The lower Broken Creek : aspects of water quality and growth of *Azolla* species.

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The Murray-Darling Freshwater Research Centre

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A report prepared for the Goulburn Broken Catchment Management Authority by the Murray-Darling Freshwater Research Centre.

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Cover Photograph: *Azolla* bloom upstream of Rices Weir wall, 10 November 2006.
(Photograph by Karina Hall)

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Executive Summary

Background and project outline

Historically, the lower Broken Creek was an ephemeral creek with water derived from natural catchment flows. The construction of a series of small weirs and changes to flow regulations have altered the creek, with the lower section now comprising a series of weir pools. Rices Weir is the last weir before the creek enters the Murray River and is noted for having reasonably poor water quality. Poor water quality (low dissolved oxygen - DO) was considered a major factor that contributed to a fish death event in 2002. In addition, other consequences of anaerobic waters, namely, potential increase in hydrogen sulfide, ammonia and other products of microbial metabolism, also were considered as possible factors compounding the problems of low dissolved oxygen.

A further characteristic of the Broken Creek is the extensive growth of the floating fern *Azolla*. The *Azolla* blooms can occur over many 100s of meters, up to kilometres and will have the capacity to greatly alter the ecological condition of the creek and to have a major impact on the overall water quality of the creek.

A conceptual model to explain aspects of *Azolla* growth and water quality involves the fixation of carbon and nutrients by Azolla, but these are deposited into sediments on death, only to be recycled into water column, thus stimulating phytoplankton growth and subsequent growth of *Azolla*.

This study addresses the following key questions to test the validity of the conceptual model:

- What standing biomass of *Azolla* in present in the Broken Creek?
- When does *Azolla* grow, at what rate does *Azolla* grow, and what factors drive the bloom and bust cycle?
- To what extent are phytoplankton important in the Broken Creek?
- How important are decomposition processes in the sediments?
- Do sediments play a role in determining water quality in the Broken Creek?
- What are the key water quality parameters and sediment conditions in the Broken Creek?
- Are water quality and sediment characteristics similar across different weir pools of the lower Broken Creek?

Key findings

The amount of *Azolla* biomass per square metre was highly variable and depended on whether multiple layers of plants were present. Estimates derived for Rices Weir showed between 27 and 277 g (dry weight)/m^2 with single layers of plants, which corresponded to 0.3 - 4.2 kg (wet weight)/m^2. *Azolla* growth rates were approximately 8 g (dry mass)/m^2/d, which is equivalent to approximately 120 g (wet weight)/m^2/d. Phytoplankton abundance, (measured as chlorophyll-*a* concentration) was generally between 40-70 µg/L, but a major bloom (240 µg/L chlorophyll-*a*) occurred toward the end of the death phase of the *Azolla*.

Oxygen profiles showed an oxygen gradient from surface to the sediment was present on almost all sampling occasions of the study and that on occasion, the DO at depth could be less than 1 mg/L. High sediment oxygen demand (SOD) were found throughout Rices Weir but the sediment oxygen demand increased up to 3 fold following the decline in *Azolla* biomass. Increases in SOD would then explain the decline in oxygen in the overlying water column. Immediately following the crash of the *Azolla* bloom (about 7th December 2007), oxygen levels in the surface layer remained relatively high, but there was clear decline in oxygen levels at depth (to less than about 2 mg O_2/L). On the next sampling occasion there had been a significant decline in the oxygen throughout the water column again with the lowest levels observed at the sediment water interface. The oxygen levels found in Rices period at this time would be toxic to many native fish. Daytime oxygen levels on the 19th of December had returned to relatively high concentrations and this may be a consequence of the formation of a significant algal bloom.

In addition to changes in oxygen levels, following the decline in *Azolla* biomass, there also an immediate increase in the dissolved phosphorus in the water column, followed by a slight delay by spikes in both ammonia and, to a lesser extent, nitrate. The pulse of nutrients corresponded to a massive algal bloom with the weir pool (with chlorophyll-*a* levels up to about 230 -240 µg Chl-a /L). A second, smaller bloom was observed in late January - early February. The December algal bloom also corresponded to the only time in the study period when gross primary production in the water column exceeded community respiration. Confirmation of the source of nutrients is required as persistent stratification would mean that diffusion of nutrients from sediment would be rather slow. It should be confirmed that nutrients were not from an upstream source.

The overall picture that emerged from the cross-weir pool study was that sediment oxygen demand, carbon loads in the sediments, and water quality are generally similar across the different weir pools. These data combine to show that the overall ecological condition, and linkages between *Azolla* and water quality (particularly DO) are the same throughout the whole of the lower section of the Broken Creek.

The sediments have a major role in determining the nutrient status of the Broken Creek. The system now undergoes regular growth and death cycles of *Azolla* and a large reserve of carbon and nutrient exists within the sediments. On occasions, the DO becomes critically low, and even when this does not occur, sediment respiration is sufficiently high to drive down the DO at depth –to a point where DO can be close to zero. Such an event occurred during and immediately after the death phase of the *Azolla*.

Ammonia levels in the creek were not high enough to be considered toxic. This study did not measure free sulfide in the water but sedimentary sulfur is relatively high and a role for sulfur in the poor water quality of Broken Creek can not be discounted. Be that as it may, O_2 is probably the main factor likely to cause stress to fish. The ability for fish to survive low DO events will be determined by that rate that the decrease in O_2 occurs e.g, if the rate of decline is sufficiently slow then the fish potentially have some capacity to move to other sections of the creek (upstream or down stream). The latter will also depend on the extent of the low DO in the creek as low DO is likely to be present over much of the weir pool.

It is difficult to quantify the carbon loads exported to the Murray. There was some evidence of increase sediment carbon down stream following *Azolla* crash, however movement of *Azolla* is also linked to wind direction as much as flow. Wind events that essentially push the *Azolla* upstream are also likely to lead to increased levels of *Azolla* within Rices Weir, as the *Azolla* will continue to grow. The overall effect of the wind will be that *Azolla* export is likely to be very variable over short time frames, but when it does happen, large "slugs" of plant material may be moved down stream.

Recommendations:

Predicting low DO events. Regular monitoring of DO and flow manipulation still appears to be the most practical approach to maintain suitable DO in Broken Creek. In situ probes should continue to be the main monitoring tool, but the possible introduction of a photographic system to monitor the spread and condition of the *Azolla* could be considered. Daily visual evaluation of the *Azolla* will assist in determining extent and spread of *Azolla* and may help in predicting critical low DO events.

The key factor responsible for the death of *Azolla* still remains to be determined. Although temperatures do get relatively high, they still do not reach levels considered the be catastrophic for *Azolla*. Ongoing temperature monitoring at fine scales may provide an answer as to why *Azolla* declines.

Azolla removal. Simple removal of the *Azolla* may not necessarily represent an ideal short-term management strategy for the Broken Creek as large algal blooms will almost certainly follow any major manipulation of the *Azolla*. Long-term removal may be of some value, but the carbon and nutrient loads within the sediments are likely to lead to poor water quality for a long period of time. Azolla has very high growth rates and any removal will have to be in excess of the rates presented in this report.

1. Background

Historically, the lower Broken Creek was an ephemeral creek with water derived from natural catchment flows. The construction of a series of small weirs and changes to flow regulations have altered the creek, with the lower section now comprising a series of weir pools. Since their first construction, the weirs have progressively been replaced by new structures, which also include fish passages.

Rices Weir is the last weir before the creek enters the Murray River. A major fish death event occurred in Rices Weir in November 2002, occurring against a backdrop of significantly reduced flow and drought conditions. Although little specific information was available on the water quality at the time, the deaths were thought to be due to very low dissolved oxygen concentrations. In addition, other consequences of anaerobic waters, namely, potential increase in hydrogen sulfide, ammonia and other products of microbial metabolism, were considered as possible factors compounding the low dissolved oxygen (DO).

A further characteristic of the Broken Creek is the extensive growth of the floating fern *Azolla*. The *Azolla* blooms can occur over many 100s of meters, up to kilometres and will have the capacity to greatly alter the ecological condition of the creek and to have a major impact on the overall water quality of the creek..

1.1 Conceptual Model.

It is possible to develop a simple conceptual model that links *Azolla* growth, water quality and fish death events. Firstly, *Azolla* goes through a growth phase, which involves fixation of carbon and nutrients into biomass. During the *Azolla* bloom, oxygen levels in the water column would vary on a diurnal basis, representing changes in the relative rates of photosynthesis and respiration. However, if the bloom catastrophically crashes, most of the fixed carbon and nutrients will be delivered to the sediments, which should then stimulate sedimentary heterotrophic respiration. Increases in sediment oxygen demand should lead simultaneously to a decline in oxygen in the overlying water column and a pulse of nutrients from the sediments. (It is possible that this pulse of nutrients may stimulate a subsequent algal or macrophyte bloom). The decline in oxygen itself can be linked to fish mortality; alternatively sediment anoxia can also release potentially noxious materials from the sediments – particularly sulfides and/or ammonia - at levels that can prove toxic to fish.

1.2 Knowledge gaps.

In a previous report, Rees (2006) reviewed the literature relevant to *Azolla* and examined the potential of *Azolla* to alter the ecological condition of Broken Creek. While collating a significant amount of information on *Azolla*, the latter report also recognized 3 key knowledge gaps in understanding the ecological condition of the Broken Creek, that ultimately limited understanding, hence the validity of the conceptual model that links *Azolla* growth, water quality and fish death events:

- *Sediment chemistry.* The sediments in the lower Broken Creek are likely to be a major source of carbon and therefore are likely to have significant oxygen demand, thus affecting the overall water quality. Furthermore, it is likely that the sediments also are a major pool of nutrients, especially phosphorus and nitrogen. Measures of sediment oxygen demand and nutrient composition should be carried out so that long-term estimates can be made of the capacity of the sediments to deliver nutrients to the water column. Sediment analyses will establish whether nitrogen, phosphorus and products of sulfate-reduction are present in sufficient quantities and appropriate form to have an important role in the nutrient status of the weirs. Sediment analyses also will help establish if there is a possible point source responsible for the low DO or whether the DO decreases are "driven" by carbon and nutrients within the sediments.

- *Nitrogen and Phosphorus dynamics*. The links between different N species, P and *Azolla* growth is not known. Since nutrients are generally high in the Broken Creek, phosphorus control may remain the only nutrient-management strategy that is likely to have any long-term effect.

- *Azolla growth dynamics. Azolla* biomass estimates have been made at irregular intervals and simply based on subjective visual estimates of the percentage cover. Quantitative measures of the *Azolla* biomass should be considered in the future, but will require reasonably rigorous sampling over a wide spatial and temporal range. Initial effort could be expended within Rices Weir, which would establish factors in the "worst case scenario", but measures also would need to be carried out up stream.

1.3 Current study

This report summarises the results of an intense sampling programme that was carried out over part of the summer of 2006-2007, in order to address the knowledge gaps outlined above and to test the conceptual model of the functioning of the Broken Creek. For the most part, the field activities concentrated on Rices Weir as this was seen as representing the "worst-case" scenario, being the last of the weirs on the creek. A single cross-weir comparison was carried out to examine whether the ecological responses were generally similar across all the weir pools in the lower section of the Broken Creek. This report addressed the following key questions:

- What standing biomass of *Azolla* in present in the Broken Creek?
- When does *Azolla* grow, at what rate does *Azolla* grow and what factors drive the bloom and bust cycle?
- To what extent are phytoplankton important in the Broken Creek?
- How important are decomposition processes in the sediments
- Do sediments play a role in determining water quality in the Broken Creek?
- What are the key water quality parameters and sediment conditions in the Broken Creek?
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Discussion external to the main focus of this report canvassed the potential for using *Azolla* as a feed stock (Wayne Tennant, Geoff Earle –personal communication). Since no data were available on the composition of Azolla in the Broken Creek system, a series of compositional analyses were carried out on Azolla harvested in December 2006.

2. Methods

2.1 Azolla biomass and growth estimates

2.1.1 Biomass estimates. *Azolla* was harvested from randomly-sampled 30cm quadrats, placed in paper bags and dried to constant weight. For wet weight measurements, harvested *Azolla* plants were placed in a 1mm sieve for 5 minutes and excess surface moisture was allowed to run through the sieve. Plants were placed in a paper bag and weighted. Harvesting methods that involved horizontal movement of traps over the surface waters were not successful in capturing *Azolla* as this method generated a small wave at the front of the harvesting device and the *Azolla* was simply pushed out of the trap.

2.1.2. *Azolla* growth rates. Experimental enclosures of known area (approximately 1 m^2) were constructed of poly-pipe (Figure 1). Enclosures were modified with a guard to prevent inadvertent transport of *Azolla* in to the enclosures once they were deployed. Experimental enclosures were deployed in pairs, all *Azolla* was removed from both enclosures then one enclosure was re-seeded with a known amount of *Azolla* while its control enclosure was left empty. Photographs were taken over time of the extent of cover in each enclosure, to allow non destructive measurement of *Azolla* biomass –biomass estimates during the growth phase were determined by conversion factors determined separately. When enclosures were full all *Azolla* plants were harvested and the amount biomass was determined by weighing. Biomass estimates and rates were scaled to derive approximate loads of *Azolla* in Rices Weir.

Figure 1. Growth enclosures designed to allow *in situ* measurement of *Azolla* growth rates.

2.2 Gross primary production and community respiration

Gross primary production (GPP) and community respiration were estimated using a modification of the diel dissolved oxygen (DO) method (Odum 1956), as described by Young and Huryn (1996). DO probes were deployed for 24 hours, recording DO and temperature at 10 minute intervals. Light meters provided measures of incident irradiation at the same time periods as the oxygen probes. The rate of change of oxygen concentration (dO_2/dt) was expresses as a function of three processes:

$$\frac{dO}{dt} = AE_t^p + kD + CR$$

Where AE_t^p describes the relationship between production and irradiance, with p = photosynthetically active radiation (PAR) at time t. The gas exchange between the atmosphere is described by the constant k at an oxygen deficit D. CR is the community respiration, and is assumed to be constant throughout the incubation period. GPP was then corrected for changes in temperature and estimates made during periods of daylight (Young and Huryn, 1996). The final presentation of GPP and CR shows GPP as a positive increase, generating O_2 through photosynthesis, whereas respiration will be shown as a negative value, indicating oxygen consumption. Net production is obtained by subtracting respiration from GPP – a negative value indicting that the system is net heterotrophic.

2.3 Physical and chemical analyses

Dissolved oxygen and temperature were obtained from hand held multi-probes. Depth profiles were obtained by manual deployment of the hand-held probes. An additional series of temperature measures was obtained directly from an *in situ* probe operated by Goulburn-Murray Water. Water quality nutrient samples were analysed using standard methods (APHA 1995) at the Murray-Darling Freshwater Research Centre, which operates under national standards of quality control and quality assurance (NATA). Sediment carbon, nitrogen, phosphorus and sulfur were carried out at the Southern Cross University (Lismore). Organic matter content was determined as sample mass loss on ignition (LOI) at 550°C for 2 hours.

3. Results and Discussion

3.1. Standing biomass of *Azolla*

3.1.1 Growth period of *Azolla*

Previous observations have been made by a range of GM-Water staff and have usually been based on simple observations of the percentage cover within the water body. These observations indicate that *Azolla* generally occurs in fringes through out autumn and early winter. *Azolla* then generally starts to show some level of proliferation towards the end of winter and reaches a peak by October/November. *Azolla* then goes through a dramatic decline, to a point where only occasional fringes are present over the hotter periods of summer, through to the next major growth cycle. The actual extent that *Azolla* grows each year has varied since regular observations have been carried out. The growth and bloom cycles in the summer of 2006-2007 generally followed the pattern reported for previous years, with *Azolla* undergoing a major decline soon after the commencement of the project (Table 1). A regrowth event occurred during January 2007, which did not lead to extensive coverage normally seen earlier in the growth phase, but was sufficient to give total coverage over areas up to several hundred square metres.

Table 1 Sampling dates and descriptions of *Azolla* coverage observed during November 2006 – February 2007.

Sampling trip date	*Azolla* coverage and condition
9-10 November 2006	Approximately 100 % *Azolla* coverage
1-17 November 2006	Approximately 100 % *Azolla* coverage.
23-24 November 2006	Approximately 90 % *Azolla* coverage. ~10% of *Azolla* in dying phase
30 November-1 Dec 2006	Approximately 90 % coverage but most of the *Azolla* in dying phase
7-8 December 2006	Most *Azolla* dead. Only remnant patches along edges.
14-15 December 2006	Most *Azolla* dead. Only remnant patches along edges.
19-20 December 2006	Most *Azolla* dead. Only remnant patches along edges.
17-18 January 2007	Post die-off phase
24-25 January 2007	Post die-off phase. Some new-growth patches
1-2 February 2007	Post die-off phase. Some new-growth patches
7-9 February 2007	Post die-off phase. Some new-growth patches
	Cross-weir survey carried out.

3.1.2 *Azolla* biomass estimates

Discharge in Broken Creek was maintained low throughout the sampling period but was sufficient to lead to down stream transport of *Azolla*. However, after the first few field trips it became clear that wind direction and intensity had a major effect on the coverage and distribution of *Azolla* within the weir itself and that these factors had a more immediate effect on the distribution of *Azolla* than flow. As a consequence, distribution of *Azolla* within the weir could change over hourly time periods, making it difficult to quantify the loads in the weir. *Azolla* plants were generally present in single or double layers, but could accumulate to several layers thick in parts of the creek where limited surface flow occurred. Variability in the degree of coverage and the constant change made experimental design difficult and capturing periods when discrete sections of the water body either had complete or no coverage of *Azolla* could not always be predicted, leading to wide ranges in the measured amounts of *Azolla* (Table 2).

Wet weights estimates provided a valuable measure of biomass per square metre and were scaled to give estimates of the amount of *Azolla* that may be in Rices Weir at any one time. For example, the biomass that would exist over an arbitrary selected 600m reach would be approximately = 69.5 tonnes[1].

Table 2. Estimates of the mass of *Azolla* present in Rices Weir, summer 2006-2007, derived from actual biomass measurement outlined in the methods.

Average mass and range of *Azolla* (kg/m^2)	Amount when *Azolla* present in single layers:	Amount when *Azolla* present with 2 layers depth*
Average dry weight of *Azolla*	0.127	0.284
Range in dry weight	0.021 – 0.277	0.210 – 0.469
Average wet weight *Azolla*	1.93	4.32
Range in wet weight	0.31 – 4.21	3.19 -7.12

* Number of layers was estimated visually.

Reported maximum yields of *Azolla* vary widely and are likely to be due to a combination of factors, such as the wide range of growth and experimental conditions in each study, the different methods used to measure growth and even possible variation between different species (Table 3). Biomass accumulation within Rices weir generally show similar values with those reported in the literature. These estimates provide a useful measure that can be scaled to provide total loads within Rices Weir and the extent that *Azolla* will be responsible for downstream transport of carbon an nutrients.

Table 3. Yields of *Azolla* spp. reported in the scientific literature.

Species	Yield - dry weight kg/m^2	Yield - wet weight kg/m^2	Reference
A. pinnata	0.24	3.4	Gopal (1967)
A. filiculoides	0.16 – 0.23	NR	Talley et al (1977)
A. pinnata	NR[1]	0.72 – 0.9	Tran and Dao (1973)

1 NR. Not reported.

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[1] Assume average width = 60m and average wet weight of 1.93 kg Azolla per m^2. Details on biomass determination appear in the methods section above.

3.2. Primary production in Rices Weir

3.2.1 *Azolla* production

This study started during the decline phase of *Azolla* and was not able to measure growth rates of *Azolla* during the main proliferation stage of the *Azolla* growth cycle. However, an unexpected regrowth period occurred in late January to early February 2007, allowing estimates of *Azolla* production to be carried out within enclosure experiments. The range and average *Azolla* production rates, calculated from the experimental enclosure experiments were:

- 0.8 – 11.9 g (dry mass) /m^2/day, with an average rate of 8.1 g (dry mass) /m^2/day.
- 12 – 180 g (wet weight) /m^2/day , with an average 123 g (wet weight) /m^2/day

Literature reports on the productivity of *A. pinnata* and *A. filiculoides* have ranged over 6.9 – 11 and 7.2 – 10 g/m^2/d respectively in outdoor mass culture experimental systems (Vincenzini et al., 1985). The values obtained in Rices Weir are well within literature values.

3.2.2. Phytoplankton biomass

Chlorophyll-*a* was very high in Rices Weir (Figure 2), ranged from 30 – 70 µg/L with a very large peak between 230 - 240 µg/L detected on the 20th Dec 2006 and a further significant peak occurred towards late January 2007. Water samples taken on 8th Dec 2006 were dominated by Euglena, with Trachelomonas also present. In addition, *Mallomonas splendens*, an unidentified Cryptomonad and Pediastrum species, and a large flagellate similar to *Merotrichia* also were present. The samples taken on 20th December 2006 were similar to the previous sampling, but the large flagellate similar to *Merotrichia* had increased in number. Also present was an unidentified Anabaena species . The dominance of flagellate inparticular is generally coincident with waters containing high levels of nutrients. By way of comparison, chlorophyll-*a* in the Murray River at Barmah forest typically range over 20 -38 µg/L (Oliver and Merrick, 2006), thus representing levels lower than the maximum in the Broken Creek.

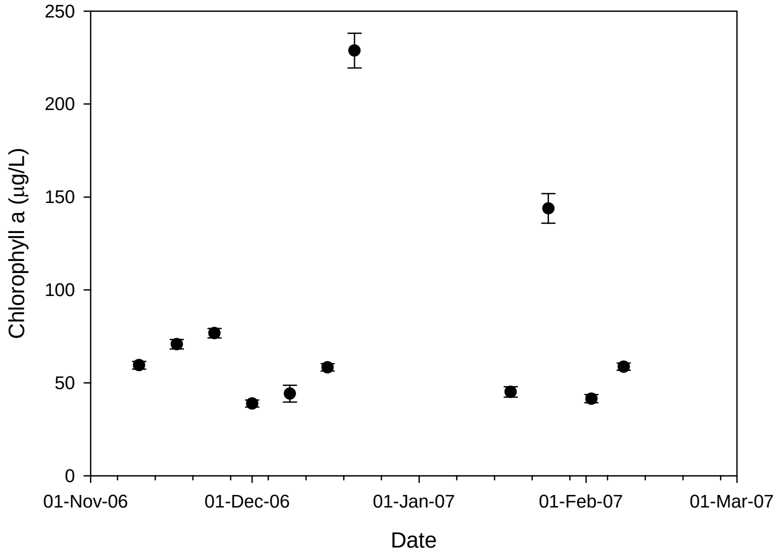


Figure 2. Chlorophyll-*a* concentrations in Rices Weir. Error bars indicate standard error.

3.2.3 Gross primary production and community respiration

Gross primary production (GPP) and community respiration (CR) within the water column were measured from November 2006 through to February 2007 (Figure 3). GPP ranged from 1 -3 g $O_2/m^3/d$ whereas respiration ranged from 1 -7 g $O_2/m^3/d$.

GPP is a measure of the total algal production within the water body and community respiration reflects the total respiratory processes within the water body, thus representing a sum of the activities of algae and bacteria within the water column and sediments. In most cases, respiration was greater than GPP, demonstrating that the system was net heterotrophic. GPP did exceed respiration on 14 Dec 2006, but was variable throughout the study and no clear trends over time were apparent. Respiration showed and increasing trend over the study.

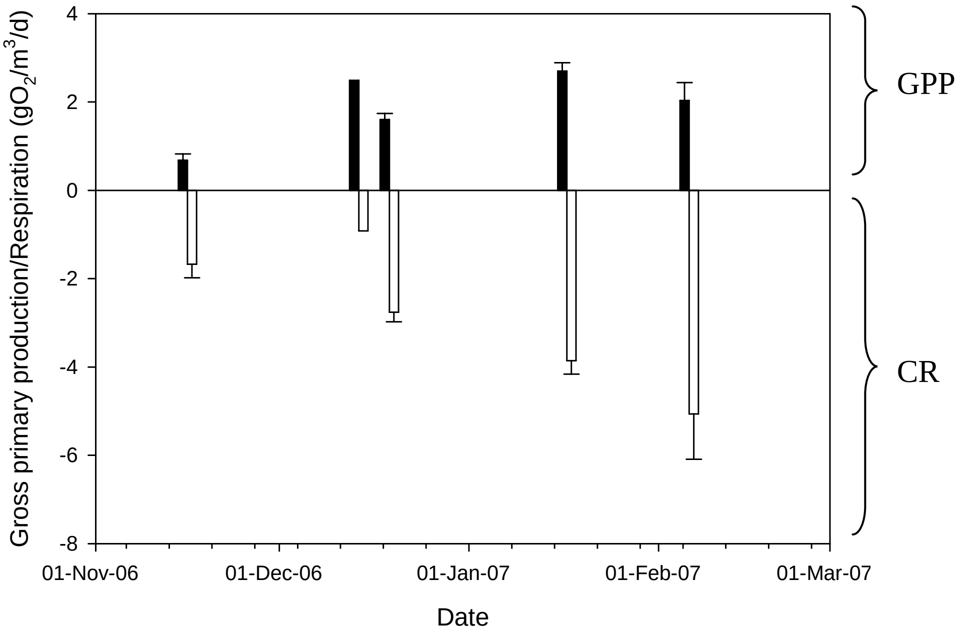


Figure 3. Gross primary production and community respiration in the water column of Rices Weir, Nov 2006-Feb 2007

GPP in the Broken Creek was slightly greater than have been reported for lowland rivers in Australia, depending on site , but the respiration rates were greater in the Broken Creek. Vink et al. (2005) found GPP in the Murrumbidgee River at Gundagai was 0.14 g $O_2/m^3/d$ in winter and increased to 0.22 g $O_2/m^3/d$ in summer. Further down stream, at Darlington Point, GPP was 1.14 g $O_2/m^3/d$ in winter and increased to 2.3 g $O_2/m^3/d$ in summer. Respiration at Gundagai was 0.31 and 0.91 g $O_2/m^3/d$ in winter and summer respectively, and 3.14 and 1.67 g $O_2/m^3/d$ respectively at Darlington Point. Similar rates have been estimated in the Murray River.

Azolla blooms complicate phytoplankton productivity measures within Rices Weir as calculations to derive GPP require measures of light intensity. *Azolla* blankets cause complete shading of the water column and phytoplankton will have reduced production during the times when they are shielded by *Azolla*, leading to underestimates of phytoplankton production.

3.3. Sediment oxygen consumption in Rices Weir

Sediment oxygen consumption in Rices Weir was highly variable, ranging from 66 mg $O_2/m^2/h$ to approximately 300 mg $O_2/m^2/h$. No trends over time were evident (Figure 4). Sediment oxygen consumption downstream of the weir wall was similarly variable and no clear patterns were seen. There was no significant difference in the sediment oxygen consumption up stream and down stream of the weir wall. These results indicate sufficient carbon loads existing within the reach downstream of the weir wall (discussed in detail later).

The rates of oxygen consumption in Rices Weir sediment were very high, compared with literature values, which include both Australian and overseas studies. Sediment oxygen consumption in the Murray River within the Barmah forest shows a seasonal pattern, ranging from 5.3 mg $O_2/m^2/h$ in winter to 90.7 mg $O_2/m^2/h$ in summer. Oxygen consumption rates in the Broken River range from 12 to 25 mg $O_2/m^2/h$, with similar values typically measured in the Ovens River (Rees et al 2005).

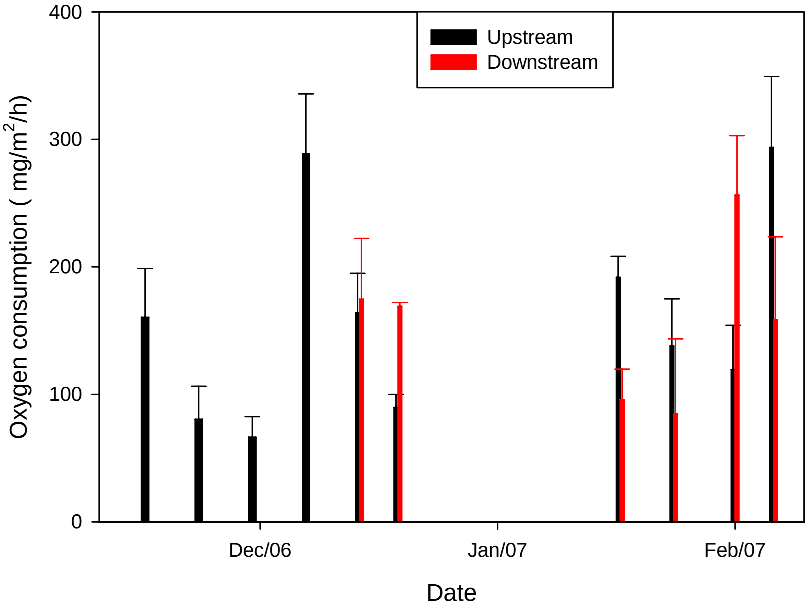


Figure 4. Sediment oxygen consumption in Rices Weir. Sediment oxygen consumption was measured above and below the weir wall from 14 Dec 07.

The overall picture emerging from the sediment respiration experiments is that sediment processes play a major role in the biogeochemistry of Rices Weir and that the sediments have a major role in determining a number of key water quality parameters.

3.4. Oxygen Profiles in Rices Weir

The actual dissolved oxygen concentrations and differences between the top and bottom waters are consistent with the high measures of sediment oxygen demand. Some degree of oxygen stratification was present at all but the last sampling period and in many cases, the difference in DO between top and bottom waters was as great as 4 mg/L (Figure 5, Figure 6). In early December, the DO in the top waters was about 5 mg/L, but strong sediment respiration and obvious lack of mixing lead to DO in the bottom waters as low as 1 -2 mg/L. Bottom waters having DO less than 1 mg/L were still present through the middle of December. Top waters were between 2 and 4 mg/L at this time. High algal activity in late Decembers gave rise to greatly increased DO, although stratification still remained.

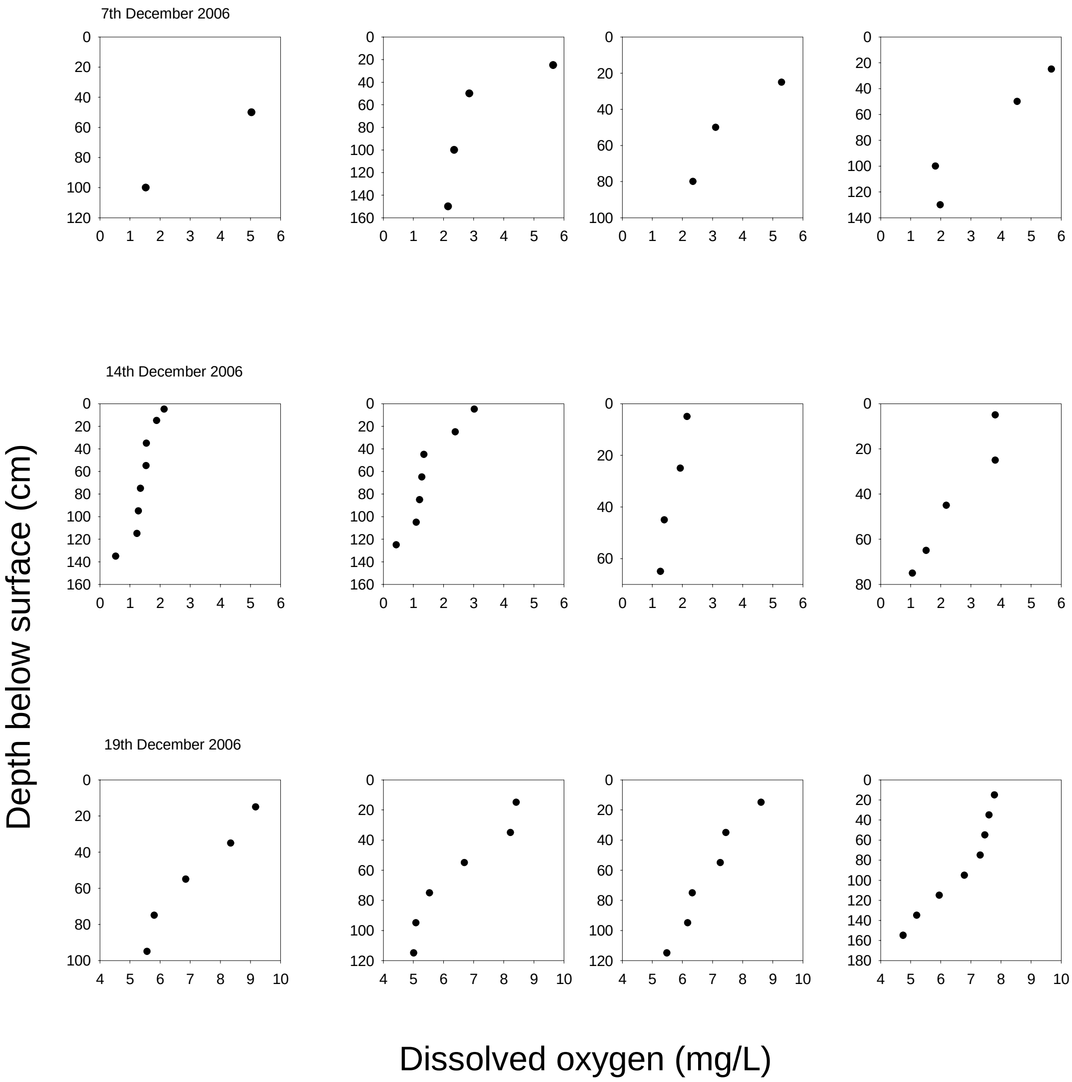


Figure 5. Oxygen depth profiles taken within Rices Weir. Four random profiles were collected at each sample time. The variation in y-axis (depth profiles) is a reflection of the different depth at each of the profiles.

Very low DO persisted at depth throughout the remainder of the study period. By early February, no strong oxygen gradient was present in Rices Weir, but importantly, the DO still remained very low.

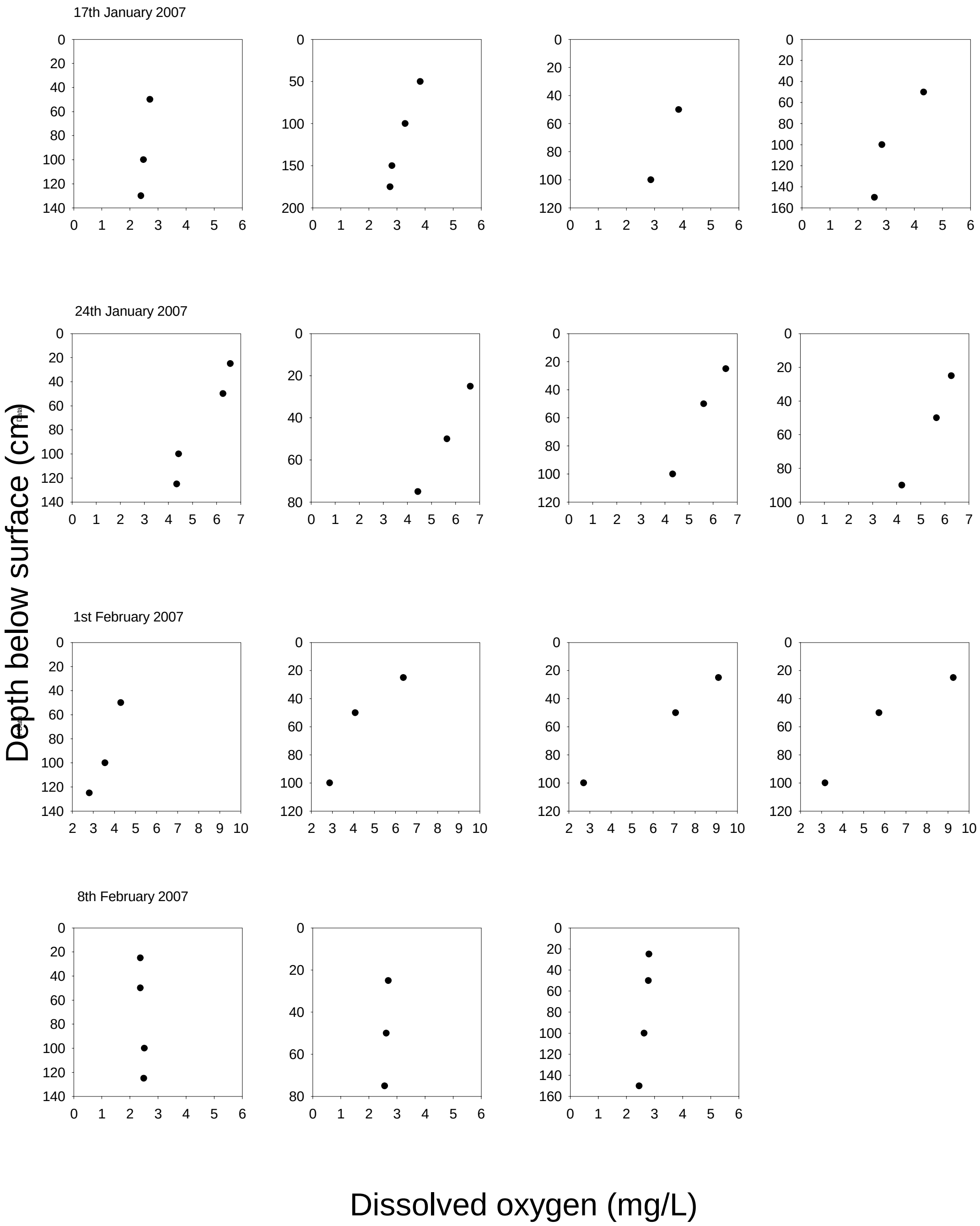


Figure 6. Oxygen depth profiles taken within Rices Weir. Four random profiles were collected at each sample time. The variation in y-axis (depth profiles) is a reflection of the different depth at each of the profiles.

3.5. Sediment chemistry in Rices Weir

3.5.1 Organic matter

Sediment organic matter in Rices Weir (determined as mass loss on ignition at 550^{o} C) was very high, making up between 8 and 16 % of the sediment content (Figure 7). A major input of organic matter occurred downstream in later December. Overall, organic matter content was significantly lower below the dam wall than above the wall (Student t test, p < 0.05).

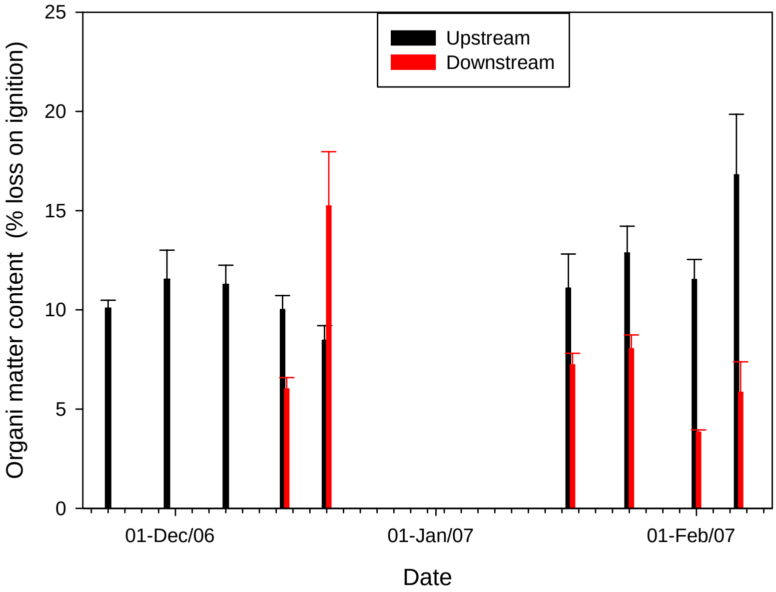


Figure 7. Organic matter content, measured as mass loss on ignition at 550°C. Organic matter content was measured above and below the weir wall from 14 Dec 07.

3.5.2. Sediment carbon, nitrogen, phosphorus and sulfur

Any potential changes in carbon, nitrogen and sulfur in sediments over time were significantly masked by large variability across the samples taken at the end of the study period (Figure 8). This is consistent with other studies where the pool of nutrients in sediments is so large that small, but significant changes in some of the nutrients can not always be seen in data. The large variability in the February samples may be due to a range of factors; some organic matter deposition may indeed be responsible for the large values, lower values may also reflect some breakdown of organic matter. Alternatively, sites with lower levels of organic matter may have been regions within the water body that were not as severely affected by large inputs of dying *Azolla*.

Sediment phosphorus at the end of the study period was significantly lower than at the start (Student t test, $p < 0.05$). A net decrease in sediment phosphorus implies a loss to the water column has occurred, which will happen when sediments become anaerobic, leading to nutrient release from sediments. Again, these data indicate that the sediment processes are having a major effect on the water column chemistry.

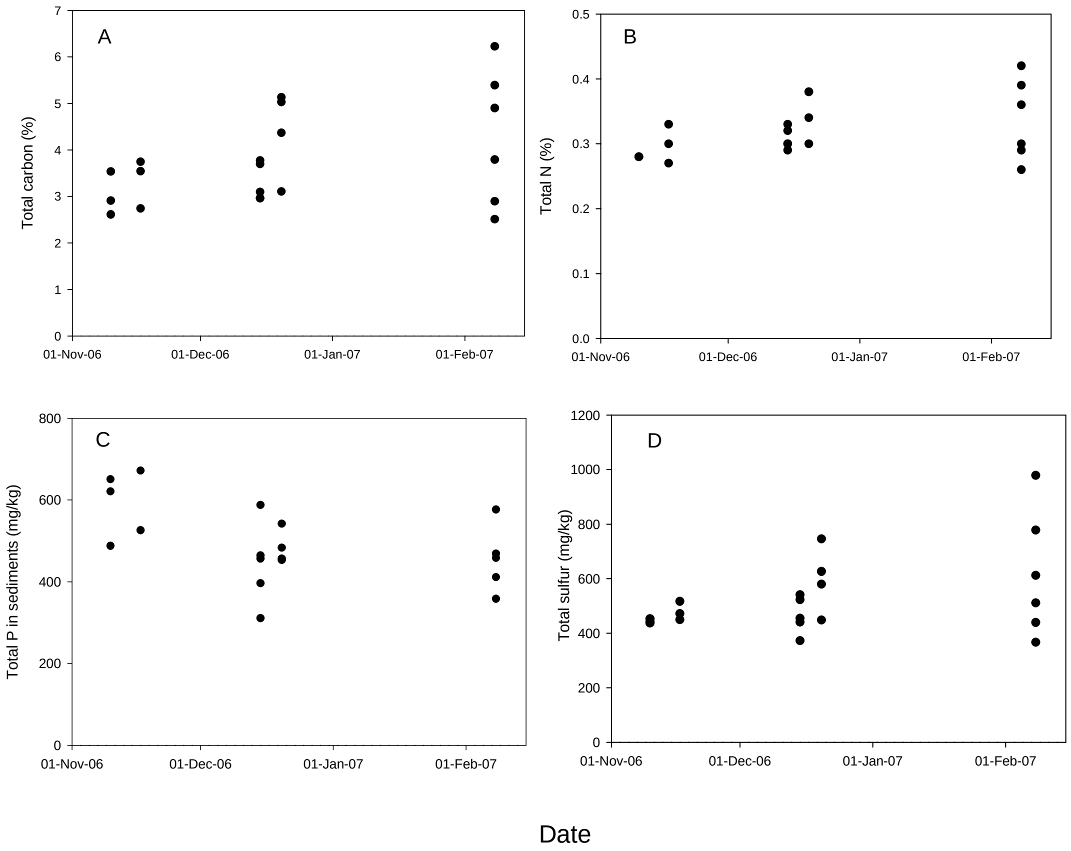


Figure 8. Key elements measured over time in Rices Weir sediments. Panel A shows total carbon, panel B shows total nitrogen, panel C show total phosphorus and panel D shows total sulfur. Samples taken at time 1 on panels B and D include 3 points, but the data points superimposed.

3.6. Water quality.

3.6.1 Water nutrient concentrations

Total nitrogen and total phosphorus were very high throughout the study period (Figure 9). Very large variability occurred between sample events and these changes don't appear to coincide with obvious events such an increase in levels of phytoplankton - reasons for the large variation remain unknown. Ammonia levels in particular were reasonably high throughout the study period, but along with nitrate, showed a major peak on the 14th December 2006. Similarly, phosphate was very high in Rices Weir and also showed a large peak, but the peak occurred in late November, prior to the ammonia and nitrate peaks. The increases in nutrients occurred soon after the most obvious death of *Azolla* and a plausible explanation is that death of *Azolla* is responsible for the increased level of nutrients. The increase may be either directly through the mineralization of organic matter from the plants or through the release of nutrients from sediments, caused by the increased oxygen demand in the sediments and resulting anaerobic conditions within the sediments – nutrients are released from sediments under anaerobic conditions (Boström et al., 1982). The extent of the ammonia and nitrate increase, along with the event occurring over a period of weeks suggests that mineralization of organic nitrogen from dying *Azolla* was an important source of the nutrients.

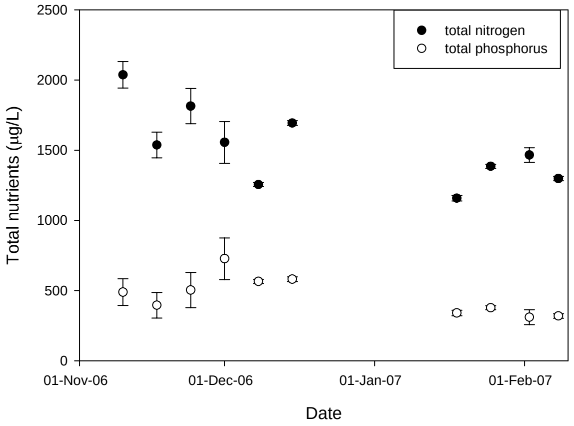


Figure 9. Total nitrogen and phosphorus in Rices Weir, Nov 2006- Feb 2007.

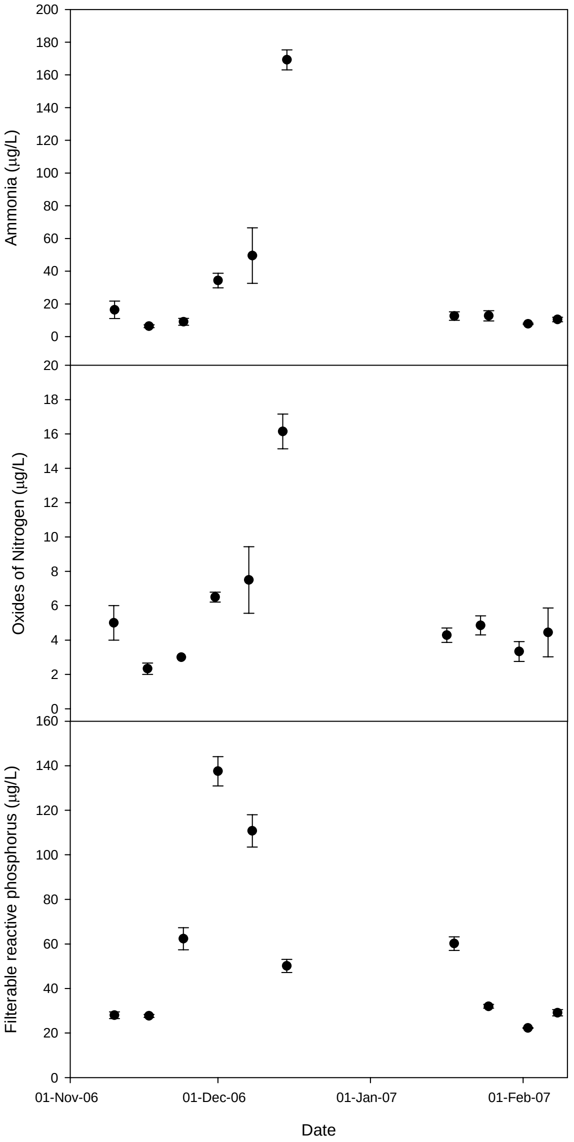
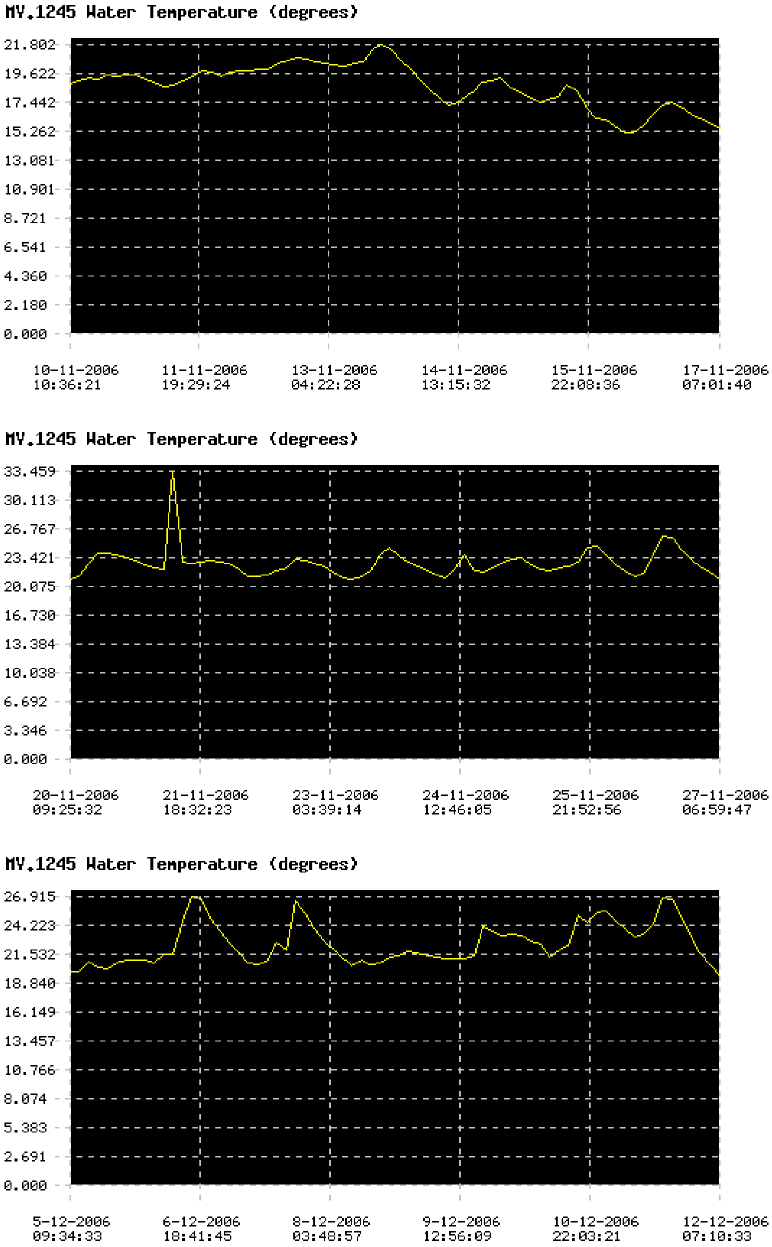


Figure 10. Dissolved nutrients in Rices Weir, Nov 2006- Feb 2007. Top panel is ammonium, central panel are the oxides of nitrogen (combined nitrate and nitrite), bottom panel is filterable reactive phosphorus (phosphate).

3.6.2 Water temperature

3.6.2.1 In situ temperature measurement

Extensive measurements are made of *in situ* temperatures in Rices Weir and a selection from 4 different weeks are highlighted in Figure 11.



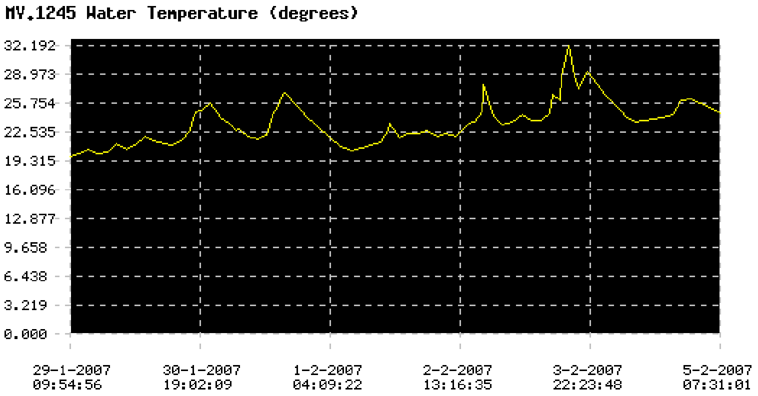


Figure 11. A selection of weekly temperature measurements made in Rices Weir. Data courtesy of Goulburn-Murray Water

There was approximately 100% *Azolla* coverage during early November, when water temperature did not exceed 21-22 °C (Figure 11 - top panel). *Azolla* started to die towards the end of November, when water temperature generally were between 20-26°C. (Figure 11 - second panel). Interestingly, a short-lived temperature increase to 33°C occurred during this stage. Remnant patches of *Azolla* survived when temperatures ranged from 19 -26 °C (Figure 11 - third panel) and a re-growth event occurred when temperatures were ranging from 19-32 °C (Figure 11 - bottom panel). The lethal temperature for *Azolla* is about 37°C and although high temperatures were recorded in Rices Weir, the lethal temperature was not reached. Although a brief high temperature did occur before the death phase, similar temperatures were present when the re-growth event occurred, indicating bulk water temperature alone (as measured with in situ or hand-held probes) is not simply responsible for the death.

Temperature measurements are invariably obtained by in situ probes at depth, or hand held discrete measurements. It became apparent that this scale of measurement may not reflect actual temperatures around the plant fronds and roots, i.e., that differences in temperature may occur over the gradient within an *Azolla* “blanket”. In essence, the *Azolla* may create its own micro-scale glass-house effect. Temperature at this scale may be more valuable in future studies of *Azolla* blooms.

3.6.2.3 Temperature profiles.

The water column showed thermal stratification on all but a few sampling occasions (Figure 12, Figure 13). Very strong stratification was preset on the 7th December, with bottom waters between 7 and 9 degrees cooler than the top waters. Over the following two weeks, the difference was reduced to between 2 and 4 degrees. Only very minor thermal stratification was present in the middle of January (less than a degree difference between top and bottom waters, but stratification did increase through until early February, where bottom waters were up to 4 degrees cooler that top waters.

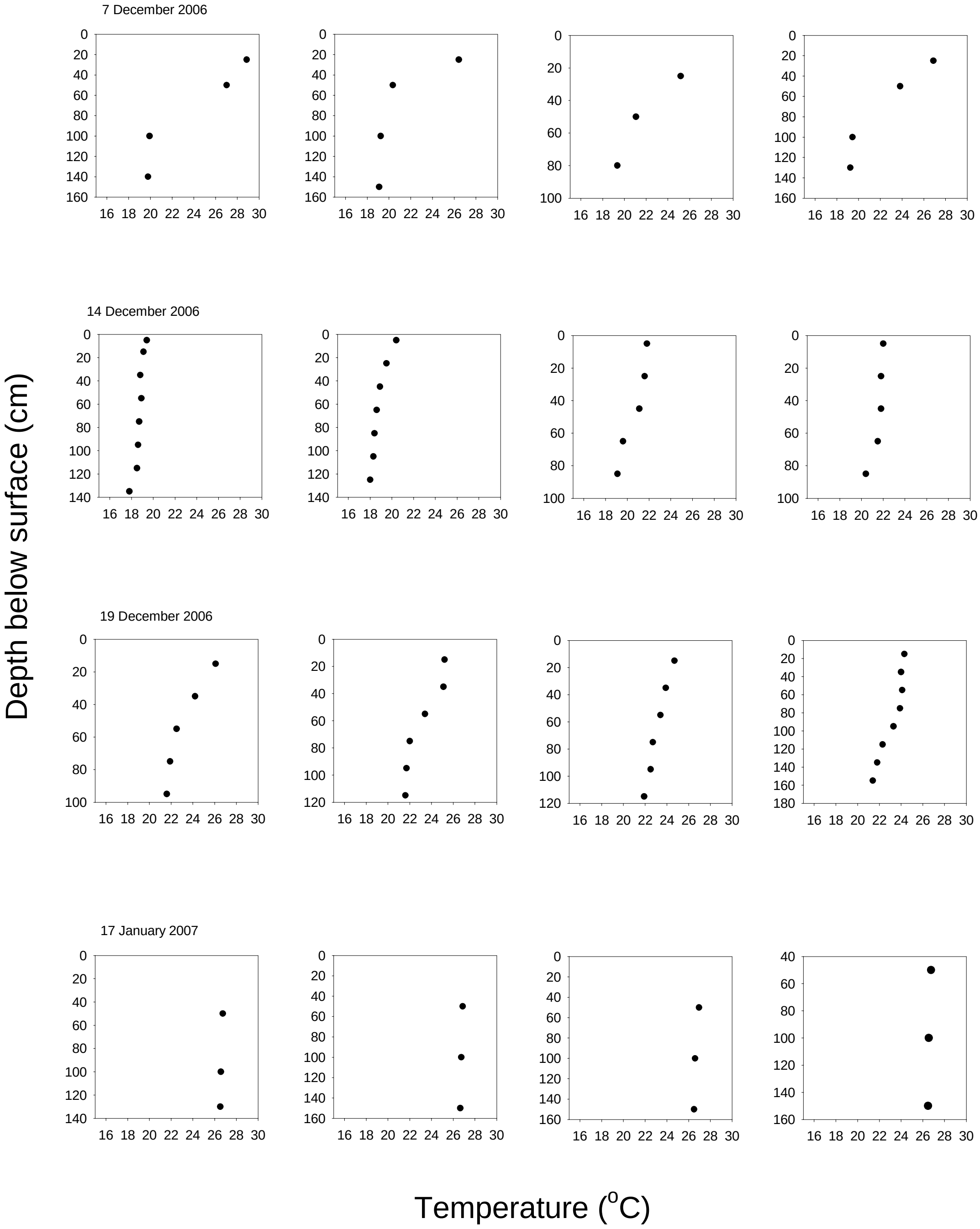


Figure 12. Temperature depth profiles taken within Rices Weir. Four random profiles were collected at each sample time. The variation in y-axis (depth profiles) is a reflection of the different maximum depth at each of the profiles.

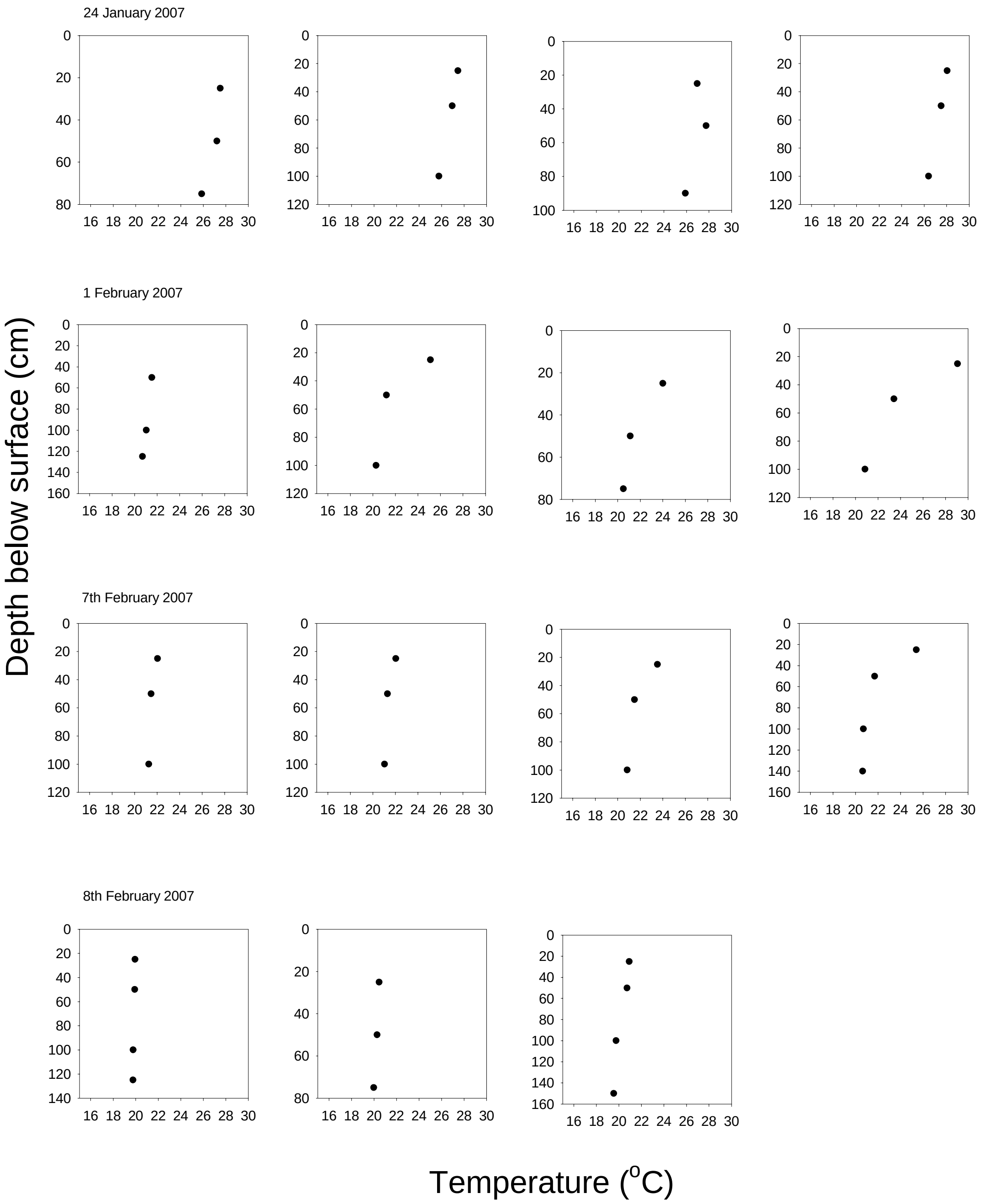


Figure 13. Temperature depth profiles taken within Rices Weir. Four random profiles were collected at each sample time. The variation in y-axis (depth profiles) is a reflection of the different maximum depth at each of the profiles.

3.7. Cross-weir comparison – Rices Weir, Kennedys Weir, Hardings Weir, Luckes Weir.

A single cross-weir comparison was carried out over the 7th -8th February 2007. The aim of the survey was to see if the overall water quality and biogeochemical responses were similar across the different weir pools.

3.7.1 Water quality

3.7.1.1 General water quality

A standard set of water quality parameters, namely, water column salinity (electrical conductivity), turbidity and pH were not significantly different across the 4 weirs (Table 4). The pH, salinity and turbidity were fairly typical of lowland creek systems. Chlorophyll-*a* content did show an increasing trend downstream. Given the magnitude and variability in phosphate concentrations (Table 5), it is likely that the Kennedys, Hardings and Luckes Weirs will show variation in their chlorophyll-*a* concentrations.

Table 4. Key water quality parameters in Rices Weir, Kennedys Weir, Hardings Weir and Luckes Weir. Brackets indicate standard error, n = 9.

	pH	EC µS/cm	Turbidity (NTU)	Chlorophyll a µg/L
Rices	6.79 (0.4)	103	130 (4)	58.7 (1.9)
Kennedys	6.88 (0.07)	117	140 (4)	62.2 (4.4)
Hardings	6.97 (0.07)	81	131 (5)	44.4 (2.5)
Luckes	7.50 (0.16)	101	104 (4)	30.7 (2.4)

3.7.1.2 Nutrients

Total nitrogen and total phosphorus show a increasing trend downstream (Table 5) and may in some part, be a reflection of the increased density of phytoplankton (Table 4). Kennedys Weir had higher concentrations of ammonia, oxides of nitrogen (nitrate) and phosphate. There seems no obvious reason for the increased concentrations of dissolved nutrients in Kennedys, other than we may have sampled this weir following an *Azolla* decaying event; dissolved nutrients in Rices Weir have previously responded to a decomposition event (described earlier in this report). The cross-weir study does show that very high levels of phosphate do exist across the weir pools in the lower sections of the Broken Creek.

Table 5. Nutrient levels in Rices Weir, Kennedys Weir, Hardings Weir and Luckes Weir. Brackets indicate standard error, n = 9 for Kennedys, Hardings and Luckes, and n = 7 for Rices Weir.

Weir	Total nitrogen µg/L	Total phosphorus µg/L	Ammonia µg/L	Oxides of nitrogen (NOx) µg/L	Filterable reactive phosphorus µg/L
Rices	1298 (16)	319 (16)	10.00 (1.42)	4.00 (1.42)	29 (1)
Kennedys	1071 (11)	273 (11)	55.00 (6.32)	12.0 (0.6)	49 (1)
Hardings	952 (6)	213 (6)	10.00 (1.61)	6.00 (0.94)	36 (1)
Luckes	808 (7)	167 (7)	7.00 (0.67)	3.00 (0.94)	31 (1)

3.7.2 Sediment Chemistry

Total carbon, nitrogen, phosphorus and sulfur in sediments were not statistically different across the 4 weir pools (Figure 14).

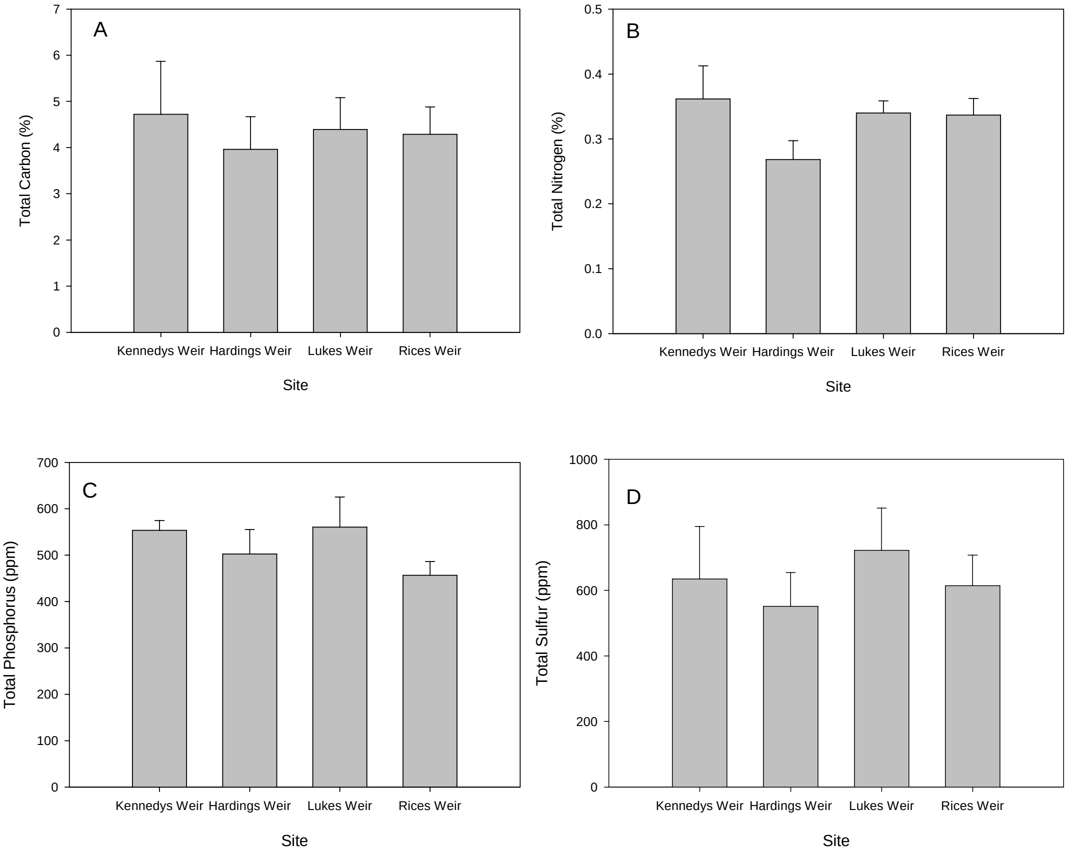


Figure 14. Total carbon (A), total nitrogen (B), total phosphorus (C) and total sulfur (D) in the sediments of Rices, Hardings, Kennedys and Luckes Weirs on 7 Feb 2007.

3.7.3 Sediment oxygen consumption and organic matter content

The sediment oxygen consumption was generally similar across 3 of the 4 weirs, the exception being a lower estimate found in Kennedys (Table 6). The more comprehensive study on Rices Weir (described earlier) showed that large variations in respiration rates could be measured over the spatial scale of Rices Weir and in all likelihood, this situation would exist within Kennedys. In this case, we believe that the sediment oxygen consumption is likely to be very similar across the weir pools, although longer term and more extensive spatial monitoring would be required to confirm this hypothesis.

The amount of organic matter in the sediments was similar in Rices, Kennedys and Luckes Weirs. Although we showed the amount was higher in Hardings, this amount is a very similar magnitude to the other weirs and we don't believe this represents a real difference between the sediments, but is more likely to demonstrate variation between samples. The ratio of respiration rate to organic matter showed considerable variation across the weirs, indicating that that organic carbon alone is not necessarily a good predictor of microbial activity. Rather, the quality or bioavailability of the carbon content is more important. For example, the presence of twigs and decaying *Azolla* in sediments would indicate similar amounts of organic matter, but clearly *Azolla* is more susceptible to decomposition as it contains much lower levels of lignin and other structural polymers.

Table 6. Sediment oxygen consumption and organic matter content in Rices, Hardings, Kennedys and Luckes Weir on 7 Feb 2007.

Weir	Sediment oxygen consumption	Organic matter (% loss on ignition)	Respiration/organic matter ration
Rices	293.75 (55.64)	16.8 (3.1)	17.5
Kennedys	85.21 (19.79)	12.2 (1.8)	6.9
Hardings	271.82 (50.30)	25.9 (2.0),	10.5
Luckes	273.83 (21.92)	13.9 (1.5).	19.7

3.7.4 Oxygen depth profiles.

At the time of sampling, Rices contained the smallest oxygen gradient, although it had the lowest actual dissolved oxygen concentrations (between 2-3 mg/L DO). No obvious trend was apparent across the individual weir pools (Figure 15). For example, the two middle weir pools had the highest dissolved oxygen and some samples within each of the weir pools showed very little in the way of an oxygen gradient at depth. Other than the lower DO in Rices Weir, there is a reasonable degree of similarity in the DO patterns among the weir pools.

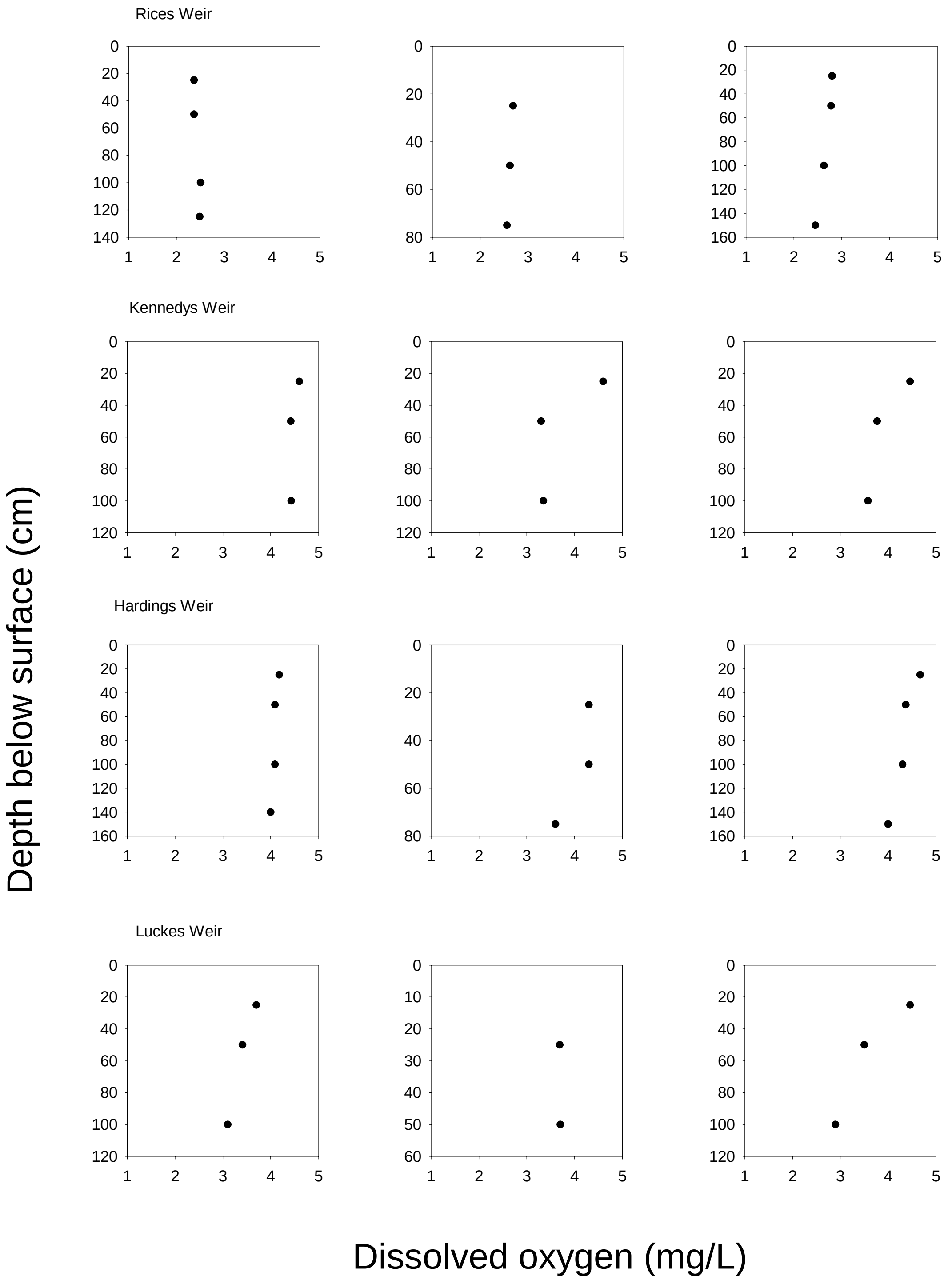


Figure 15. Oxygen depth profiles in Rices, Kennedys, Hardings and Luckes Weir, February 7th, 2007. Variation in the y-axis among different profiles reflects the maximum depth at each of the sample sites.

3.7.5 Temperature depth profiles

Overall, the upper weir pools had higher surface temperatures than Rices Weir, with Luckes Weir surface waters up to 29 °C (Figure 16). Rices Weir showed only minor stratification, unlike the weir pools further upstream. However, significant variation did occur across different samples. For example, in Luckes Weir, differences between top and bottom waters was as high as 7 °C in one sample, but less than 1 °C in a further sample.

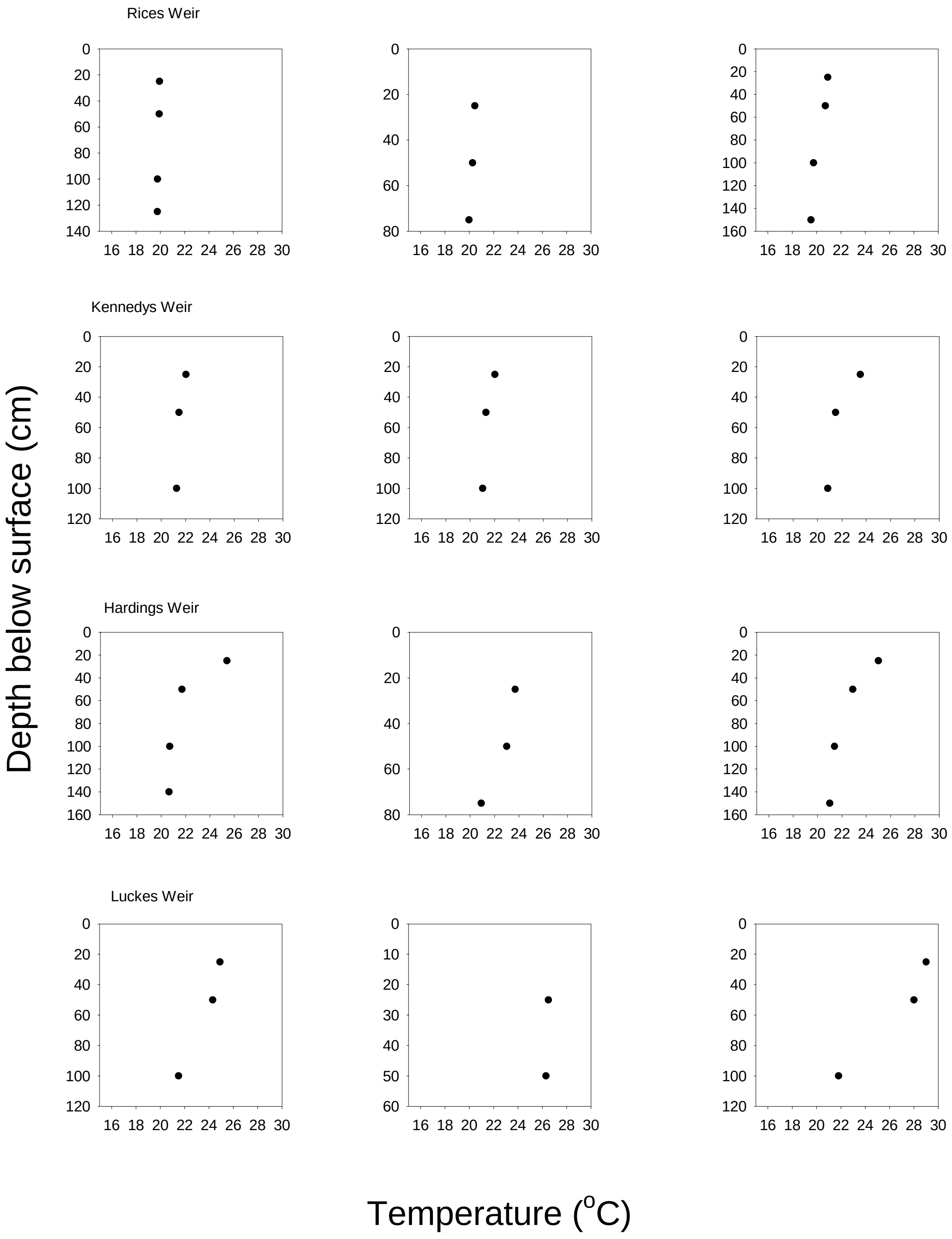


Figure 16. Temperature depth profiles in Rices, Kennedys, Hardings and Luckes Weir, February 7th, 2007. Variation in the y-axis among different profiles reflects the maximum depth at each of the sample sites.

The degree of stratification was generally more consistent within Hardings Weir but in Kennedys, only one of the three samples showed a gradient greater than 1 °C.

3.7.6 Summary of cross-weir study

The cross weir survey showed that some small variations exist between the different weir pools, but in general, the overall water quality and biogeochemical processes within the weir pools are not greatly different. One clear difference was the increase in total nitrogen and phosphorus down stream. Reason for this are not known at this stage, but may be reflecting the capture of nutrients within phytoplankton biomass.

The cross-weir study shows very high levels of phosphate are present across the lower sections of the Broken Creek. *Azolla* has a nitrogen fixing symbiont and it is less reliant on external sources nitrogen for growth. The high phosphate concentration in the waters will be one of the main factors contributing to the growth of *Azolla*.

4. Synthesis of results

4.1 Implications for the conceptual model

The results of the current study are consistent with the conceptual model described at the beginning of this report. *Azolla*, dominated the water column at the beginning of the study period, with kilometre reaches of Rices Weir being covered by a mat of *Azolla*. (Table 1). However, by the beginning of December the bloom was in decline and by the end of the first week of December only remanent patches of *Azolla* remained in the weir pool. Currently it is not possible to ascribe the exact cause for the dramatic decline in the bloom, as, at least on first appearances, bulk conditions in the weir pool did not exceed the reported physiological tolerances of *Azolla* (Lumpkin and Plunkett, 1980). However, there is some evidence, that while the net temperature in the weir pool water column did not exceed the temperature tolerance of *Azolla*, the temperature in the micro layer immediately below the *Azolla* bloom (depths less than a few centimetres) may have been higher than in the water column. It is hypothesised that this increase in temperature is caused by a combination of the insulating properties of the continuous *Azolla* mat, minimizing heat exchange across the air water inter face and, given the incredible amount of biomass present in the weir pool, the heat generated by the bloom through physiological activity (respiration and photosynthesis). It is recommended that an additional study be undertaken during the next summer period to examine micro-scale shifts in water column temperature under dense *Azolla* mats to determine if temperature may in fact be the principle mortality agent.

Notwithstanding the cause of the blooms demise, the crash of the bloom coincided with significant changes in sediment and water chemistry within the weir pool. While it was not possible to detect increases in total nutrient concentrations within the sediments in the weir pool, there was some evidence for an increase in sedimentary carbon concentration downstream of the weir (see latter). More importantly there were significant changes in the sediment oxygen demand and nutrient levels in the overlying water column.

Sediment oxygen demand increased up to 3 fold following the decline in *Azolla* biomass (Figure 4). Increases in SOD would then explain the decline in oxygen in the overlying water column. Immediately following the crash of the *Azolla* bloom (7 December 2007), oxygen levels in the surface layer remained relatively high, but there was clear decline in oxygen levels at depth (to less than about 2 mg O_2/L). On the next sampling occasion there had been a substantial decline in the oxygen throughout the water column again with the lowest levels observed at the sediment water interface. The oxygen levels found in Rices period at this time would be toxic to many native fish. Daytime oxygen levels on the 19th of December had returned to relatively high concentrations and this may be a consequence of the formation of a substantial algal bloom. A further consideration is the physical shielding offered by the Azolla blankets. Under such conditions, the Azolla will be very effective is preventing wind action from mixing the water column, this contributing to an enhanced oxygen gradient at depth.

In addition to changes in oxygen levels, following the decline in *Azolla* biomass, there also an immediate increase in the dissolved phosphorus in the water column, followed by a slight delay by spikes in both ammonia and, to a lesser extent, nitrate. Release of nutrients from sediments under anoxic conditions is well documented in the literature (Boström et al, 1982). The pulse of nutrients corresponded to a massive algal bloom with the weir pool (to with chlorophyll-*a* levels up to about 230 µg Chl-a /L) . A second, smaller bloom was observed in late January - early February. The December algal bloom also corresponded to the only time in the study period when gross primary production in the water column exceeded community respiration – hence explaining the reoxygenation of the water column in late December (see above).

4.2 Comparisons between weir pools

The overall picture that emerged from the cross-weir pool study was that sediment oxygen demand, carbon loads in the sediments, and water quality are generally similar across the different weir pools. These data combine to show that the overall ecological condition, and linkages between *Azolla* and water quality (particularly DO) are the same throughout the whole of the lower section of the Broken Creek.

4.3 Implications for fish deaths

The sediments have a major role in determining the nutrient status of the Broken Creek. The system now undergoes regular growth and death cycles of *Azolla* and a large reserve of carbon and nutrient exists within the sediments. On occasions, the DO becomes critically low, and even when this does not occur, sediment respiration is sufficiently high to drive down the DO at depth –to a point where DO can be close to zero. Such an event will occur during and immediately after the death phase of the *Azolla*.

Ammonia levels in the creek were not high enough to be considered toxic. This study did not measure free sulfide in the water but sedimentary sulfur is relatively high and a role for sulfur in the Broken Creek can not be discounted. Be that as it may, O_2 is probably the main factor likely to cause stress to fish. The ability for fish to survive low DO events will be determined by that rate that the decrease in O_2 occurs e.g, if the rate of decline is sufficiently slow then the fish potentially have some capacity to move to other sections of the creek (upstream or down stream). The latter will also depend on the extent of the low DO in the creek as low DO is likely to be present over many meters up stream.

4.4 Carbon export to the Murray River

It is difficult to quantify the carbon loads exported to the Murray. There was some evidence of increase sediment carbon down stream following *Azolla* crash, however movement of *Azolla* is also linked to wind direction as much as flow. Wind events that essentially push the *Azolla* upstream are also likely to lead to increased levels of *Azolla* within Rices Weir, as the *Azolla* will continue to grow. The overall effect of the wind will be that *Azolla* export is likely to be very variable over short time frames, but when it does happen, large “slugs” of plant material may be moved down stream.

This report also shows that *Azolla* growth rate was approximately 120 g/m^2/d. For the ultimate removal of *Azolla* from the Broken Creek, flows have to be greater than the growth rate. It is conceivable that very low rates of transport down stream may not in fact lead to a great reduction in *Azolla* biomass within Rices Weir, but that the surface area created by slow removal is colonized by the rapidly growing *Azolla*. In either event, *Azolla* will represent a major source of carbon transport from the creek.

Phytoplankton are a potential source of carbon to the Murray River, however we calculate that this will only be a minor contribution. If it is assumed that 70 µg/L chlorophyll-*a* is present in the Broken Creek, with minimum flow of 30 ML/d (flow over the fish ladder) and a chlorophyll to carbon conversion factor of 20 (Reynolds 1984), this chlorophyll-*a* concentration represents approximately 40 kg/d of carbon exported as phytoplankton. Literature values for the Murray River at Barmah indicate that phytoplankton comprise approximately 4000 kg carbon/day (Oliver and Merrick 2006) so the Broken Creek would be contributing only 1% of the carbon as phytoplankton.

5.Recomendations

The Broken Creek is a highly modified water body that suffers from long term nutrient input and managed water flow. Historically, the growth and death cycles of *Azolla* (dying *Azolla* returned to the sediments) has given rise to organic sediments rich in nutrients. The sediment oxygen demand have the capacity to drive down the dissolved oxygen levels within the water body, to a point that will be harmful to fish. The most catastrophic changes occur during the period where *Azolla* dies. Therefore determining the factors that lead to its death is still a major gap in our knowledge that needs to be addressed. Although temperatures do get relatively high, temperatures still do not reach levels considered the be catastrophic for *Azolla*. Ongoing temperature monitoring at fine scales may provide an answer as to why *Azolla* declines.

Predicting low DO events. Regular monitoring of DO coupled with flow manipulation still appears to be the most practical approach to maintain suitable DO. In situ probes should continue to be the main monitoring tool, but the possible introduction a photographic system to monitor the spread and condition of the *Azolla* could be considered. Daily visual evaluation of the *Azolla* will certainly assist and may help to predict critical low DO events. Once any indication of an oxygen decline is detected, combined with complete coverage of Azolla, particularly id any signs of decomposition occur, then remedial action can be taken.

Azolla removal. For any success removal programme, the removal rates clearly will have to be greater than the combined effect of the high *in situ* growth rate of the *Azolla* (which are very high) and the transport of *Azolla* from the upstream weir pools. Simple removal of the *Azolla* may not represent an ideal short-term management strategy for the Broken Creek as large algal blooms will almost certainly follow any major manipulation of the *Azolla*. Long-term removal may be of some value, but the carbon and nutrient loads within the sediments are likely to lead to poor water quality for a long period of time.

5.1 Possible future improvement : emerging ideas for improved research or monitoring of Rices Weir

- *Azolla distribution, measurement.*
    - Grab sampling methods were the only successful methods to collect *Azolla* clumps. Samples procedures involving dragging a net through the *Azolla* were not successful as the plants were simply pushed out of the net.
    - Wind had a major effect on the distribution of *Azolla*. On occasions, wind blowing upstream was able to reduce and temporarily eliminate downstream movement of *Azolla*. The consequence is that rates of downstream movement may be rather variable and even spasmodic Improved understanding on the distribution of *Azolla* within Rices Weir could be gained by using on site photography. Photography would be possible through small digital security style camera system that takes images over hourly/few hourly intervals.
    - Deploy a basic weather station to monitor localised wind direction and speed.
- *Azolla movement down stream.* Inferred measures of downstream movement may be possible once real-time estimates have been obtained. Two real options seem to exist
    - collect *Azolla* directly in nets placed at the weir wall. This will only be possible for short term sampling periods, but easy deployment would mean that many sampling event could be carried out in a short period of time.
    - collect *Azolla* downstream of the wall. The logistics of this approach are more intensive and would require deployment of larger nets. The approach may be useful to get integrated, or longer term samples of *Azolla*.
    - Coordinate measures of downstream movement with biomass measures (% coverage) within the weir and physical properties (flow, wind, etc).
- *Azolla growth response to temperature.* Although lethal temperatures are not achieved, a satisfactory explanation for the death phase of the *Azolla* blooms is

yet to be found. One possibility is that micro-sale temperatures may actually be higher than those measured with hand-held or in situ probes. The increased temperature caused my micro-scale green house effect of *Azolla* mass. Further studies should incorporate temperature measures of millimetre scales, through an *Azolla* bloom

- *Nitrogen cycling.* Nitrogen dynamics in Broken Creek sediments remains a key knowledge gap. Total N load are very high, but the source has not been demonstrated. This report identified Azolla as a key source of nitrogen, but load can not be determined at this stage.
    - To what extent does down stream transport of N occur.
    - Questions involve understanding the role of nitrogen fixation (within Azolla) a major source of nitrogen?
    - Since anoxic sediment almost certainly exist, does denitrification occur, thus have a role in removing excess nitrogen from the system?

6. Azolla compositional analysis

At the completion of the project field work, discussion between the project team and GBCMA (Wayne Tennant and Geoff Earl) considered whether *Azolla* could be used as a possible stock feed. In response to these discussion, total nutrients, salts and metals were determined from 4 replicate samples collected in December 2006. (Table 7). These data can be used to decide on the viability of further study into whether *Azolla* would be useful as a stock food. The authors of this report suggest wider studies, involving research personal involved in the stock feed industry, as well as end users be consulted before *Azolla* is used as a stock feed.

Initial analyses indicate that Azolla from the Broken Creek contains about 16% protein, which is similar to protein content reported in other studies (Lumpkin and Plunkett 1980).

Table 7 Major nutrients, salts and metals in Azolla collected from the Broken Creek, December 2006.

Part A *Major nutrients and salts*	Average composition (% w/w)	Standard Error
Carbon	38.39	0.83
Nitrogen	2.62	0.13
Phosphorus	0.26	0.02
Potassium	2.46	0.15
Sulfur	0.56	0.03
Calcium	0.56	0.02
Magnesium	0.37	0.01
Sodium	0.64	0.04

Part B *Total Metals*	Average composition (ppm)	Standard Error
Copper	22.475	5.38
Zinc	111.08	42.17
Manganese	526.93	23.26
Iron	8609.67	1418.03
Boron	37.39	4.46
Molybdenum	1.92	0.20
Cobalt	2.80	0.16
Silica	3834.68	2002.54
Silver	0.17	0.04
Arsenic	1.15	0.08
Lead	2.78	0.48
Cadmium	<0.01	
Chromium	8.48	0.80
Nickel	8.18	0.73
Mercury	<0.5	
Aluminium	7745	846.91

Part D *Nutrient ratios*	
Carbon :Nitrogen	14.8
Nitrogen:Phosphorus	9.98
Nitrogen:Potassium	1.08
Nitrogen:Sulfur	4.72
Crude Protein*	16.4 % (w/w)

* by calculation:- crude protein = %N x 6.25

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