
  14 TP 
 15 TN 
Global Test 
Sample statistic (Rho): 0.585 
Significance level of sample statistic: 0.1% 
Number of permutations: 999 (Random sample) 
Number of permuted statistics greater than or equal to Rho: 0 
Best results 
No.Vars Corr. Selections 
       3 0.585 3,5,6 
       3 0.570 3,6,13 
       4 0.566 3,5,6,12 
       5 0.561 3,5,6,8,13 
       4 0.560 3,6,12,13 
       4 0.553 3,5,6,13 
       4 0.542 3,5,6,9 
       4 0.538 3,6,8,13 
       5 0.538 3,5,6,9,13 
       5 0.538 3,5,6,12,13
```


EDGE

Appendix E –

Taxa predicted to occur with >50% probability but were not collected in the spring samples

APPENDIX E. Taxa expected, but not collected in the riffle habitat. The number in each cell is the probability of collection

*np = Not predicted at 50%

APPENDIX E (cntd.). Taxa expected, but not collected in the edge habitat spring 2010

Appendix F–

Point Hut Pond Hydrograph: 2010

Appendix F. Point Hut Pond and Lobb's Hole Hydrograph showing mean daily flows (in Cumecs) for 2010. Sampling seasons are highlighted (blue for autumn and green for spring)

