Invertebrate survey of Mulloon Creek 2015-16

Biological and physical parameters of water quality in the Mulloon Creek catchment, New South Wales

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Introduction

Macroinvertebrates of freshwater can serve as an indicator of water quality as well as the changes that occur to water over a season (Rosenberg and Resh 1993). The types of macroinvertebrates are considered to vary in water as a result of differences in species in their ability to cope with the differing physical parameters of the water in which they dwell (Resh and Rosenberg 1984). The major physical changes in water are those that can affect the life cycle of the invertebrates, as well as their ability to survive over the short term (Johnson et al., 1993).

Although many studies have examined macroinvertebrates in Australia (*e.g.* (Thomson et al., 2012, Verkaik et al., 2014), few studies have considered the smaller catchments that may change as farming practices change. The review by Lester and Boulton (2008) on placing timber into the waterways of agricultural streams was one of the rare papers to indicate ways of improving the water conditions following many years of agricultural activity. However, no studies in Australia have reported how water quality changes over time with restoration of agricultural landscapes, although a large literature exists in North America regarding the same question.

The study of macroinvertebrates can examine changes in either taxonomic groups or functional groups (usually based on feeding). These patterns can be compared to a number of stream conditions, such as the water temperature, dissolved oxygen, pH, conductivity and flow rate. Each of the environmental aspects affects some aspect of the macroinvertebrate life cycle (Johnson *et al.* 1993). For example, temperature determines the rate of growth of macroinvertebrates, but also influences the oxygen that can be dissolved in the water. As temperature increases, the capacity of water to hold oxygen decreases. Oxygen is necessary to meet the macroinvertebrate metabolic requirements. There is also a relation between temperature and metabolic rate, as the metabolic requirements of macroinvertebrates increase with temperature (Harrison *et al.* 2012). so not only does the requirement for oxygen increase as temperature increases, but the availability of oxygen typically decreases at the same time in water.

The dissolved salts in water can also affect the amount of oxygen that is present, and conductivity is an indication of dissolved salts in water (Randall *et al.* 2002). As conductivity increases, the amount of oxygen that is held in water decreases, but freshwater is typically defined as conductivity that is below the threshold for reducing oxygen concentration. However, salts and pH can affect the ability of invertebrates to regulate ions and water within their body, so that changes in these parameters can be reflected in the invertebrates that are present in various locations where these parameters change (Cooper 2004).

Finally the rate at which water flows and the substrate that forms the anchor for invertebrates can cause a change in the composition of observed invertebrates. As vegetation increases on the substrate, the invertebrates have a greater ability to find locations to adhere, so that any change in water flow is limited in causing their movement down the stream (Lester and Boulton 2008).

In this report, we present information regarding the macroinvertebrate communities that are present at six sites along Mulloon and Reedy Creeks over 9 months (October 2015-May 2106) along with the physical conditions that were present along the stream at that time. Mulloon Creek originates in the Tallagandra State Forest, flows in a northerly direction through agricultural land, and joins Reedy Creek before entering the Shoalhaven River system near Braidwood. We examined how the physical parameters can influence the composition of invertebrates at various locations as well as how stable the communities were over the period of measurement. This study will be a baseline for understanding how future work and development along Mulloon Creek may affect the various macroinvertebrate communities.

Methods and sites

Study sites

Sites along Mulloon Creek were selected in consultation with Luke Peel and Peter Hazell. Six sites were chosen and are shown relative to the catchment in Figure 1.
Invertebrates were collected by either kicking substrate (pebbles and small rocks) or by sweeping above other substrates. At least 10 kicks or 10 sweeps per location to standardize amount of water sampled, with three collections made per site. Although no effort was made to determine substrate type in detail, notes of the substrate were made to indicate potential variation among samples at each site (see Appendix 1 for site details). Invertebrates were collected at each site in October 2015, January 2016 and April 2016. No attempt was made to take physical or invertebrate samples in winter 2016 because of the rain during that time.

Physicochemical analysis of water

Samples were made monthly at the various locations listed above for the parameters of water temperature, dissolved oxygen, conductivity, pH and water flow. Water temperature and dissolved oxygen measurements were made using battery operated electronic thermometer (°C) (WP-90, TPS Australia) and oxygen electrode (% saturated) (WP-82, TPS Australia). Thermometer was calibrated against a digital thermometer traceable to NBS standards and oxygen electrode calibrated as directed in the instructions from TPS Australia. The conductivity was measured using a portable system (Activon) and measured to the nearest 0.001 mS cm^{-1}. pH was determined using pH paper (Merck) capable of measuring from pH 0-14. Water flow rate (± 0.01 m s^{-1}) was measured using an electronic flow metre (Flo-Mate model 2000, Marsh-McBirney). The flow head was submerged at least 1 cm below the surface at each location. The maximum and minimum temperatures and monthly rainfall were obtained from weather stations at Mulloon Farm (courtesy of Tony Bernardi).

Macroinvertebrate analysis

Invertebrates were returned to the laboratory and ethanol added to preserve specimens until separation from the mixed substrate-specimen material could be made. Invertebrates were removed under microscope and re-stored in individual vials containing 80% ethanol. The three samples from each site were then combined for counting and identification of macroinvertebrates. Insects were classified to family, while other invertebrates were only described to order. Identifications were made using keys (Cairns *et al.* 2017, Gooderham and Tsyrlin 2002, Hawking 1986, Hawking and Smith 1997, http://keys.lucidcentral.org/keys/lwrrdc/public/Aquatics/main.htm)

Statistical analysis

Statistical analyses of macroinvertebrate and physical parameters have several alternative techniques to determine what is occurring within a stream (lotic) environment (Norris and Georges 2003). We used two techniques: principal component analysis and SIGNAL scores.

Principal component analysis on the physicochemical data (no data transformation) was used to determine how similar the various sites were based upon those parameters.

Principal component analysis was used to determine how similar the various sites were with respect to composition and quantity of invertebrates. This analysis was done on the data using a natural log transformation ($\ln(x+1)$), where x is the number of individuals collected in a family. Comparing the grouping of the sites was used to determine whether similarities existed between the physicochemical and biological information.

Signal scores were determined according to the procedures outlined by Chessman (2003). The current Signal analysis was then compared to the studies made in 2006-8 to see how sites along Mulloon Creek may have changed in the intervening years.

Statistical analysis was done using Microsoft Excel and JMP 13 (SAS Corp).

Results

Physicochemical measurements

Water temperature at the various sites differed among the sampling months increasing from around 15 °C in October up to 23-24°C in summer, with the highest measured values at Palerang Crossing (Figure 2). Temperatures started decreasing in April, and by May most sites were around 10°C.

Dissolved oxygen values were typically higher in sites along Mulloon Creek from Black Jackie to Palerang Crossing, but tended to be lower at Sandhills and Reedy Creeks (Figure 3). The seasonal changes were less obvious, although in December lower values were present in the water as the various sites did not have a continuous water flow. Oxygen values increased again in January, but then decreased again in April and May.

Conductivity was lower in the four Mulloon Creek sites than in the much higher conductivities present at the Sandhills and Reedy Creek sites (Figure 4). Water input tended to cause a decrease in conductivity in both Sandhills and Reedy Creek sites, although the response of these two sites did not always mirror each other.

Very little difference was observed in pH across both the sites and seasons, as nearly all measurements indicated that the sites were circumneutral (pH 5-7) (Figure 5). However, the values did vary either as a result of changes in flow or following input of organic acids.

Water flow differed markedly among the sites and with season, partly in response to the rainfall and also to the structure of the substrate and site (Figure 6). Reedy Creek often had high flows, as it had a rockier substrate leading to fast flowing channels in various parts of the stream. The wider sites had a reduced flow pattern compared to the narrower sites, but the quantity of water passing through had to be nearly the same as sites upstream.

Rain fell in every month of the sampling period, with heavy falls in January (about twice long term average) and June (about three times long term average) (Figure 7). The rain in January resulted in Mulloon Creek flowing again, after becoming a chain of ponds in mid-December. The periodic fall after that ensured that during the rest of the study, the creek continued to flow. Seasonal maximum and minimum temperatures were similar to long-term average values (Bureau of Meteorology data from Braidwood).

To determine what physicochemical parameters may affect each site, a principal component analysis was performed on the data in Figures 2-6, with the graphical result presented in Figure 8 and eigenvectors in Table 1. The analysis indicates that dissolved oxygen and conductivity are the most important variables defining the sites, although water flow rate is nearly as important (70% of variation in first 2 vectors). Figure 8 shows that Reedy Creek and Sandhills Creek formed a group as a result of their high conductivity, Palerang and Triple Ponds formed a group, a result of flow rate, and Black Jackie and Peter's Pond clustered together, presumably because of conductivity and dissolved oxygen values.

Macroinvertebrates

Overall, 3439 macroinvertebrates were identified, falling into 60 different taxa (see Appendix 2 for complete list). The largest number of invertebrates was collected in January (Figure 9), although the pattern differed somewhat among the sites (Figure 10). The number of taxa tended to increase as number of individuals increased, but that was dependent upon site (Figure 10). Peter's Pond was the source of the highest number of individuals collected (January) and the most taxa collected (April). Sandhills Creek had the fewest number of individuals collected over the study period, and was generally depauperate. Triple Ponds and Palerang also yielded relatively few individuals in April.

Even though the sites differed in the numbers of individuals collected, some taxa were commonly collected in all sites and accounted for at least 10% of the collected individuals in at least once (Table 2). These common taxa were the chironomid sub-families Chironominae and Tanypodinae, and the mayflies in the family Leptophlebiidae (although the latter were not collected from Sandhills Cr.). Amphipods in the family Ceinidae made up more than 10% of the collection from Peter's Pond, Palerang, Sandhills Creek and Reedy Creek.

To see how the macroinvertebrate collections indicated the state of the various sites, two different methods for comparing the sites were undertaken. The first method was the calculation of SIGNAL scores, as that which was done previously in 2006-08, where sampling was done at the sites Below pump shed (compared with Black Jackie), William's Wallow (compared with Triple Pond), Peter's Pond and Palerang. The scores depend upon the quality of water grade of the macroinvertebrate family and the weighting based upon the number of individuals collected in that family (Chessman 2003). Comparison of the previous scores with the current analyses at the various sites is presented in Figure 11. The current scores for Black Jackie are much higher than the earlier scores, but both Triple Pond and Peter's Pond are similar to the earlier scores. The summer measurement for Palerang in 2016 is also much higher than the same measurement in December 2007. The winter measurements for the earlier period do appear to show an increase in water quality, but no comparable measurement was made in this study.

The principal component analysis (PCA) gives a slightly different picture of the sites (Figure 12) compared with the SIGNAL analysis. In the PCA, Triple Ponds and Palerang have exactly the same vector, suggesting similarities in species and number. Black Jackie and Peter's Pond also have a similar trajectory for their vectors, again suggesting some similarity, as these two sites tended to have the most invertebrates. Sandhills Creek and Reedy Creek are not grouped together and have opposite trajectories, as the numbers of macroinvertebrates are quite different in the two locations. A seasonal pattern is present in all the data as shown by the anticlockwise change in the vectors from spring through to autumn (see Appendix 3 for similar pattern in earlier data). Clearly, that indicates that when taken together, the taxa change with season, as would be expected as insects moult into adults and new species emerge from eggs with time.

Comparison with figure 8 derived from the physicochemical characteristics of the sites shows that Triple Ponds and Palerang formed one pair and Black Jackie and Peter's Pond another pair, similar to what was found in the biological analysis. The physicochemical and biological analyses did differ with respect to Sandhills and Reedy Creek, but that may be a result of the differences in water flow and dissolved oxygen levels in the two sites.

Discussion

Differences were found among the study sites with respect to both physicochemical characteristics and biological macroinvertebrate assemblages using the principal component analyses. Surprisingly, the patterns that emerged indicated that at least for the upper four sites, there were some similarities among the sites. However, the similarities were not between adjacent sites, but instead alternate sites, so that Black Jackie and Peter's Pond grouped together in both physicochemical and biological analyses, as did Triple Ponds and Palerang. However, exactly what was driving the similarities was not clear from any of the univariate measurements for the physicochemical patterns, or the assemblages of the macroinvertebrates. However, since both analyses gave the same pattern, some characteristic must be in common. The measurements may reflect the overall structure of the two pairs of sites, as Triple Ponds and Palerang were sampled below gravel crossings, while Black Jackie and Peter's Pond had a greater vegetation structure surrounding the sampling region. These habitat structural components could lead to subtle patterns in physicochemical and biological patterns that are not easily discernable in univariate analyses.

The SIGNAL system gave a somewhat different pattern of site structure with respect to water quality. In the spring collection, Peter's Pond had the lowest SIGNAL score of 3.5 with an increase in score either above or below that location. Using an AUSRIVAS analysis, good quality water would be anything above a SIGNAL score of 5 for this type of creek, and Black Jackie and Reedy Creek were approaching that value. In summer, Black Jackie has a score that exceeds 5, while Triple Pond and Peter's Pond show a reduction in their score, suggesting a higher input of nutrient levels at that time into Triple Pond and Peter's Pond. However, both Palerang and Reedy Creek have increases in score, suggesting that these locations have an improvement in their water quality. This response is surprising given that Reedy Creek has a higher conductivity, but then the score for Sandhills Creek is nearly as high and is higher than either Triple Pond or Peter's Pond despite Sandhills Creek having a high conductivity. These anomalies demonstrate that some care must be taken when interpreting the results of the SIGNAL scores and that following the pattern of macroinvertebrates overtime may be the best method for understanding how the water quality varies (Chessman 2003).

The differences observed with the pairings of sites also do not appear to be overly influenced by the taxa that contributed excessively to the overall abundance (Table 2). If the chironomid larvae determined the overall pattern of the vectors for the various sites, then distinguishing the sites would have been difficult. However, despite the preponderance of chironomids and other taxa, it does not appear that influences the outcome of the PCA of the macroinvertebrates. Unfortunately, determining what taxa did influence the separation of the sites was not easy, as shown in figure 13. As the first two eigenvalues only explain 38% (22.7% + 15.1%), and six eigenvalues are needed to explain more than 70% of the variance (Table 3), the ability to distinguish individual taxa as important for the analysis is complex. However, those sites (TPC, PaC and SHG) that were taxa poor in at least one season do group together in the negative-negative quadrant of the PCA, suggesting that the seasonal effects may play an important role in determining the role of the taxa for distinguishing site characteristics.

Underwater timber can affect stream flow patterns, so that water flow slows around timber allowing colonisation by macroinvertebrate larvae, especially shredders (McKie and Cranston 2001). Although the flow is slower, it also becomes more turbulent ensuring higher oxygenation of water. As future changes with Mulloon Creek may include placing more weirs along the water path, inclusion of timber could result in an increase in natural habitat for macroinvertebrates. The presence of allochthonous wood and leaves can be used by a variety of macroinvertebrate functional groups, especially shredders and grazers, to enhance the biodiversity within streams (McKie and Cranston 2001), but may also permit feeding or mating sites for larger macroinvertebrates (Starrs *et al.* 2015). Black Jackie was similar to Peter's Pond, a much larger water body, potentially a result of the presence of leaves, wood and rocks that contribute to that macroinvertebrate diversity. The leaves and wood result from vegetation addition into that stream section as a result of the surrounding and overhanging plants that will periodically add to the local carbon supply. The increased carbon supply then permits a greater number and more diverse macroinvertebrate population to become established, even in a smaller region.

The overall result suggests that little change has occurred over the last decade in macroinvertebrate diversity, but Black Jackie represents a "good" site in the SIGNAL system. As restoration continues, ways of including more overhanging natural vegetation should be considered in other sites as well. Regions of gravel crossings (Triple Ponds and Palerang) may present difficult areas for restoration, but the hardest location for restoration will be Sandhills Creek as its flow is much more periodic (usually only during flood periods) compared with the rest of the Mulloon-Reedy system.

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Table 1. Eigenvectors derived from the principal component analysis of the physicochemical parameters that were measured during the course of this work. The large values of the first eigenvectors indicate that dissolved oxygen and conductivity were important for separating the sites, but that water flow rate was also an important variable. The first two vectors account for 70% of the variation, and vector 3 accounts for an additional 16.9% of the variation. Although temperature and pH did not appear to account for much of the variation in the univariate plots, they appear to play a role in characterising the sites as shown by the large values in the second and third eigenvectors.

	Eigenvector 1	Eigenvector 2	Eigenvector 3
Conductivity (mS/cm)	-0.4943	0.4250	0.1978
Temp (°C)	0.3287	0.5074	-0.6334
Dissolved O_2 (%)	0.6220	0.1661	-0.0102
pH	-0.1346	0.7269	0.2543
H_2O flow (m/s)	0.4926	0.0769	0.7035

Table 2. Taxa that made up more than 10% of the individuals collected at any one collection for the various sites.

Site	Month	Taxa	Percent of total
Black Jackie	October, January	Chironominae	19.4, 10.9
	October, January	Tanypodinae	37.9, 26.2
	April	Baetidae	13.5
	October, January, April	Leptophlebiidae	11.7, 17.5, 28.0
Triple Ponds	October, April	Ceinidae	19.4, 18.6
	April	Copepoda	25.6
	January	Ostracoda	36.9
	October	Chironominae	14.5
	October, January	Tanypodinae	19.4, 16.6
	October	Leptophlebiidae	21.0
	January, April	Oligochaeta	11.5, 16.3
Peter's Pond	October, January, April	Ceinidae	22.1, 23.5, 17.9
	October	Chironominae	28.8
	October, April	Tanypodinae	27.8, 11.3
	January	Leptophlebiidae	12.3
	January, April	Coenagrionidae	12.5, 15.0
Palerang	October	Ceinidae	22.0
	October, January, April	Chironominae	18.3, 28.2, 67.7
	October, January	Tanypodinae	17.1, 14.4
	January	Simulidae	26.2
	October	Leptophlebiidae	23.2
Sandhills Creek	January	Ceinidae	11.1
	April	Copepoda	22.5
	January	Atyidae	11.1
	January, April	Chironominae	14.8, 27.5
	January	Tanypodinae	11.1
	January	Leptoceridae	11.1
	April	Temnocephalidae	15.0
Reedy Creek	January	Ceinidae	15.6
	January	Atyidae	25.4
	October, April	Chironominae	18.5, 17.4
	October, April	Tanypodinae	34.5, 38.5
	January	Leptophlebiidae	13.9
	April	Ecnomidae	10.1

Table 3. Eigenvalues and cumulative variation explained from the principal component analysis on the individual taxa relative to site. As shown by the cumulative percent column, six scores are needed to account for 70% of the variation indicating that the taxa have complex relationships for direct explanation of the site relationships.

Number of eigenvalues	Eigenvalue	Cumulative Percent
1	10.9164	22.742
2	7.2426	37.831
3	5.694	49.694
4	4.7553	59.6
5	3.7113	67.332
6	3.2294	74.06
7	2.6763	79.636
8	2.5165	84.879
9	2.1009	89.255
10	1.4063	92.185

Figure 1. Sites of sampling for physical attributes and macroinvertebrates along Mulloon Creek (courtesy of Luke Peel).

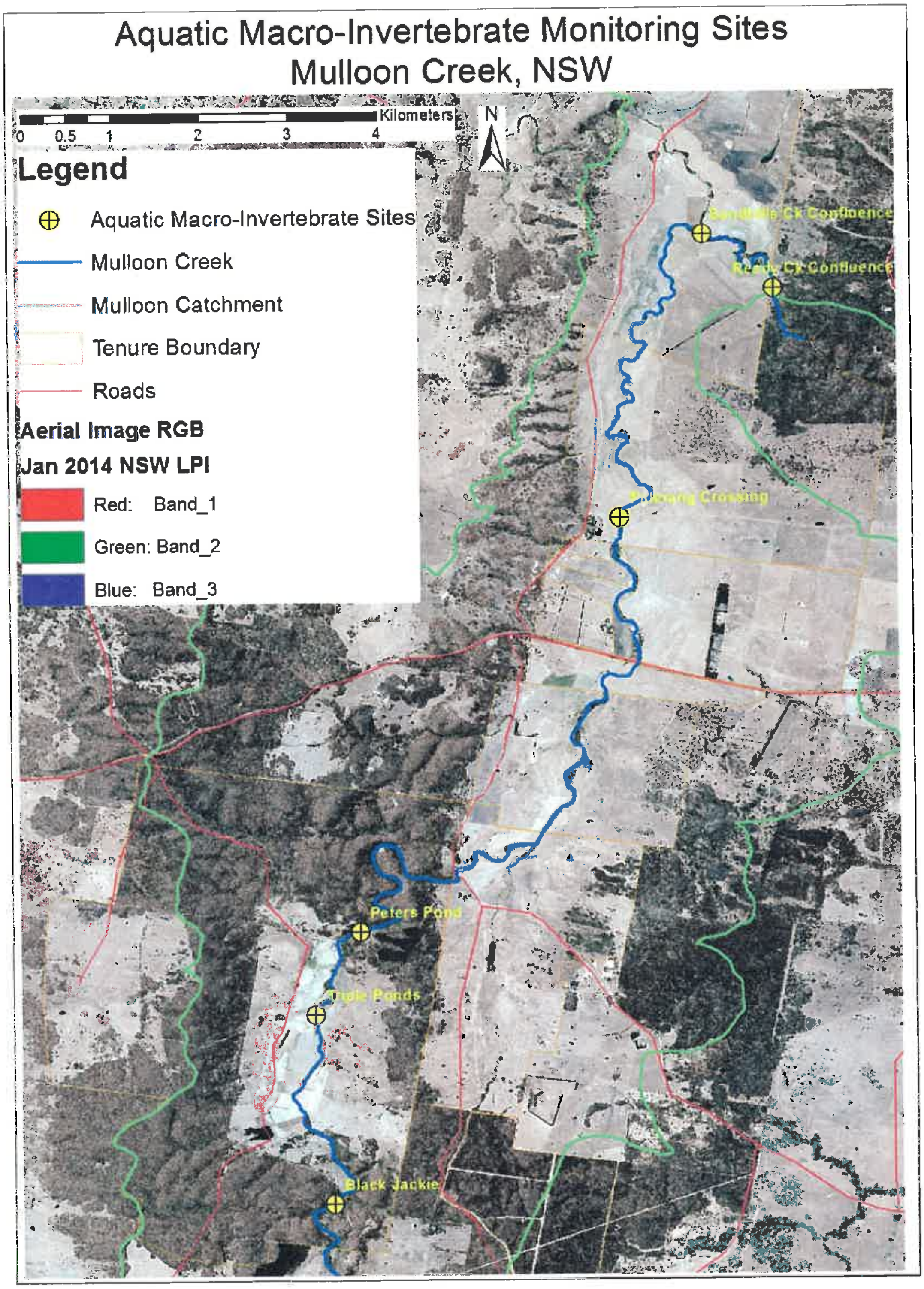


Figure 2. Water temperature at six sites along Mulloon Creek from Oct 2015-May 2016. Temperature is near 20 °C except as entering April and May when temperatures are dropping to 10 °C.

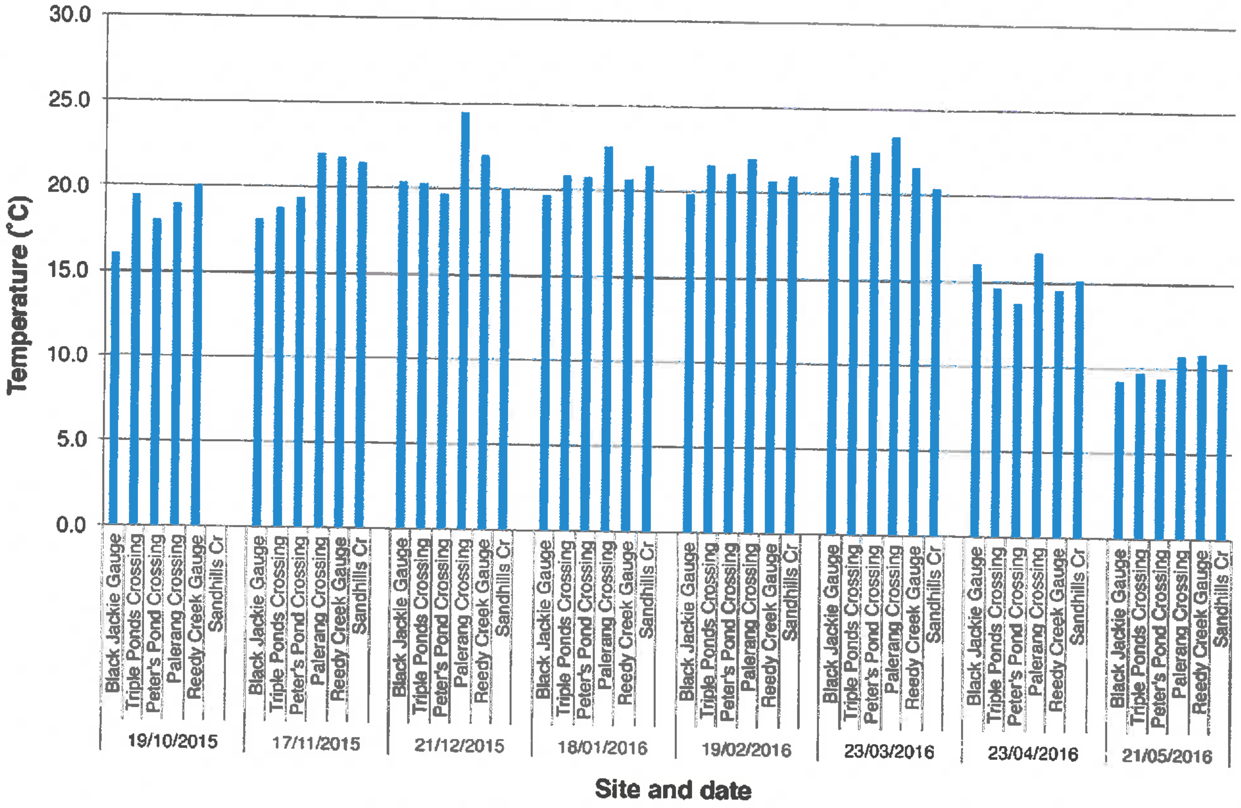


Figure 3. Dissolved oxygen (%saturation at measured temperature) for the six sites along Mulloon Creek from October 2015 –May 2016. Dissolved oxygen is usually lowest in Sandhills, although low oxygen was measured in all sites in December when water was not flowing.

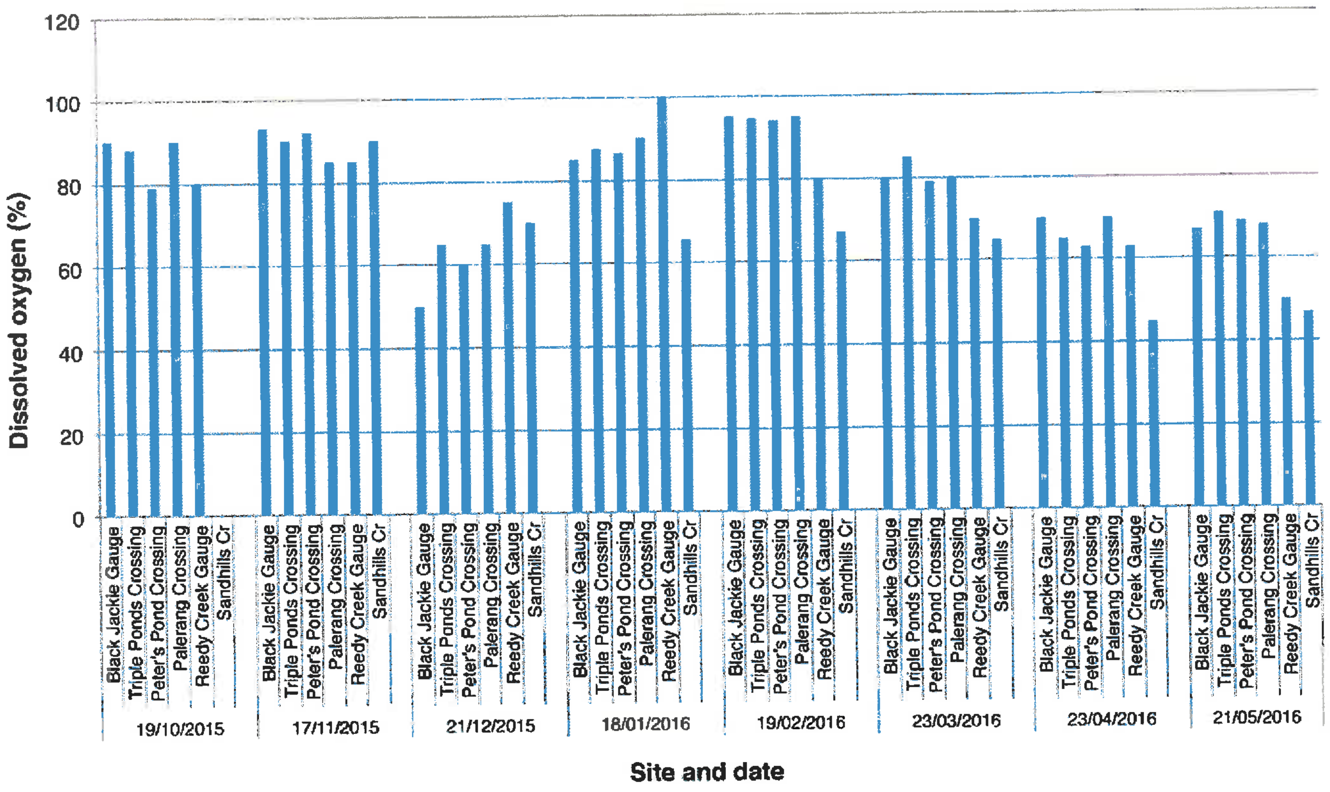


Figure 4. Changes in conductivity at 6 sites along Mulloon Creek from Oct 2015-May2016. Reedy Creek and Sandhills are much higher in conductivity than the other sites.

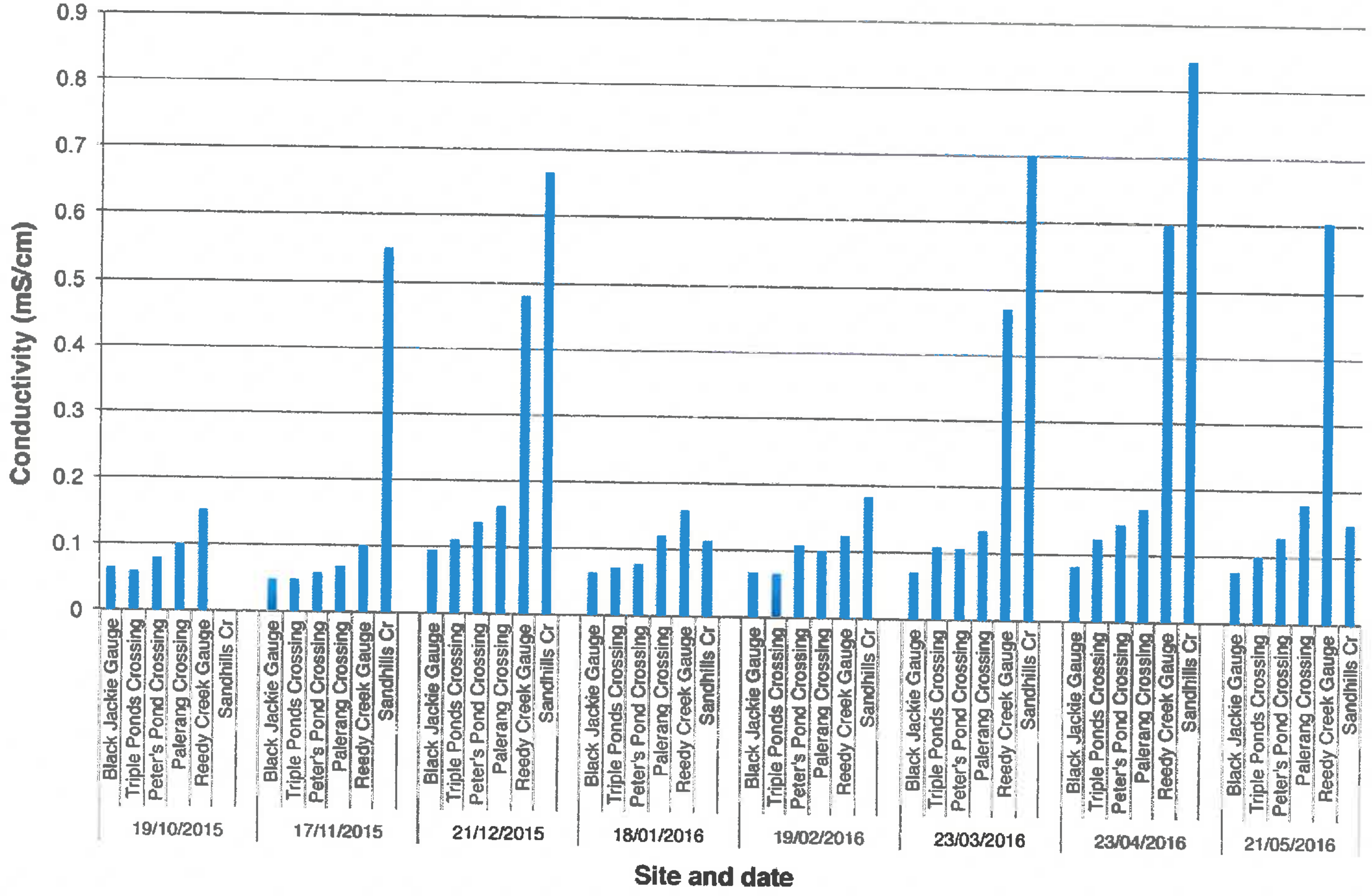


Figure 5. pH was circumneutral in all sites ranging from 5-7 throughout the sampling period.

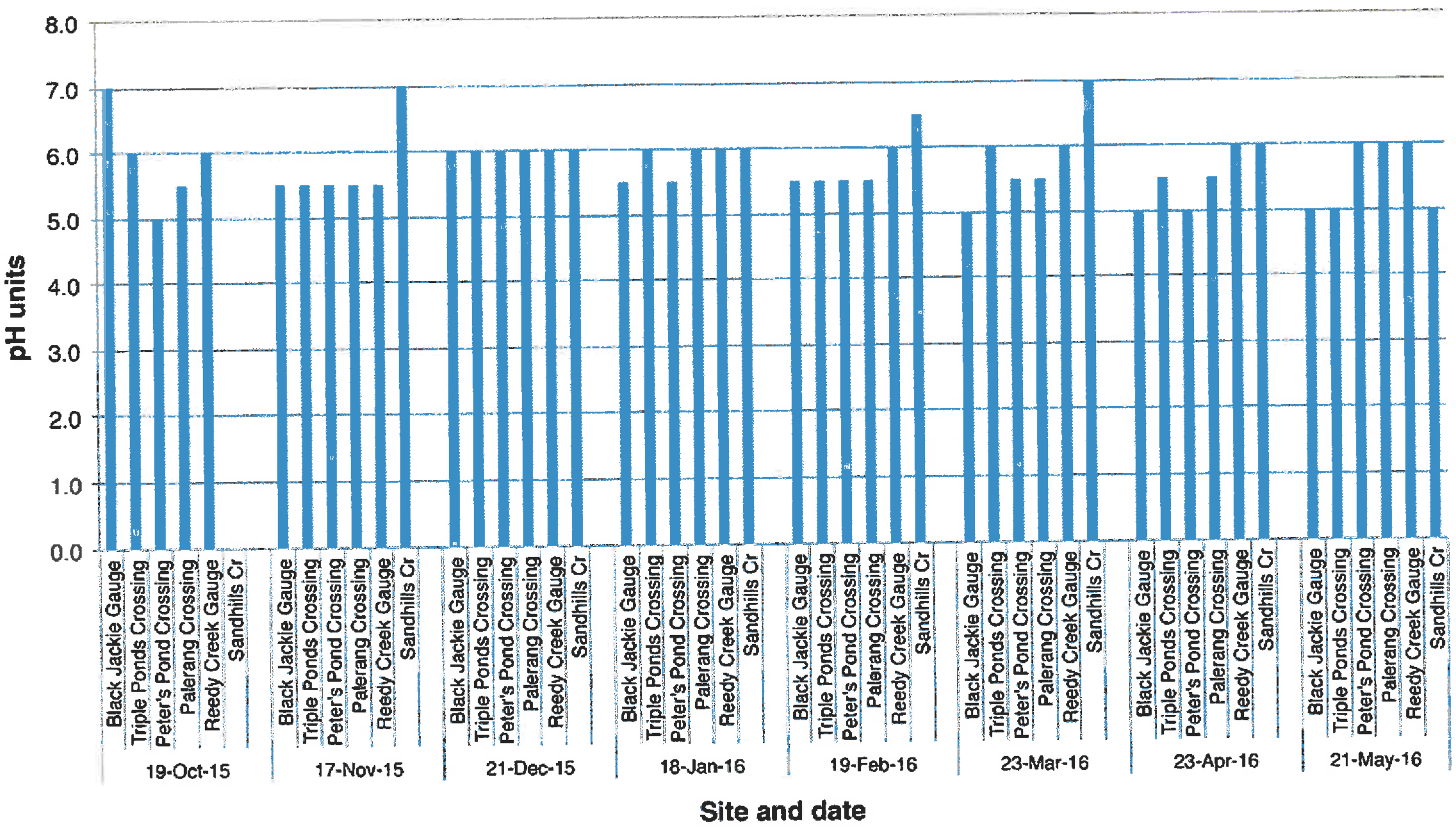


Figure 6. Rate of water flow (ms-1) measured at the various sites during the study period. No measurement was made at any site in October 2015, but measurements were made at all other times and sites, so the results in December indicate that water flow was nil at all sites.

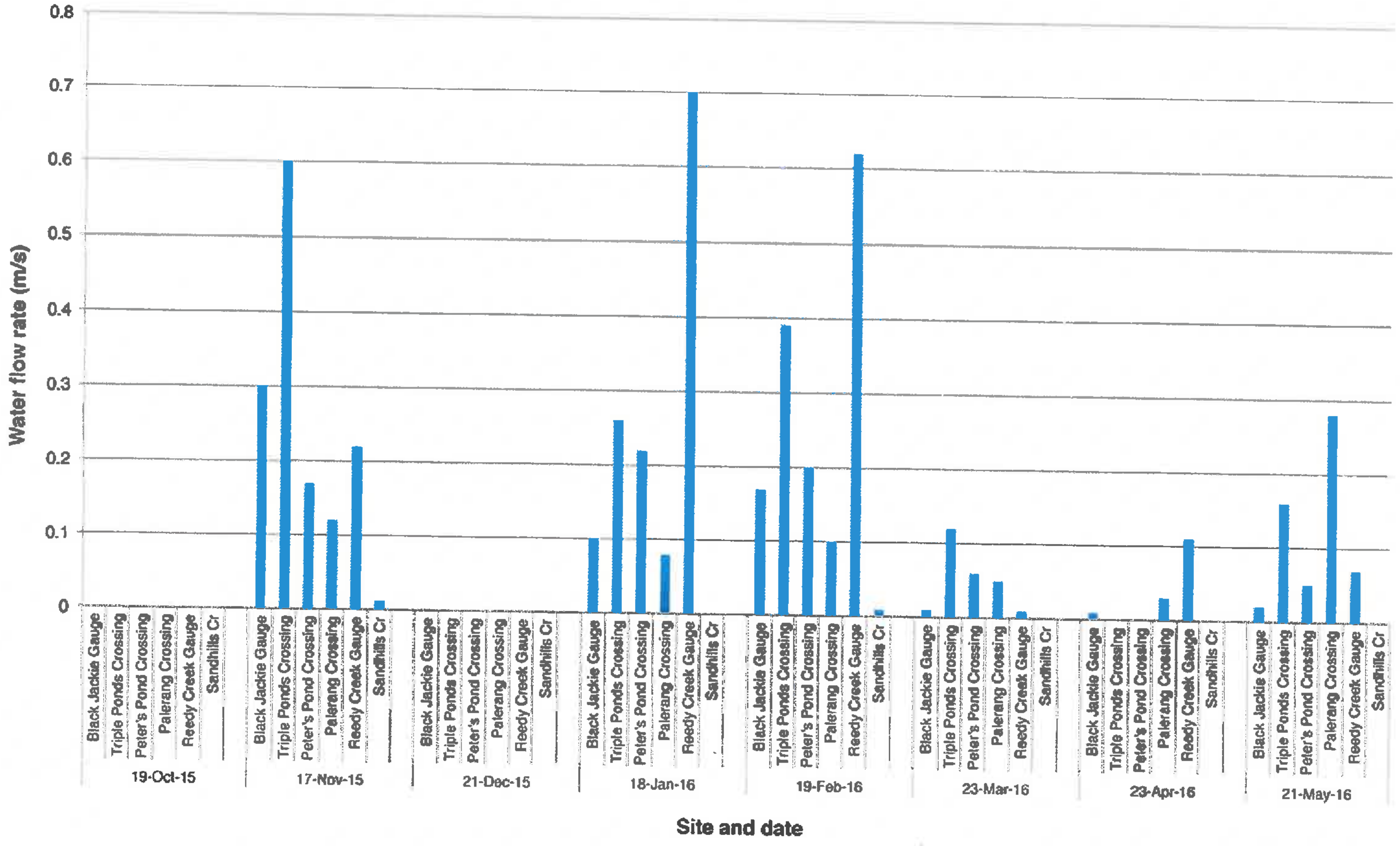


Figure 7. Maximum and minimum temperatures and rainfall taken from Mulloon Creek weather information courtesy of Tony Bernardi.

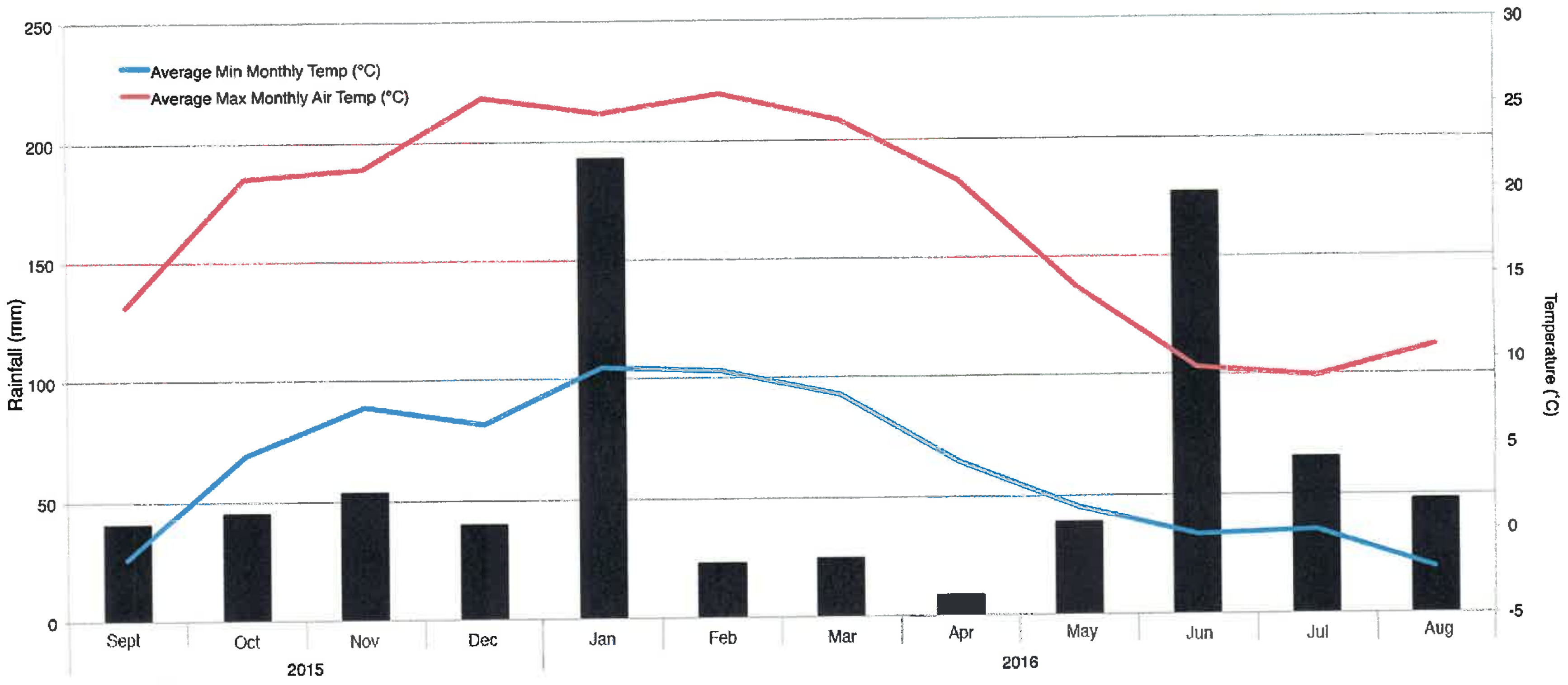


Figure 8. Principal component analysis of the physicochemical measurements shows how the individual parameters determine differences among the sites. The eigenvectors indicate that dissolved oxygen and conductivity are the major aspects differing among sites, but an important secondary effect is the rate of water flow. Sandhills Cr is the dotted blue line along the conductivity vector that is off scale. (BJG=Black Jackie Gauge, TPC = Triple Ponds Crossing, PPC= Peter's Pond Crossing, PaC= Palerang Crossing, SHG= Sandhills Creek Gauge, RCG= Reedy Creek Gauge)

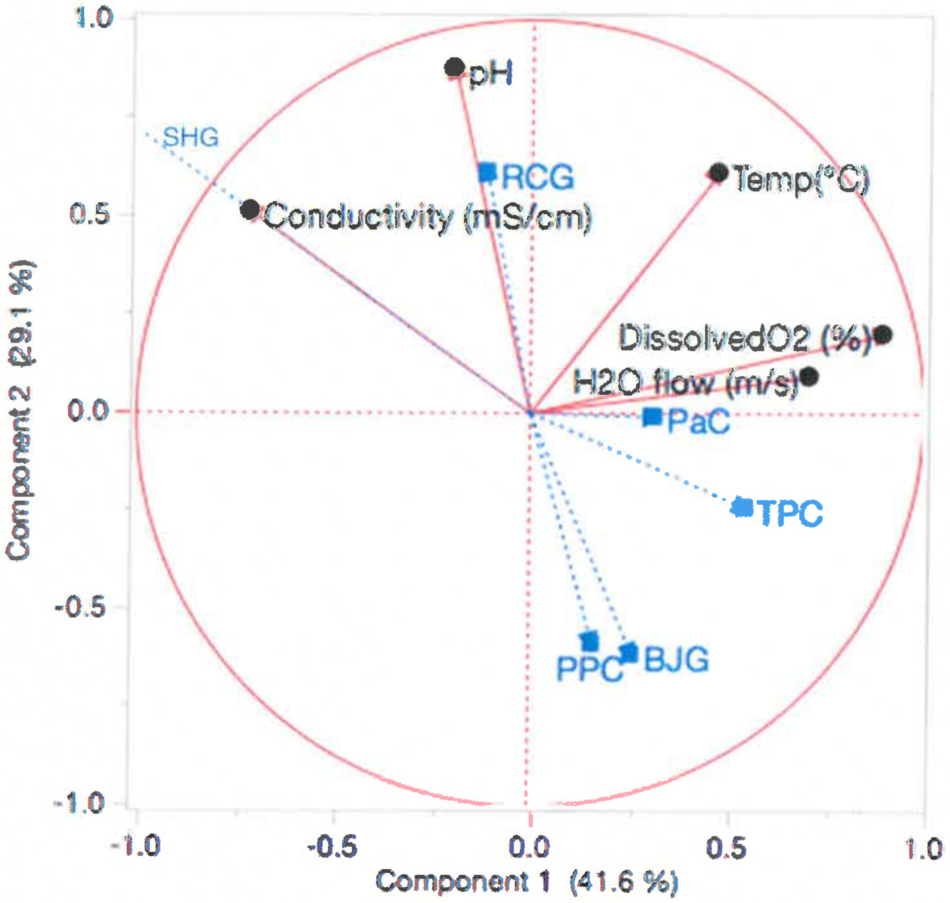


Figure 9. Distribution of total number and taxa of macroinvertebrates collected during the study. January had both the greatest number of invertebrates collected and the most number of taxa represented.

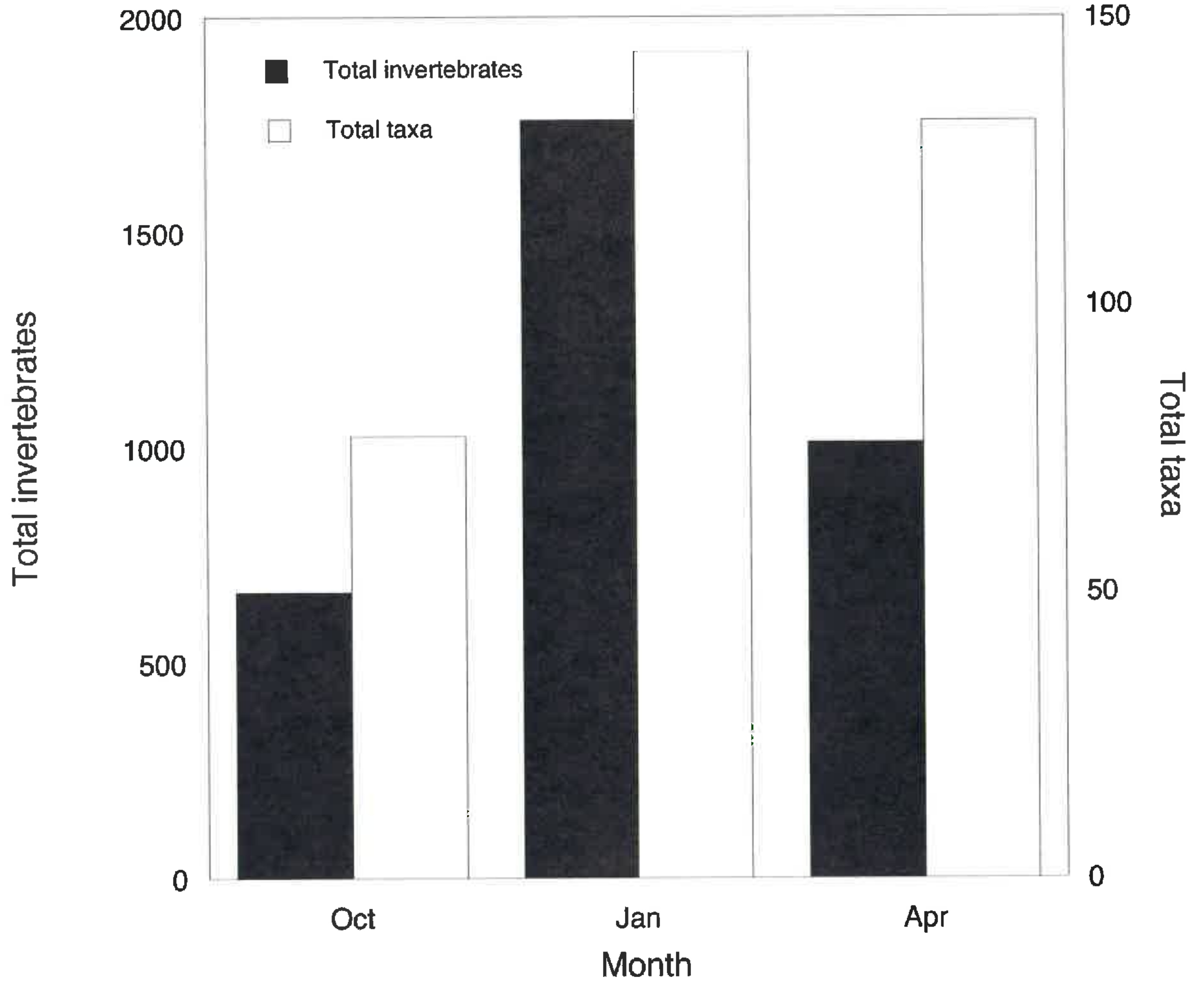


Figure 10. Monthly distribution of numbers on invertebrates and taxa collected at each site. Peter's Pond yielded the greatest number of individuals of all sites in January and highest number of taxa in April.

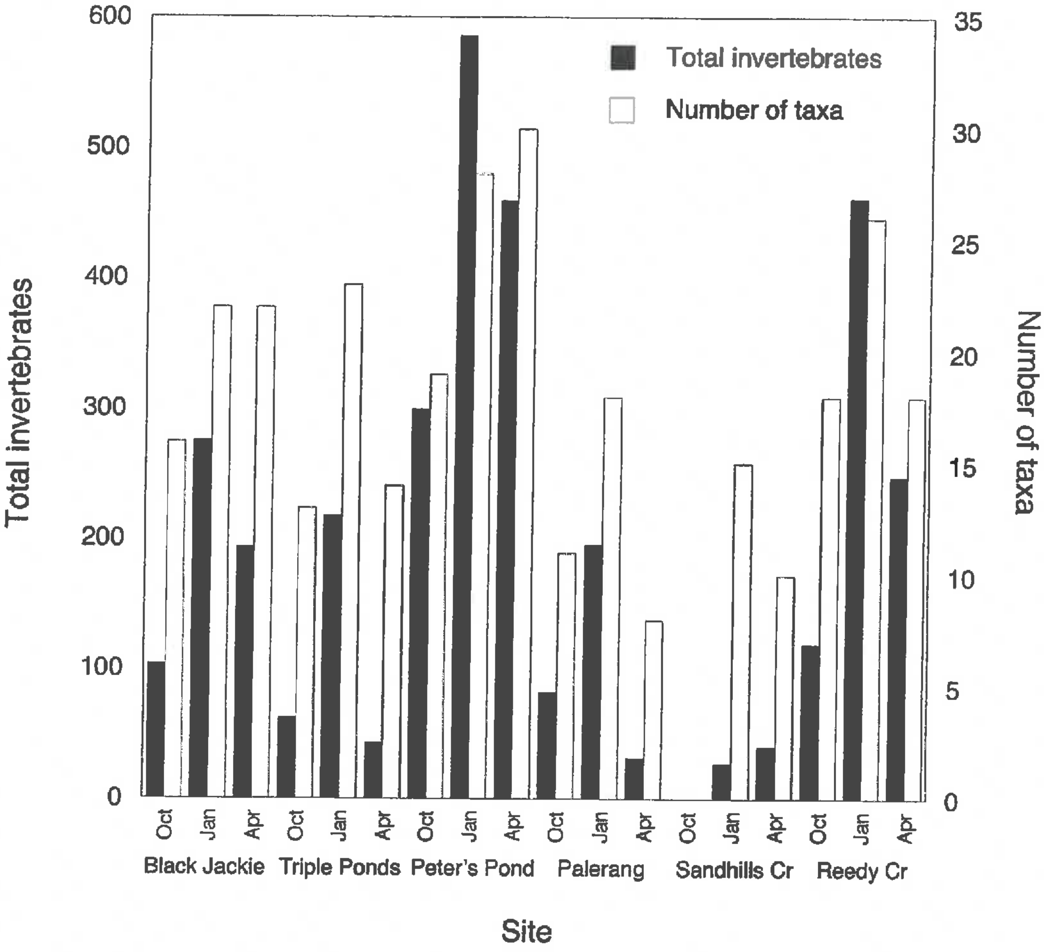


Figure 11. SIGNAL scores for 2006-08 compared with recent scores for macroinvertebrates collected in 2015-16. Scores for upper part of Mulloon Creek are higher, but lower sections are similar.

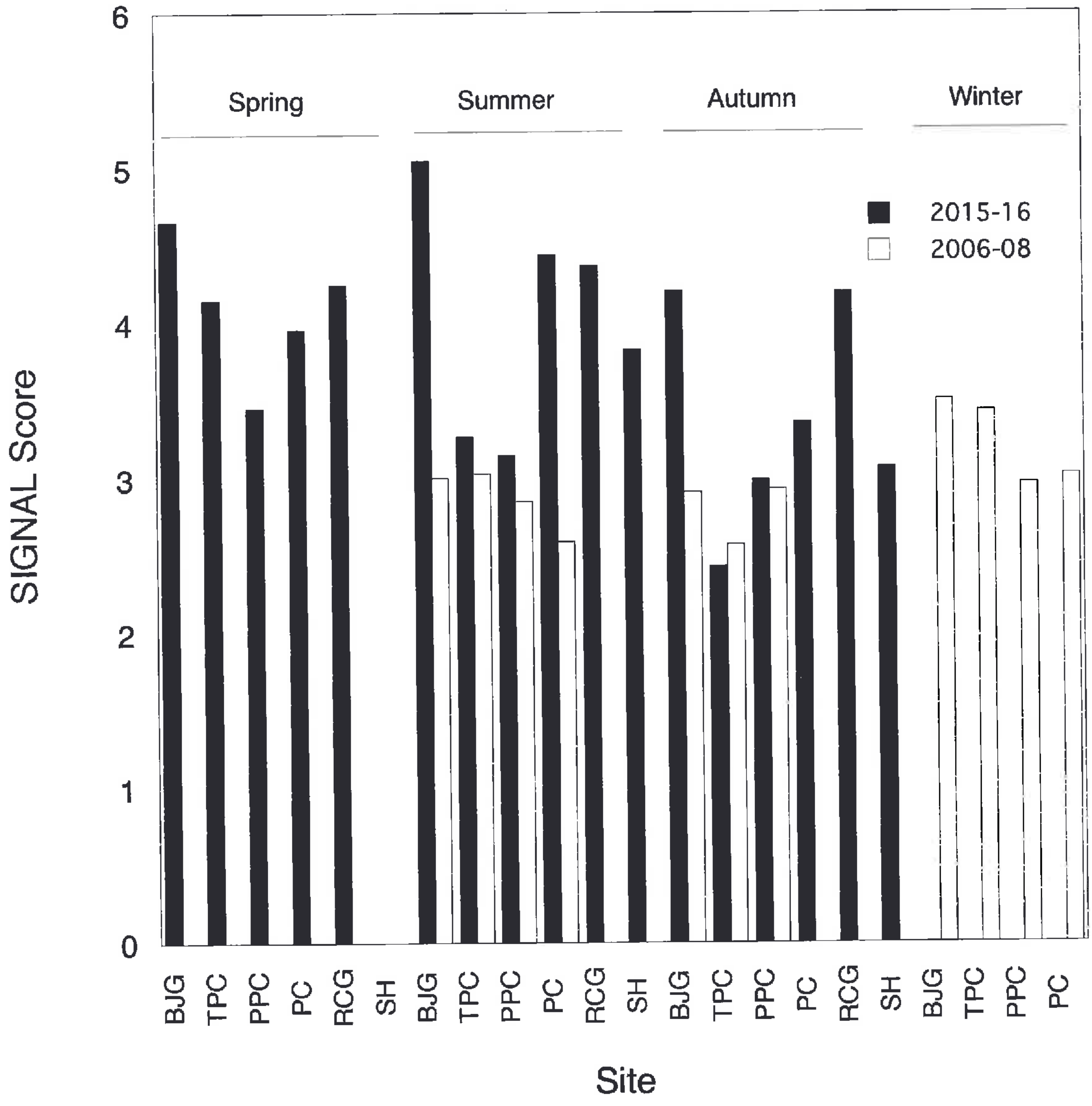


Figure 12. Site comparison across the seasons based upon the macroinvertebrate collection. Triple Pond and Palerang were similar and grouped together, while Black Jackie and Peter's Pond also formed a group. Sandhills and Reedy Creek were separated as a result of the differences in number of macroinvertebrates collected. (BJG=Black Jackie Gauge, TPC = Triple Ponds Crossing, PPC= Peter's Pond Crossing, PaC= Palerang Crossing, SHG= Sandhills Creek Gauge, RCG= Reedy Creek Gauge)

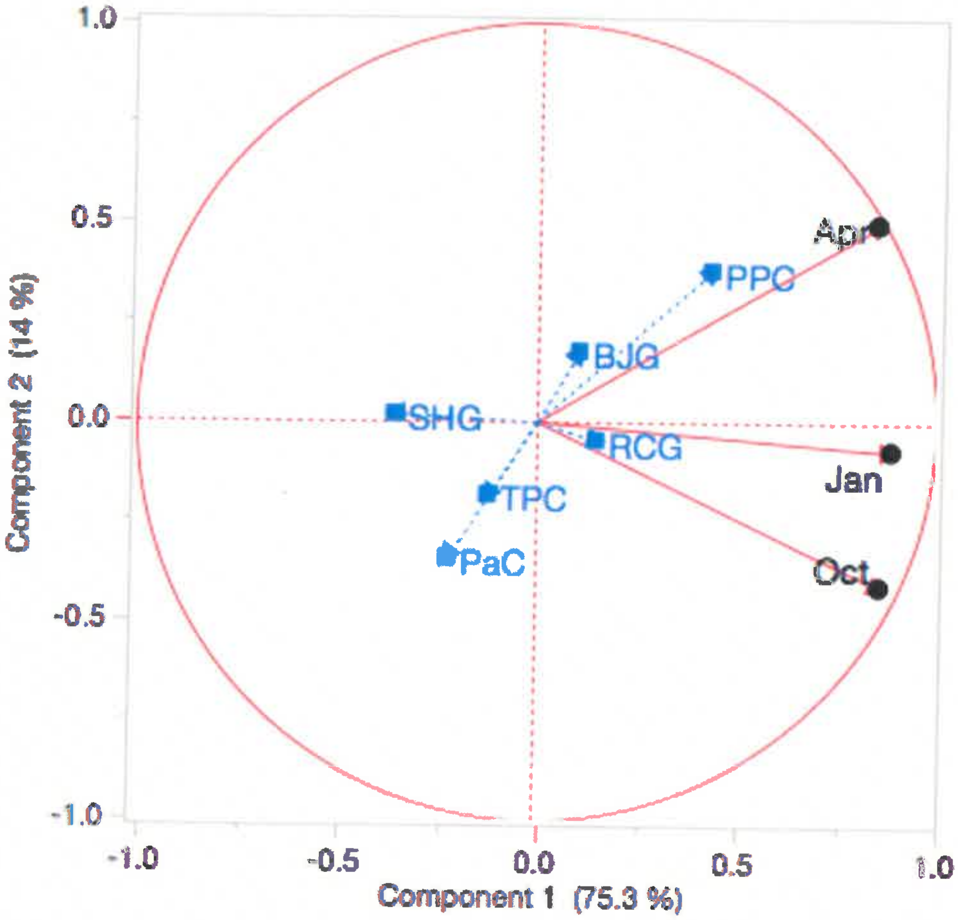
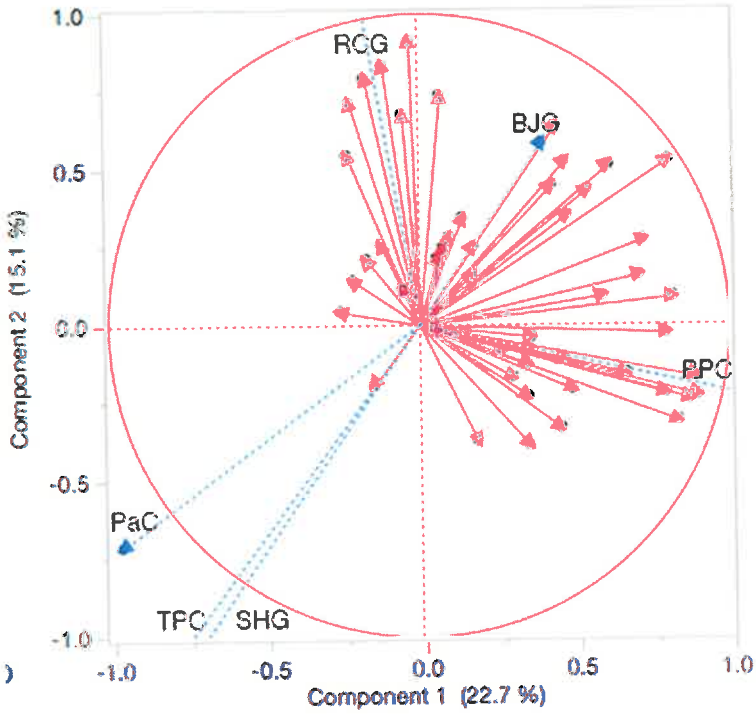


Figure 13. Principal component analysis to determine which taxa had the greatest effect on the variation among the sites. Examination of the vectors indicated that several taxa had equal weighting in the structure of the data and that 10 taxa had equal weighting in the positive direction. Comparison of these 10 PCA scores with SIGNAL grades for the same taxa indicated that no relationship was present. However, the taxa-rich sites in all seasons are RCG, BJG and PPC, while those sites that are taxa-poor in at least one season are in the negative-negative quadrant. (BJG=Black Jackie Gauge, TPC = Triple Ponds Crossing, PPC= Peter's Pond Crossing, PaC= Palerang Crossing, SHG= Sandhills Creek Gauge, RCG= Reedy Creek Gauge)



Appendix 1.

Description of sites

Black Jackie- Cool, shady location. Medium size rocks and mud substrate with vegetation growing in the water as well as dropping from overhanging trees. 3 samples from path, below path and above path (Figure 14)

Triple Pond – Shallow, reedy substrate below crossing. Pebbles were present at crossing, but rare elsewhere. Invertebrate collection was done about 25 m below crossing with mud and reeds present as substrate (Figure 15).

Peter's Pond – Three collection locations 1) above the pond near rain gauge below rocks, 2) in lily pads and reeds near spillway, 3) at exit of water near willows (Figure 16)

Palerang – Pebbly substrate (riffle region), very wide with some vegetation at upstream at top of pond, and some vegetation and mud below crossing (Figure 17)

Sandhills Creek – Mostly static pond with vegetation from overhanging trees. Sampling across pond. Gauge not in water most of year.

Reedy Creek – Downstream from gauge where rocks in water present. Sampled in reeds, at rocks and slightly upstream of rocks, reeds had mud present. Gauge in nearly static water upstream from region of sampling (Figure 18).

Figure 14. Black Jackie sampling site looking downstream towards Mulloon Farm

Figure 15. Triple Pond crossing, above where sampling was done, although at low flows, water physical measurements were made just below branch on left side of picture (arrow).

Figure 16. Peter's Pond with sampling areas 2 and 3 shown (arrows). Sampling area 1 was just below Weather station crossing on the pond side.

Figure 17. Palerang crossing with 3 sampling sites indicated at top of pond, near spillway and below spillway.

Figure 18. Reedy Creek sampling region above and below rocks in middle of creek.

Appendix 2. Identified aquatic invertebrates collected during the study for each site for each month collection was undertaken

Site	Taxon		October	January	April
Black Jackie	**Acarina**	**Acarina**			
Black Jackie	**Amphipoda**	**Ceinidae**		7	12
Black Jackie	**Bivalvia**	**Sphaeriidae**		2	1
Black Jackie	**Coleoptera**	**Psephenidae**	1		
Black Jackie	**Coleoptera**	**Dytiscidae**			
Black Jackie	**Coleoptera**	**Gyrinidae**			
Black Jackie	**Coleoptera**	**Haliplidae**			
Black Jackie	**Coleoptera**	**Hydrophilidae**			
Black Jackie	**Coleoptera**	**Scirtidae**			
Black Jackie	**Megaloptera**	**Corydalidae**	1	4	
Black Jackie	**Collembola**	**sp.**			
Black Jackie	**Crustacea**	**Cladocera**			3
Black Jackie	**Crustacea**	**Copepoda**			
Black Jackie	**Crustacea**	**Ostracoda**			
Black Jackie	**Decapoda**	**Atyidae**	5	12	
Black Jackie	**Decapoda**	**Parastacidae**			2
Black Jackie	**Diptera**	**Ceratopogonidae**			
Black Jackie	**Diptera**	**Culicidae**	1		
Black Jackie	**Diptera**	**s-f Chironominae**	20	30	11
Black Jackie	**Diptera**	**s-f Orthocladiinae**	6	18	5
Black Jackie	**Diptera**	**s-f Tanypodinae**	39	72	16
Black Jackie	**Diptera**	**Simulidae**		25	
Black Jackie	**Diptera**	**Stratiomyidae**			
Black Jackie	**Plecoptera**	**Gripopterygidae**	5	3	
Black Jackie	**Ephemeroptera**	**Baetidae**	4	10	26
Black Jackie	**Ephemeroptera**	**Caenidae**	1	3	9
Black Jackie	**Ephemeroptera**	**Leptophlebiidae**	12	48	54
Black Jackie	**Gastropoda**	**Lymnaeidae**			
Black Jackie	**Gastropoda**	**Physidae**			
Black Jackie	**Gastropoda**	**Planorbidae**			
Black Jackie	**Hemiptera**	**Corixidae**			7
Black Jackie	**Hemiptera**	**Naucoridae**			
Black Jackie	**Hemiptera**	**Notonectidae**			
Black Jackie	**Hemiptera**	**Veliidae**			4
Black Jackie	**Hirudinea**	**Glossiphoniidae**			
Black Jackie	**Odonata**	**Aeshnidae**	1	2	1
Black Jackie	**Odonata**	**Coenagrionidae**	3		
Black Jackie	**Odonata**	**Gomphidae**			
Black Jackie	**Odonata**	**Hemicorduliidae**			
Black Jackie	**Odonata**	**Lestidae**			4
Black Jackie	**Odonata**	**Libellulidae**			
Black Jackie	**Odonata**	**Telephlebiidae**			
Black Jackie	**Odonata**	**Synlestidae**	1	1	2

Black Jackie	**Oligochaeta**	**Oligochaeta**			12
Black Jackie	**Trichoptera**	**Calamoceratidae**		1	1
Black Jackie	**Trichoptera**	**Calosidae**		7	
Black Jackie	**Trichoptera**	**Conosucidae**		1	
Black Jackie	**Trichoptera**	**Ecnomidae**	2	8	2
Black Jackie	**Trichoptera**	**Glossosomatidae**			
Black Jackie	**Trichoptera**	**Helicophidae**		1	1
Black Jackie	**Trichoptera**	**Hydribiosiidae**			3
Black Jackie	**Trichoptera**	**Hydropsychidae**		5	
Black Jackie	**Trichoptera**	**Hydroptilidae**		2	
Black Jackie	**Trichoptera**	**Leptoceridae**	1	13	16
Black Jackie	**Trichoptera**	**Philoreithridae**			
Black Jackie	**Turbellaria**	**Dugesiidae**			
Black Jackie	**Turbellaria**	**Temnocephalidae**			1
		Total invertebrates	103	275	193
		Total taxa	16	22	22
Triple Ponds	**Acarina**	**Acarina**			1
Triple Ponds	**Amphipoda**	**Ceinidae**	12	6	8
Triple Ponds	**Bivalvia**	**Sphaeriidae**		2	2
Triple Ponds	**Coleoptera**	**Psephenidae**			
Triple Ponds	**Coleoptera**	**Dytiscidae**			
Triple Ponds	**Coleoptera**	**Gyrinidae**			
Triple Ponds	**Coleoptera**	**Haliplidae**		1	
Triple Ponds	**Coleoptera**	**Hydrophilidae**			
Triple Ponds	**Coleoptera**	**Scirtidae**			
Triple Ponds	**Megaloptera**	**Corydalidae**			
Triple Ponds	**Collembola**	**sp.**			
Triple Ponds	**Crustacea**	**Cladocera**			
Triple Ponds	**Crustacea**	**Copepoda**		2	11
Triple Ponds	**Crustacea**	**Ostracoda**		80	1
Triple Ponds	**Decapoda**	**Atyidae**	2	1	
Triple Ponds	**Decapoda**	**Parastacidae**			
Triple Ponds	**Diptera**	**Ceratopogonidae**		2	
Triple Ponds	**Diptera**	**Culicidae**			
Triple Ponds	**Diptera**	**s-f Chironominae**	9	13	2
Triple Ponds	**Diptera**	**s-f Orthocladiinae**	3	1	
Triple Ponds	**Diptera**	**s-f Tanypodinae**	12	36	1
Triple Ponds	**Diptera**	**Simulidae**		5	
Triple Ponds	**Diptera**	**Stratiomyidae**			
Triple Ponds	**Plecoptera**	**Gripopterygidae**	2		
Triple Ponds	**Ephemeroptera**	**Baetidae**		2	
Triple Ponds	**Ephemeroptera**	**Caenidae**	1	4	1
Triple Ponds	**Ephemeroptera**	**Leptophlebiidae**	13	16	2
Triple Ponds	**Gastropoda**	**Lymnaeidae**			
Triple Ponds	**Gastropoda**	**Physidae**			

Triple Ponds	**Gastropoda**	**Planorbidae**			
Triple Ponds	**Hemiptera**	**Corixidae**			
Triple Ponds	**Hemiptera**	**Naucoridae**			
Triple Ponds	**Hemiptera**	**Notonectidae**		3	2
Triple Ponds	**Hemiptera**	**Veliidae**			
Triple Ponds	**Hirudinea**	**Glossiphoniidae**	1	4	1
Triple Ponds	**Odonata**	**Aeshnidae**		1	
Triple Ponds	**Odonata**	**Coenagrionidae**		1	
Triple Ponds	**Odonata**	**Gomphidae**			
Triple Ponds	**Odonata**	**Hemicorduliidae**			
Triple Ponds	**Odonata**	**Lestidae**			
Triple Ponds	**Odonata**	**Libellulidae**		2	
Triple Ponds	**Odonata**	**Telephlebiidae**			
Triple Ponds	**Odonata**	**Synlestidae**			
Triple Ponds	**Oligochaeta**	**Oligochaeta**	2	25	7
Triple Ponds	**Trichoptera**	**Calamoceratidae**			
Triple Ponds	**Trichoptera**	**Calosidae**			
Triple Ponds	**Trichoptera**	**Conosucidae**			
Triple Ponds	**Trichoptera**	**Ecnomidae**	3		2
Triple Ponds	**Trichoptera**	**Glossosomatidae**			
Triple Ponds	**Trichoptera**	**Helicophidae**			
Triple Ponds	**Trichoptera**	**Hydribiosiidae**			
Triple Ponds	**Trichoptera**	**Hydropsychidae**	1	1	
Triple Ponds	**Trichoptera**	**Hydroptilidae**			
Triple Ponds	**Trichoptera**	**Leptoceridae**		8	2
Triple Ponds	**Trichoptera**	**Philoreithridae**	1	1	
Triple Ponds	**Turbellaria**	**Dugesiidae**			
Triple Ponds	**Turbellaria**	**Temnocephalidae**			
		Total invertebrates	62	217	43
		Total taxa	13	23	14
Peter's Pond	**Acarina**	**Acarina**	1		1
Peter's Pond	**Amphipoda**	**Ceinidae**	66	138	82
Peter's Pond	**Bivalvia**	**Sphaeriidae**			
Peter's Pond	**Coleoptera**	**Psephenidae**			
Peter's Pond	**Coleoptera**	**Dytiscidae**		2	
Peter's Pond	**Coleoptera**	**Gyrinidae**			
Peter's Pond	**Coleoptera**	**Haliplidae**			
Peter's Pond	**Coleoptera**	**Hydrophilidae**			
Peter's Pond	**Coleoptera**	**Scirtidae**			
Peter's Pond	**Megaloptera**	**Corydalidae**			
Peter's Pond	**Collembola**	**sp.**			
Peter's Pond	**Crustacea**	**Cladocera**		55	3
Peter's Pond	**Crustacea**	**Copepoda**		8	38
Peter's Pond	**Crustacea**	**Ostracoda**	17	3	7
Peter's Pond	**Decapoda**	**Atyidae**	2	22	2

Peter's Pond	**Decapoda**	**Parastacidae**			1
Peter's Pond	**Diptera**	**Ceratopogonidae**	2		1
Peter's Pond	**Diptera**	**Culicidae**			2
Peter's Pond	**Diptera**	**s-f Chironominae**	86	52	39
Peter's Pond	**Diptera**	**s-f Orthocladiinae**	4	18	11
Peter's Pond	**Diptera**	**s-f Tanypodinae**	83	39	52
Peter's Pond	**Diptera**	**Simulidae**	1	2	1
Peter's Pond	**Diptera**	**Stratiomyidae**		1	
Peter's Pond	**Plecoptera**	**Gripopterygidae**	1		
Peter's Pond	**Ephemeroptera**	**Baetidae**	4	35	40
Peter's Pond	**Ephemeroptera**	**Caenidae**	2		
Peter's Pond	**Ephemeroptera**	**Leptophlebiidae**	17	72	18
Peter's Pond	**Gastropoda**	**Lymnaeidae**		1	
Peter's Pond	**Gastropoda**	**Physidae**		4	10
Peter's Pond	**Gastropoda**	**Planorbidae**			
Peter's Pond	**Lepidoptera**	**Pyralidae**			1
Peter's Pond	**Hemiptera**	**Corixidae**			1
Peter's Pond	**Hemiptera**	**Naucoridae**		5	5
Peter's Pond	**Hemiptera**	**Notonectidae**		11	
Peter's Pond	**Hemiptera**	**Veliidae**		1	
Peter's Pond	**Hirudinea**	**Glossiphoniidae**			27
Peter's Pond	**Odonata**	**Aeshnidae**		3	1
Peter's Pond	**Odonata**	**Coenagrionidae**	5	73	69
Peter's Pond	**Odonata**	**Gomphidae**		5	5
Peter's Pond	**Odonata**	**Hemicorduliidae**			
Peter's Pond	**Odonata**	**Lestidae**	3	13	12
Peter's Pond	**Odonata**	**Libellulidae**		2	2
Peter's Pond	**Odonata**	**Telephlebiidae**			
Peter's Pond	**Odonata**	**Synlestidae**		3	1
Peter's Pond	**Oligochaeta**	**Oligochaeta**	1	2	4
Peter's Pond	**Trichoptera**	**Calamoceratidae**	2		
Peter's Pond	**Trichoptera**	**Calosidae**			
Peter's Pond	**Trichoptera**	**Ecnomidae**	1	1	13
Peter's Pond	**Trichoptera**	**Glossosomatidae**			
Peter's Pond	**Trichoptera**	**Helicophidae**			
Peter's Pond	**Trichoptera**	**Hydribiosiidae**			
Peter's Pond	**Trichoptera**	**Hydropsylidae**			
Peter's Pond	**Trichoptera**	**Hydropsychidae**			
Peter's Pond	**Trichoptera**	**Hydroptilidae**	1	4	
Peter's Pond	**Trichoptera**	**Leptoceridae**		11	4
Peter's Pond	**Turbellaria**	**Dugesiidae**			
Peter's Pond	**Turbellaria**	**Temnocephalidae**			6
		Total invertebrates	299	586	459
		Total taxa	19	28	30
Palerang	**Acarina**	**Acarina**			

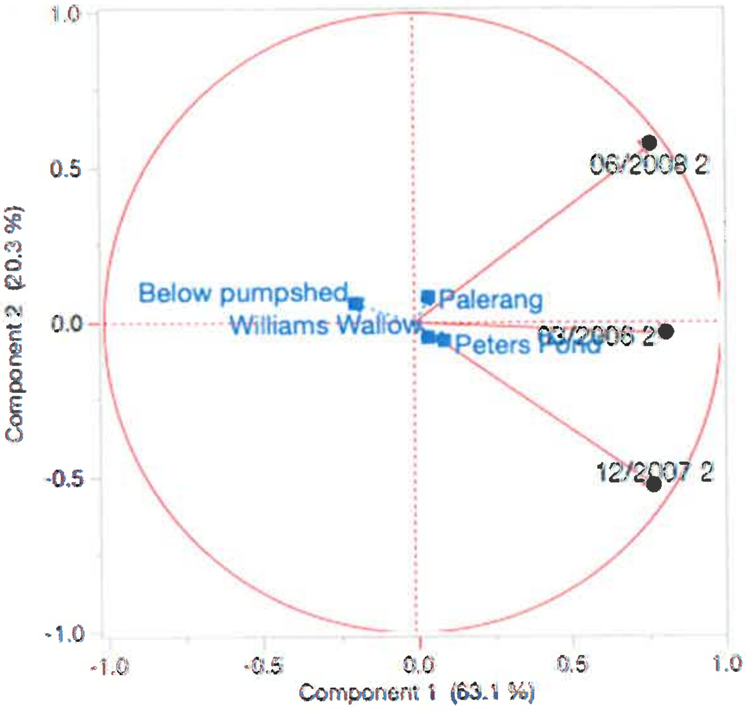
Palerang	**Amphipoda**	**Ceinidae**	18	4	
Palerang	**Bivalvia**	**Sphaeriidae**		1	
Palerang	**Coleoptera**	**Psephenidae**			
Palerang	**Coleoptera**	**Dytiscidae**	2	1	
Palerang	**Coleoptera**	**Gyrinidae**			
Palerang	**Coleoptera**	**Haliplidae**			
Palerang	**Coleoptera**	**Hydrophilidae**			
Palerang	**Coleoptera**	**Scirtidae**		3	
Palerang	**Megaloptera**	**Corydalidae**			
Palerang	**Collembola**	**sp.**		1	
Palerang	**Crustacea**	**Cladocera**			
Palerang	**Crustacea**	**Copepoda**			3
Palerang	**Crustacea**	**Ostracoda**			
Palerang	**Decapoda**	**Atyidae**		1	
Palerang	**Decapoda**	**Parastacidae**	1		
Palerang	**Diptera**	**Ceratopogonidae**		6	1
Palerang	**Diptera**	**Culicidae**			
Palerang	**Diptera**	**s-f Chironominae**	15	55	21
Palerang	**Diptera**	**s-f Orthocladiinae**			1
Palerang	**Diptera**	**s-f Tanypodinae**	14	28	2
Palerang	**Diptera**	**Simulidae**		51	
Palerang	**Diptera**	**Stratiomyidae**			
Palerang	**Diptera**	**Syrphidae**		1	
Palerang	**Plecoptera**	**Gripopterygidae**			
Palerang	**Ephemeroptera**	**Baetidae**	1	1	
Palerang	**Ephemeroptera**	**Caenidae**	6	3	
Palerang	**Ephemeroptera**	**Leptophlebiidae**	19	16	
Palerang	**Gastropoda**	**Lymnaeidae**			
Palerang	**Gastropoda**	**Physidae**			
Palerang	**Gastropoda**	**Planorbidae**			
Palerang	**Hemiptera**	**Aphididae**			1
Palerang	**Hemiptera**	**Corixidae**			
Palerang	**Hemiptera**	**Naucoridae**			
Palerang	**Hemiptera**	**Notonectidae**			
Palerang	**Hemiptera**	**Veliidae**			
Palerang	**Hirudinea**	**Glossiphoniidae**			
Palerang	**Odonata**	**Aeshnidae**	1		
Palerang	**Odonata**	**Coenagrionidae**	1		1
Palerang	**Odonata**	**Gomphidae**		1	
Palerang	**Odonata**	**Hemicorduliidae**			
Palerang	**Odonata**	**Lestidae**			
Palerang	**Odonata**	**Libellulidae**			
Palerang	**Odonata**	**Telephlebiidae**			
Palerang	**Odonata**	**Synlestidae**			
Palerang	**Oligochaeta**	**Oligochaeta**	4	7	
Palerang	**Trichoptera**	**Calamoceratidae**			

Palerang	**Trichoptera**	**Calosidae**			
Palerang	**Trichoptera**	**Ecnomidae**		3	1
Palerang	**Trichoptera**	**Glossosomatidae**			
Palerang	**Trichoptera**	**Helicophidae**			
Palerang	**Trichoptera**	**Hydribiosiidae**			
Palerang	**Trichoptera**	**Hydropsychidae**		12	
Palerang	**Trichoptera**	**Hydroptilidae**			
Palerang	**Trichoptera**	**Leptoceridae**			
Palerang	**Turbellaria**	**Dugesiidae**			
Palerang	**Turbellaria**	**Temnocephalidae**			
		Total invertebrates	82	195	31
		Total taxa	11	18	8
Sandyhills Cr	**Acarina**	**Acarina**	NO samples		
Sandyhills Cr	**Amphipoda**	**Ceinidae**	NO samples	3	1
Sandyhills Cr	**Bivalvia**	**Sphaeriidae**	NO samples		2
Sandyhills Cr	**Coleoptera**	**Psephenidae**	NO samples		
Sandyhills Cr	**Coleoptera**	**Dytiscidae**	NO samples		
Sandyhills Cr	**Coleoptera**	**Elmidae**		1	
Sandyhills Cr	**Coleoptera**	**Gyrinidae**	NO samples	1	
Sandyhills Cr	**Coleoptera**	**Haliplidae**	NO samples		
Sandyhills Cr	**Coleoptera**	**Hydrophilidae**	NO samples		
Sandyhills Cr	**Coleoptera**	**Scirtidae**	NO samples		
Sandyhills Cr	**Megaloptera**	**Corydalidae**	NO samples		
Sandyhills Cr	**Collembola**	**sp.**	NO samples		
Sandyhills Cr	**Crustacea**	**Cladocera**	NO samples	1	1
Sandyhills Cr	**Crustacea**	**Copepoda**	NO samples	1	9
Sandyhills Cr	**Crustacea**	**Ostracoda**	NO samples		
Sandyhills Cr	**Decapoda**	**Atyidae**	NO samples	3	
Sandyhills Cr	**Decapoda**	**Parastacidae**	NO samples		
Sandyhills Cr	**Diptera**	**Ceratopogonidae**	NO samples		
Sandyhills Cr	**Diptera**	**Culicidae**	NO samples		
Sandyhills Cr	**Diptera**	**s-f Chironominae**	NO samples	4	11
Sandyhills Cr	**Diptera**	**s-f Orthocladiinae**	NO samples		2
Sandyhills Cr	**Diptera**	**s-f Tanypodinae**	NO samples	3	3
Sandyhills Cr	**Diptera**	**Simulidae**	NO samples		
Sandyhills Cr	**Diptera**	**Stratiomyidae**	NO samples		
Sandyhills Cr	**Plecoptera**	**Gripopterygidae**	NO samples		
Sandyhills Cr	**Ephemeroptera**	**Baetidae**	NO samples		
Sandyhills Cr	**Ephemeroptera**	**Caenidae**	NO samples		
Sandyhills Cr	**Ephemeroptera**	**Leptophlebiidae**	NO samples	2	
Sandyhills Cr	**Gastropoda**	**Lymnaeidae**	NO samples		
Sandyhills Cr	**Gastropoda**	**Physidae**	NO samples		
Sandyhills Cr	**Gastropoda**	**Planorbidae**	NO samples		
Sandyhills Cr	**Hemiptera**	**Corixidae**	NO samples	1	
Sandyhills Cr	**Hemiptera**	**Naucoridae**	NO samples		

Sandyhills Cr	**Hemiptera**	**Notonectidae**	NO samples	1	2
Sandyhills Cr	**Hemiptera**	**Veliidae**	NO samples		
Sandyhills Cr	**Hirudinea**	**Glossiphoniidae**	NO samples		
Sandyhills Cr	**Odonata**	**Aeshnidae**	NO samples	1	
Sandyhills Cr	**Odonata**	**Coenagrionidae**	NO samples	1	3
Sandyhills Cr	**Odonata**	**Gomphidae**	NO samples		
Sandyhills Cr	**Odonata**	**Hemicorduliidae**	NO samples		
Sandyhills Cr	**Odonata**	**Lestidae**	NO samples		
Sandyhills Cr	**Odonata**	**Libellulidae**	NO samples		
Sandyhills Cr	**Odonata**	**Telephlebiidae**	NO samples		
Sandyhills Cr	**Odonata**	**Synlestidae**	NO samples		
Sandyhills Cr	**Oligochaeta**	**Oligochaeta**	NO samples		
Sandyhills Cr	**Trichoptera**	**Calamoceratidae**	NO samples		
Sandyhills Cr	**Trichoptera**	**Calosidae**	NO samples		
Sandyhills Cr	**Trichoptera**	**Ecnomidae**	NO samples	1	
Sandyhills Cr	**Trichoptera**	**Glossosomatidae**	NO samples		
Sandyhills Cr	**Trichoptera**	**Helicophidae**	NO samples		
Sandyhills Cr	**Trichoptera**	**Hydribiosiidae**	NO samples		
Sandyhills Cr	**Trichoptera**	**Hydropsylidae**	NO samples		
Sandyhills Cr	**Trichoptera**	**Hydroptilidae**	NO samples		
Sandyhills Cr	**Trichoptera**	**Leptoceridae**	NO samples	3	
Sandyhills Cr	**Turbellaria**	**Dugesiidae**	NO samples		
Sandyhills Cr	**Turbellaria**	**Temnocephalidae**	NO samples		6
		Total invertebrates		27	40
		Total taxa		15	10
Reedy Creek	**Acarina**	**Acarina**			
Reedy Creek	**Amphipoda**	**Ceinidae**	10	72	1
Reedy Creek	**Bivalvia**	**Sphaeriidae**			2
Reedy Creek	**Coleoptera**	**Psephenidae**			
Reedy Creek	**Coleoptera**	**Dytiscidae**			
Reedy Creek	**Coleoptera**	**Gyrinidae**			
Reedy Creek	**Coleoptera**	**Haliplidae**			
Reedy Creek	**Coleoptera**	**Hydrophilidae**	1	1	
Reedy Creek	**Coleoptera**	**Scirtidae**	1		
Reedy Creek	**Megaloptera**	**Corydalidae**			
Reedy Creek	**Collembola**	**sp.**		1	
Reedy Creek	**Crustacea**	**Cladocera**			
Reedy Creek	**Crustacea**	**Copepoda**		9	20
Reedy Creek	**Crustacea**	**Ostracoda**			
Reedy Creek	**Decapoda**	**Atyidae**	3	117	
Reedy Creek	**Decapoda**	**Parastacidae**			
Reedy Creek	**Diptera**	**Ceratopogonidae**		1	1
Reedy Creek	**Diptera**	**Culicidae**			
Reedy Creek	**Diptera**	**s-f Chironominae**	22	30	43
Reedy Creek	**Diptera**	**s-f Orthocladiinae**	2	2	4

Reedy Creek	Diptera	s-f Tanypodinae	41	39	95
Reedy Creek	Diptera	Psychodidae		3	
Reedy Creek	Diptera	Sciomyzidae			1
Reedy Creek	Diptera	Simulidae		1	
Reedy Creek	Diptera	Stratiomyidae			
Reedy Creek	Plecoptera	Gripopterygidae	3		
Reedy Creek	Ephemeroptera	Baetidae	8	37	4
Reedy Creek	Ephemeroptera	Caenidae	3	7	21
Reedy Creek	Ephemeroptera	Leptophlebiidae	7	64	11
Reedy Creek	Gastropoda	Lymnaeidae			
Reedy Creek	Gastropoda	Physidae			
Reedy Creek	Gastropoda	Planorbidae		1	
Reedy Creek	Hemiptera	Corixidae			
Reedy Creek	Hemiptera	Naucoridae			
Reedy Creek	Hemiptera	Notonectidae			
Reedy Creek	Hemiptera	Saldidae			6
Reedy Creek	Hemiptera	Veliidae			
Reedy Creek	Hirudinea	Glossiphoniidae			
Reedy Creek	Lepidoptera	Pyralidae		1	
Reedy Creek	Odonata	Aeshnidae	1		3
Reedy Creek	Odonata	Gomphidae		3	
Reedy Creek	Odonata	Coenagrionidae	1	3	3
Reedy Creek	Odonata	Amphipterygidae		1	
Reedy Creek	Odonata	Hemicorduliidae			
Reedy Creek	Odonata	Lestidae	4	3	
Reedy Creek	Odonata	Libellulidae			
Reedy Creek	Odonata	Telephlebiidae			
Reedy Creek	Odonata	Synlestidae			
Reedy Creek	Oligochaeta	Oligochaeta	3	5	2
Reedy Creek	Trichoptera	Calamoceratidae			
Reedy Creek	Trichoptera	Calosidae		17	1
Reedy Creek	Trichoptera	Coenosuchidae		1	
Reedy Creek	Trichoptera	Ecnomidae	6	6	25
Reedy Creek	Trichoptera	Glossosomatidae			
Reedy Creek	Trichoptera	Helicophidae			
Reedy Creek	Trichoptera	Hydribiosiidae	2		
Reedy Creek	Trichoptera	Hydropsylidae	1		
Reedy Creek	Trichoptera	Hydroptilidae			
Reedy Creek	Trichoptera	Leptoceridae		34	4
Reedy Creek	Turbellaria	Dugesiidae			
Reedy Creek	Turbellaria	Temnocephalidae		2	
		Total invertebrates	119	461	247
		Total taxa	18	26	18

Appendix 3. Principal component analysis of the collections done in 2006-08. The difficulty with this analysis is two-fold: 1) the collections were done in different years and different seasons and 2) the numbers of taxa and individuals identified were much reduced compared with the current study. As a result, less variation is found between sites as all are close to the origin. Peter's Pond and William's Wallow do show some similarity as they are nearly identical in vector direction and length. As William's Wallow is relatively close to Peter's Pond, water flows may have been similar, and the similarity could reflect that situation. The seasonal pattern is apparent though as the shift is in the same anticlockwise pattern as the current data.



Appendix 4. Differences in SIGNAL score and principal component analysis of macroinvertebrates.

In this report, we show differences among the various sites on the basis of two mathematical ways of determining macroinvertebrate numbers and taxa, the SIGNAL score and a principal component analysis.

The SIGNAL (Stream Invertebrate Grade Number – Average Level) score is derived from a simplified system to give an indication of water quality (Chessman 2003). The score is based on a table that gives each taxon a grade from 1-10 that is derived from the perception of how representative that taxon is for indicating high quality water. The higher the grade the less tolerant that taxon is considered to be to pollution, the lower the grade the more pollution tolerant. The grade for the taxon is then multiplied by a weighting associated with the numbers of that taxon collected, but the weighting only varies from 1-5 as shown in Table 4.1.

Table A4.1. Relationship between number of any single taxon collected and weighting for that number in SIGNAL calculation. This table shows that collecting more than 20 from any taxon is not considered in the calculation of a SIGNAL score.

Number of taxon collected	Weighting
1-2	1
3-5	2
6-10	3
11-20	4
>20	5

As the SIGNAL score is determined by the calculation in equation 1, a few high grade macroinvertebrates can have a much higher influence on the overall score than many lower grade macroinvertebrates, especially as no more than 21 are considered in the actual calculation.

$$\text{SIGNAL score} = \frac{\sum Grade \times weighting}{\sum taxa\ weight} \qquad \text{(Equation 1)}$$

The SIGNAL score is easy to determine using an Excel spread sheet and also minimises the time for sorting macroinvertebrates as only 21 individuals from a taxon are considered in the calculation, therefore counting more than that is unnecessary (for grades used in this report, see Table A4.2).

In contrast, a principal component analysis does not consider any taxon different from another, but only considers how many taxa and how many individuals for each taxa were collected. The comparison is therefore not weighted towards any single taxa, but makes the comparison as though nothing is known about which taxa may represent a certain freshwater condition. Because of this lack of precondition expectation, the analysis will be more robust towards comparing biodiversity at different locations and a few specimens will not unduly bias the analysis. The data is also natural log transformed to reduce the influence of highly common species on the analysis as mentioned in the results. A principal component analysis is not as easily calculated as a SIGNAL score, but requires specialised computer/statistical programs, but for making site-by-site comparisons as required for ecological studies presents a more complete picture of what is happening in each location.

Table A4.2. Grades of taxa used in this report taken from Chessman (2003). No grades are given for Acari or Collembola, but these taxa were rare in the collections. No grades are given for Copepoda, Cladocera or Ostracoda as grades were omitted in Chessman (2003).

Taxon	Family	Signal Grade
Acarina	Acarina	
Amphipoda	Ceinidae	2
Bivalvia	Sphaeriidae	5
Coleoptera	Dytiscidae	2
Coleoptera	Gyrinidae	4
Coleoptera	Haliplidae	2
Coleoptera	Hydrophilidae	2
Coleoptera	Psephenidae	6
Coleoptera	Scirtidae	6
Collembola	sp.	
Crustacea	Cladocera	
Crustacea	Copepoda	
Crustacea	Ostracoda	
Decapoda	Atyidae	3
Decapoda	Parastacidae	4
Diptera	Ceratopogonidae	4
Diptera	Culicidae	1
Diptera	s-f Chironominae	3
Diptera	s-f Orthocladiinae	4
Diptera	s-f Tanypodinae	4
Diptera	Simulidae	5
Diptera	Stratiomyidae	2
Ephemeroptera	Baetidae	5
Ephemeroptera	Caenidae	4
Ephemeroptera	Leptophlebiidae	8
Gastropoda	Lymnaeidae	1
Gastropoda	Physidae	1
Gastropoda	Planorbidae	2
Hemiptera	Corixidae	2
Hemiptera	Naucoridae	2
Hemiptera	Notonectidae	1
Hemiptera	Veliidae	3
Hirudinea	Glossiphoniidae	1
Megaloptera	Corydalidae	7
Odonata	Aeshnidae	4
Odonata	Coenagrionidae	2
Odonata	Gomphidae	5
Odonata	Hemicorduliidae	5
Odonata	Lestidae	1
Odonata	Libellulidae	4

Odonata	Synlestidae	7
Odonata	Telephlebiidae	9
Oligochaeta	Oligochaeta	3
Plecoptera	Gripopterygidae	8
Trichoptera	Calamoceratidae	7
Trichoptera	Calosidae	9
Trichoptera	Conosucidae	7
Trichoptera	Ecnomidae	4
Trichoptera	Glossosomatidae	9
Trichoptera	Helicophidae	10
Trichoptera	Hydribiosiidae	8
Trichoptera	Hydropsychidae	6
Trichoptera	Hydroptilidae	4
Trichoptera	Leptoceridae	6
Trichoptera	Philoreithridae	8
Turbellaria	Dugesiidae	2
Turbellaria	Temnocephalidae	5