Invertebrate survey of Mulloon Creek during a drought 2019-20

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

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Mulloon Institute
For environment, farming and society.

Australian Government National Landcare Programme

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Table of Contents

Acknowledgements **2**
Executive Summary **3**
Introduction **4**
Methods and sites **5**
Results **7**
Discussion **9**
References **12**
Appendices **30**

Acknowledgements
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Front cover pictures (clockwise):
Black Jackie pond in May 2019
Two Hydroptilidae from Black Jackie Pond in January 2020
Veliidae from Black Jackie Pond in October 2019

Executive Summary

1. Physicochemical components of six sites along Mulloon, Sandhills and Reedy Creeks are similar during drought except for the higher conductivity in Sandhills and Reedy Creeks.
2. According to SIGNAL scores for macroinvertebrates, no site during the drought would be considered pristine as all sites were at most near 3, indicating a less diverse taxa.
3. Principal component analysis of macroinvertebrates indicated that sites were similar except for Sandhills Creek and Palerang Crossing which appeared to be depauperate.
4. Future modifications of the sites should include greater overhanging vegetation to increase allochthonous input to enhance certain functional groups of macroinvertebrates.

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 (Thomson *et al.* 2012, Verkaik *et al.* 2014), few studies have considered the smaller catchments that may change as farming practices change, especially during droughts. 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), a situation that is exacerbated in droughts as vegetation increases and flow completely ceases. All these physical components can become more important as drought extends the time between renewal of water movements among the series of ponds that exist during droughts, potentially benefitting some taxa, but decreasing populations of other taxa (Boulton 2003, White *et al*. 2012).

In this report, we present information regarding the macroinvertebrate communities that are present at six sites along Mulloon and Reedy Creeks over 12 months (April 2019-March 2020) along with the physical conditions that were present in the separate ponds located at each site 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, however during the extended drought conditions, little to no flow occurred to connect these sites. 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 can be compared to the previous study when rain occurred in each month of the survey and the creek was flowing continuously. The effects of drought conditions measured over this year are important for understanding how future work and development along Mulloon Creek can influence the macroinvertebrate communities when rain input may be limiting.

Methods and sites

Study sites

Six sites along Mulloon Creek were chosen and are shown relative to the catchment in Figure 1 and are the same sites examined in the previous work (Cooper and Wallenius 2017).

Invertebrates were collected only by sweep net, as no location had water flow. At least 10 sweeps were done 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 April, July, and October 2019 and January 2020. Physical measurements were made month from April 2019-March 2020, with forest fires potentially affecting measurements between December 2019-February 2020.

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. No water flow was measured throughout this year study, until February 2020 after all invertebrate collections had been done, as rain only began to fall then. 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. Collections included large quantities of vegetation, as more vegetation was present in all sites as water content decreased. 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 (Gooderham and Tsyrlin 2002, Hawking 1986, Hawking and Smith 1997, Lucid Australian Aquatic Invertebrates).

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 1993). We used two techniques: principal component analysis and SIGNAL scores as done previously (Cooper and Wallenius 2017).

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 compared with the 2006-08 and 2016-17 data 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 decreased in May following April, but then progressively increased into summer (Figure 2). Temperatures then decreased with the rain in February and March.

Dissolved oxygen values progressively decreased from April through to January, then increased with rain in February (Figure 3). Sandhills had the lowest dissolved oxygen values throughout the study compared with the other sites.

Conductivity was lower in the four Mulloon Creek sites than the conductivities present at the Sandhills and Reedy Creek sites (Figure 4), and all sites increased throughout the study. Water input in February tended to cause a decrease in conductivity in both Sandhills and Reedy Creek sites to nearly the same values as other sites.

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 lower pH values may be associated with organic acids with leaf falls.

Water flow was nil until the rain in February, although some movement within ponds as wind stirred the top surface water around.

Rain fell in most months (Figure 7). The rain in February resulted in Mulloon Creek flowing again, after becoming a chain of ponds. The periodic fall before that was not adequate to cause the creek 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, 8992 macroinvertebrates were identified, falling into 30 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 (July and January) Triple Ponds (January) and Reedy Creek (April) were the most diverse in number of taxa collected. Sandhills Creek had the fewest number of individuals collected over the study period, and was generally depauperate. Palerang, Reedy Creek and Sandhills all appeared to have fewer numbers of macroinvertebrates collected compared with the upstream sites.

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 at least once (Table 2). The most common taxon was the Chironominae in all seasons for all locations except Triple Pond and Peter's Pond, where they were only collected in abundance in April (TP) and July (TP and PP). Although large numbers of other taxa were collected, the number of sites or seasons did not correspond to what was collected.

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 and subsequently in 2015-16 at the same sites as here. 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 are lower than the scores in 2015-16, but comparable to those in 2006-08. The winter measurements for 2006-08 are slightly better than the scores made in this study, but whether that difference is important requires further research.

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, Black Jackie Reedy Creek and Peter's Pond have similar trajectories for their vectors, suggesting some similarity for these four sites. Sandhills Creek and Palerang Crossing have vectors towards the negative on the first eigenvector. A slight seasonal pattern is present as the vectors vary from autumn to summer, but summer is the only time when the second vector becomes positive.

Comparison with figure 8 derived from the physicochemical characteristics of the most sites are similar and not dependent upon physicochemical structure except for Sandhills and Reedy Creek, where the salinity increases but that seems to have only slight effects on the taxa that are present.

Discussion

Differences were found among the study sites with respect to both physicochemical characteristics and biological macroinvertebrate assemblages using the principal component analyses. The patterns that were present between sites in the study of 2015-16 are not found in this study, as four of the sites were similar and two (Sandhills and Palerang) were clearly different. Sandhills Creek was the most depauperate site, as observed previously (Cooper and Wallenius 2017), and Palerang Crossing was subjected to excavation midway through the study. How the excavation affected the studied populations is unclear, as prior information regarding the excavation was not available, so allowance was not made in the collection of samples. As the ponds were all isolated units throughout the collection period, the similarity indicated among the sites represented those taxa that did best when water was not flowing. The similarities among the sites indicated by the PCA of the physicochemical composition suggested that despite the separation, differences were still present, such as the salinity at Sandhills Gauge and Reedy Creek.

The SIGNAL system gave a somewhat different pattern of site structure with respect to water quality. In all collections, only 6 samples were greater than 3; Reedy Creek in all four seasons and Black Jackie Gauge in summer and autumn. In contrast, Peter's Pond in 2015-16 had the lowest SIGNAL score of 3.5. Using an AUSRIVAS analysis, good quality water would be anything above a SIGNAL score of 5 for this type of creek, no site in any season even approached that, indicating that drought does decrease the quality of diversity, with the reduction in diversity similar to previous work on Cotter River in a 2006-07 drought period (White *et al*. 2012). Reedy Creek has the highest scores despite having the highest conductivity, while Sandhills Gauge has some of the lowest SIGNAL scores in Spring and Winter, but also has a similar conductivity. Presumably, conductivity is not limiting the diversity, but other aspects of habitat, such as vegetation, may be influencing the taxa diversity during drought. 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).

Taxa appear to have an overwhelming similarity in all sites, as the first eigenvector accounts for nearly 79% of the variation (Figure 12, Table 2). The dipteran larvae may have played an extreme role in this analysis as that vector is almost perfectly aligned with the first eigenvector in the positive direction (Figure 13). The Odonata, Trichotera and Coleoptera appear to have some effect as well, as they all align with the negative axis. However, those sites (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, but given the excavation at PaC, that may have been a confounding effect.

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) and freshwater crayfish (Parastacidae) were present in both Black Jackie and Peter's Pond in January 2020 despite the lack of flow. Black Jackie was similar to Peter's Pond, a much larger water body, according to the PCA, potentially a result of the presence of leaves, wood and rocks that contribute to that macroinvertebrate diversity, again a result similar to the earlier study (Cooper and Wallenius 2017). 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 in both locations, although not all taxa appear to benefit.

The overall result suggests that little change has occurred over the 25 years in macroinvertebrate diversity, but drought is associated with a reduction in taxa, especially those dependent upon flowing water such as Trichoptera. 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, a result that was even apparent when water flow was more consistent (Cooper and Wallenius 2017).

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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, especially Sandy Hills and Reedy Creek, but pH was also an important variable. Water flow is important as no flow occurred at most sites except for the last two measurments (February and March 2020). The first two vectors account for 71% of the variation, and vector 3 accounts for an additional 12.9% of the variation. Although temperature did not appear to account for much of the variation in the univariate plots, it appears 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)	0.5442	0.0913	0.4982
Temp (˚C)	0.1213	0.7280	-0.5929
Dissolved O_2 (%)	-0.5480	0.1861	0.4106
pH	0.4722	0.3814	0.3446
H_2O flow (m/s)	-0.4073	0.5306	0.3361

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

Site	Month	Taxa	Percent of total
Black Jackie	April, July, October, January	Chironominae	13.6, 15.8, 18.5, 25.3
	April, July	Copepods	26.4, 13.3
	April	Ostracod	11.1
	October	Cladocera	16.6
	April	Baetidae	11.3
	January	Notonectidae	13.3
	July	Calocidae	26.9
Triple Ponds	January	Ceinidae	18.7
	January	Acarina	14.9
	July, October	Cladocera	15.2,10.9
	April	Ostracoda	10.1
	July	Copepoda	15.7
	April, July	Chironominae	11.7, 22.5
	October	Tanypodinae	36.8
	April, October	Ceratopogonidae	20.2, 10.3
	April, October	Coenagrionidae	23.6, 12.0
Peter's Pond	April, July, October, January	Ceinidae	32.6, 45.8, 27.2, 57.0
	July	Cladocera	10.2
	April	Ceratopogonidae	17.1
	July	Chironominae	10.8
	April, October	Tanypodinae	10.1, 19.3
	January	Leptophlebiidae	12.3
	April, October	Coenagrionidae	14.5, 14.0
Palerang	July	Copepoda	21.6
	July, October	Ostracoda	14.7, 26.2
	April, July, October	Ceratopogonidae	19.5, 15.4, 15.0
	April, July, October, January	Chironominae	36.3, 30.5, 11.6, 41.9
	October, January	Tanypodinae	17.2, 13.4
Sandhills Creek	July	Collembola	36.4
	July, October	Copepoda	23.1, 15.6
	April, July, October, January	Chironominae	62.1, 18.2, 50.0, 77.1
	October	Oligochaeta	10.9
Reedy Creek	April, July, January	Copepoda	13.0, 14.8, 20.4
	April, July, October, January	Chironominae	34.9, 38.6, 21.2, 16.3
	October, January	Tanypodinae	29.0, 15.7

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, one eigenvalue accounts for 79% of the variation indicating that the taxa are responding similarly in the various sites.

Number of eigenvalues	Eigenvalue	Cumulative Percent
1	3.1621	79.053
2	0.3582	88.009
3	0.3091	95.735

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

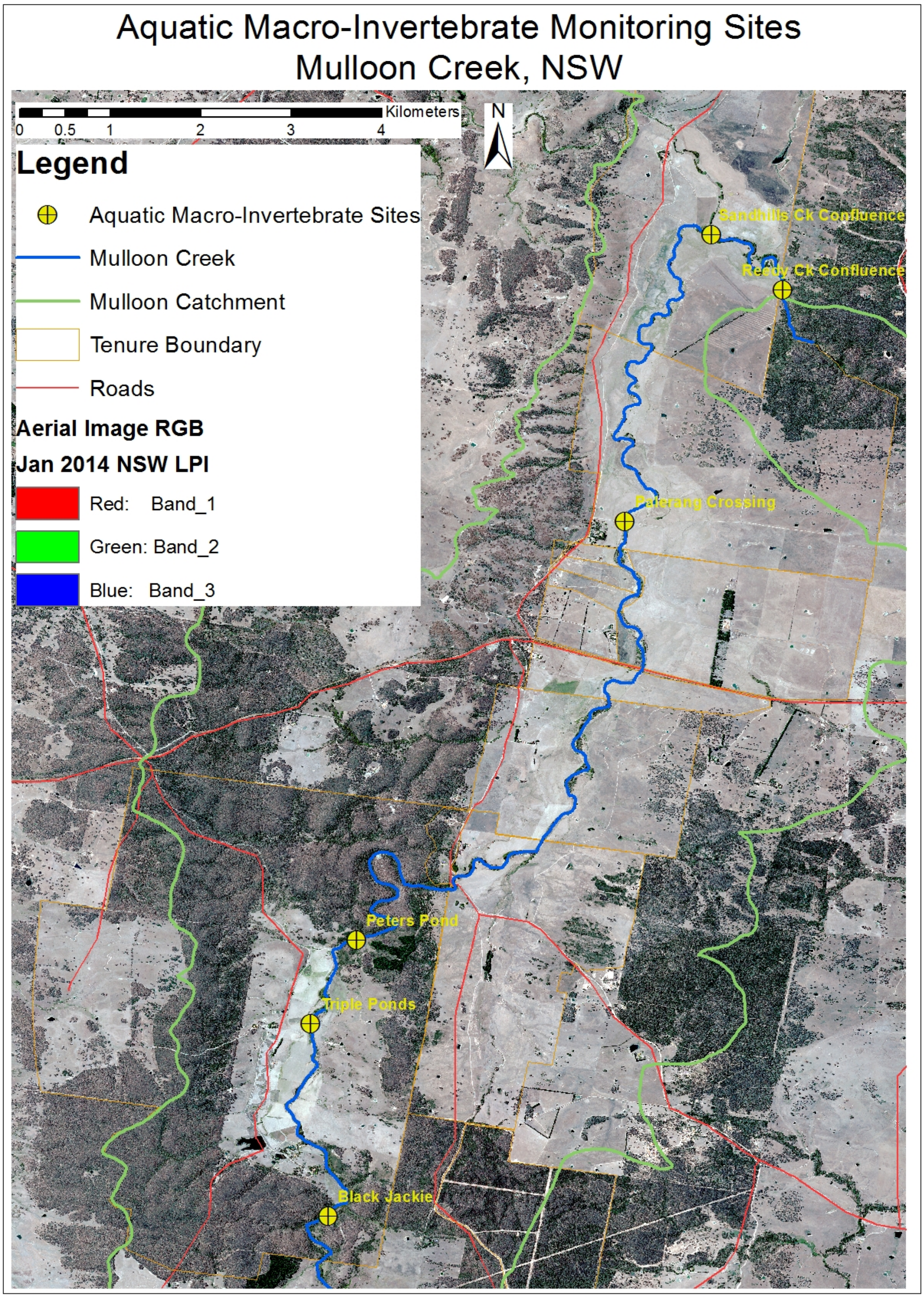


Figure 2. Water temperature at six sites along Mulloon Creek from Oct 2019-May 2020. Temperature is near 20 ˚C from December to February. Temperatures were only between 5-10˚C May to September then started increasing above 10˚C in October and November.

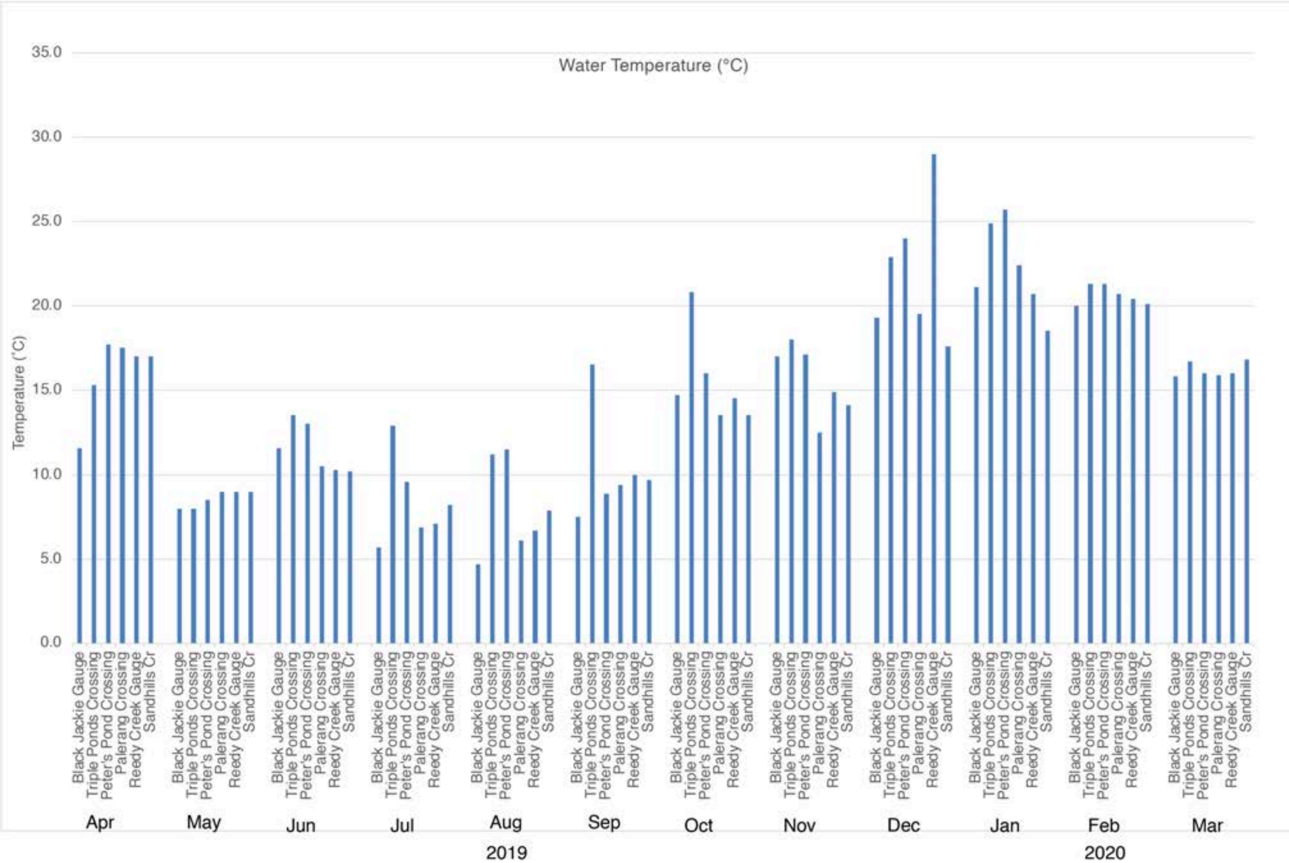


Figure 3. Dissolved oxygen (%saturation at measured temperature) for the six sites along Mulloon Creek from April 2019 –March 2020. Oxygen decreased as temperatures increased from April to January, but increased again after the rain. Dissolved oxygen is usually lowest in Sandhills, however oxygen in most ponds would have been maintained by both wind and vegetation within the ponds producing oxygen during the day.

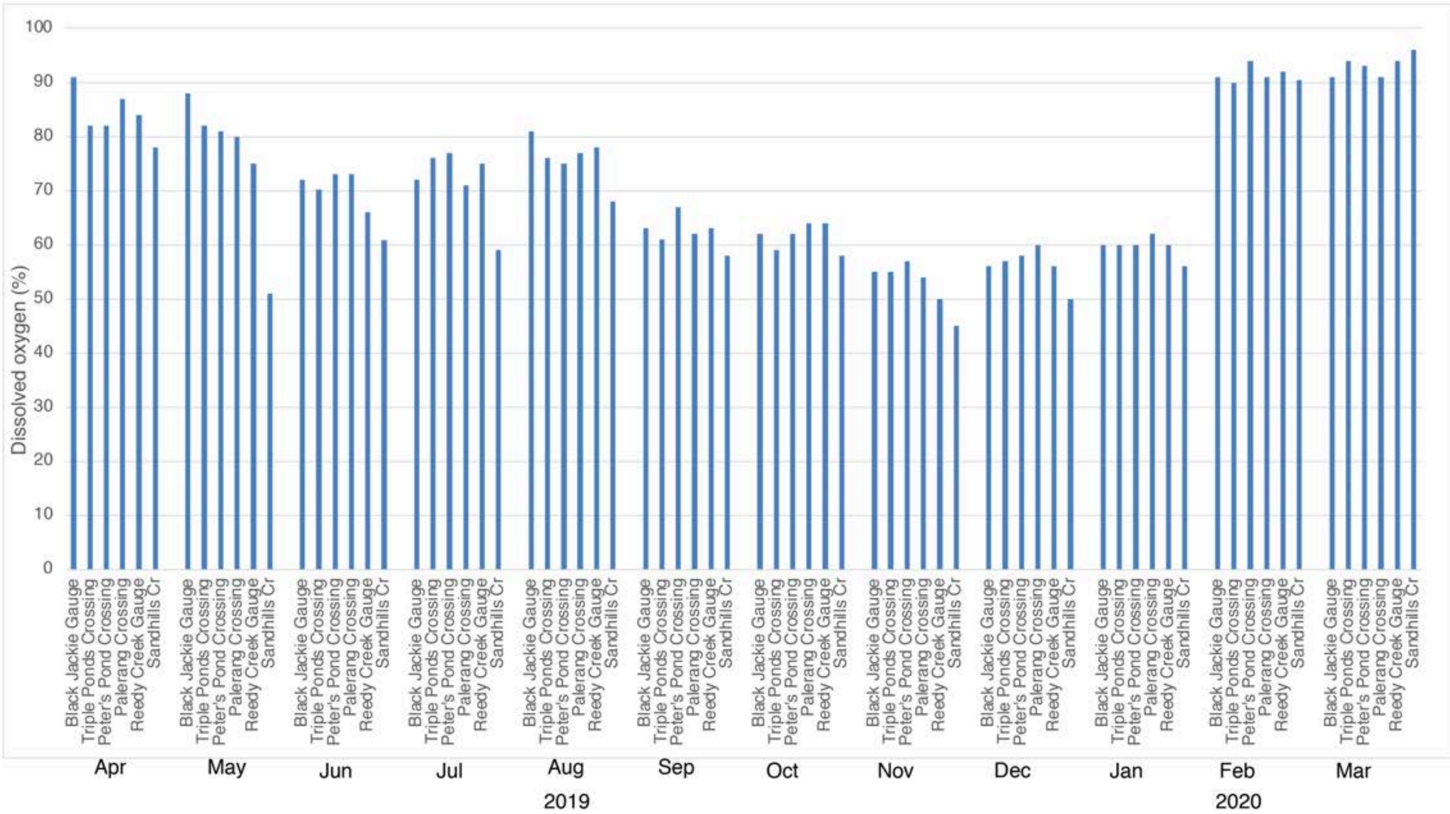


Figure 4. Changes in conductivity at 6 sites along Mulloon Creek from Apr 2019-March 2020. Reedy Creek and Sandhills are much higher in conductivity than the other sites, until rain occurred in February and March.

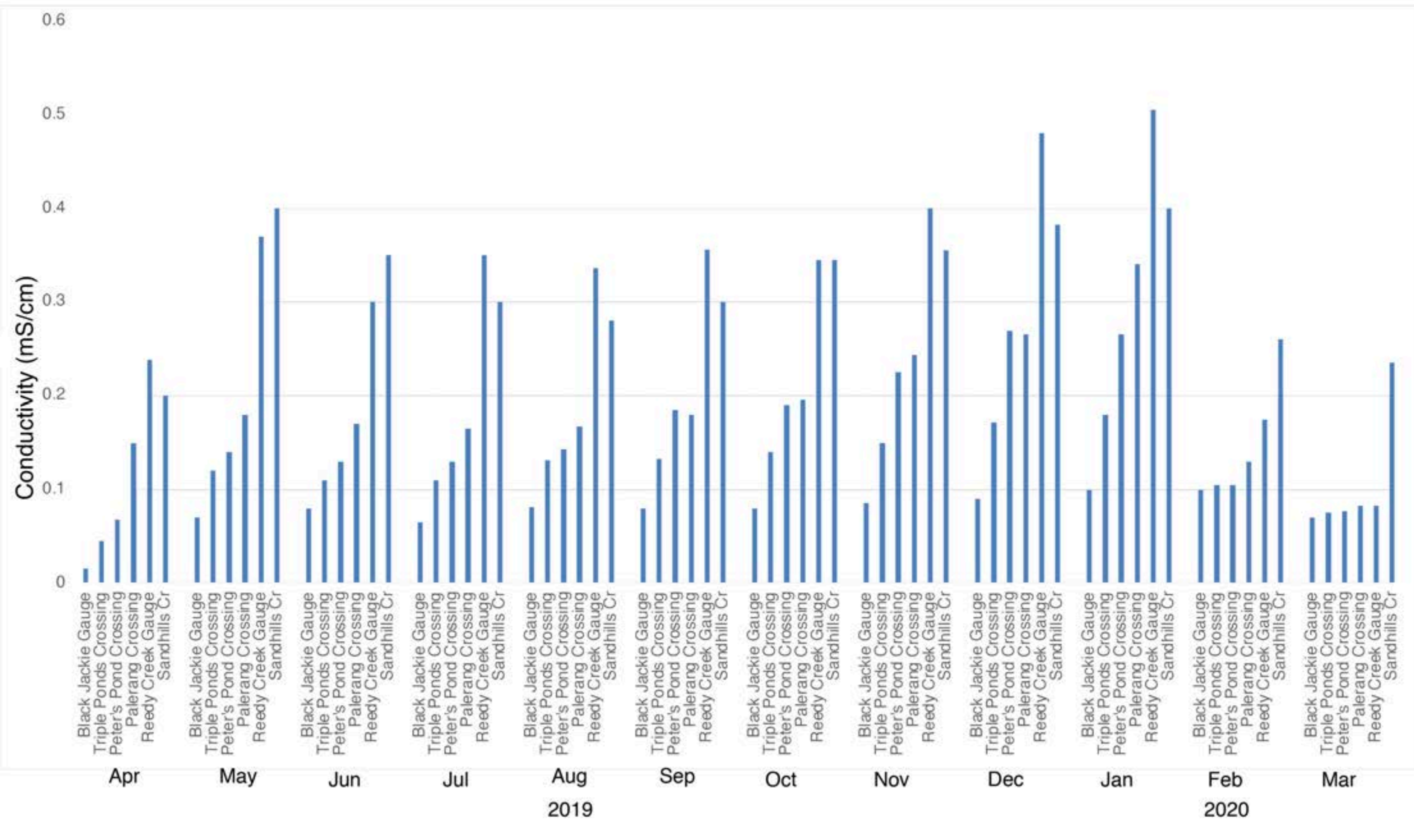


Figure 5. pH was circumneutral in all sites ranging from 5-7 throughout the sampling period.

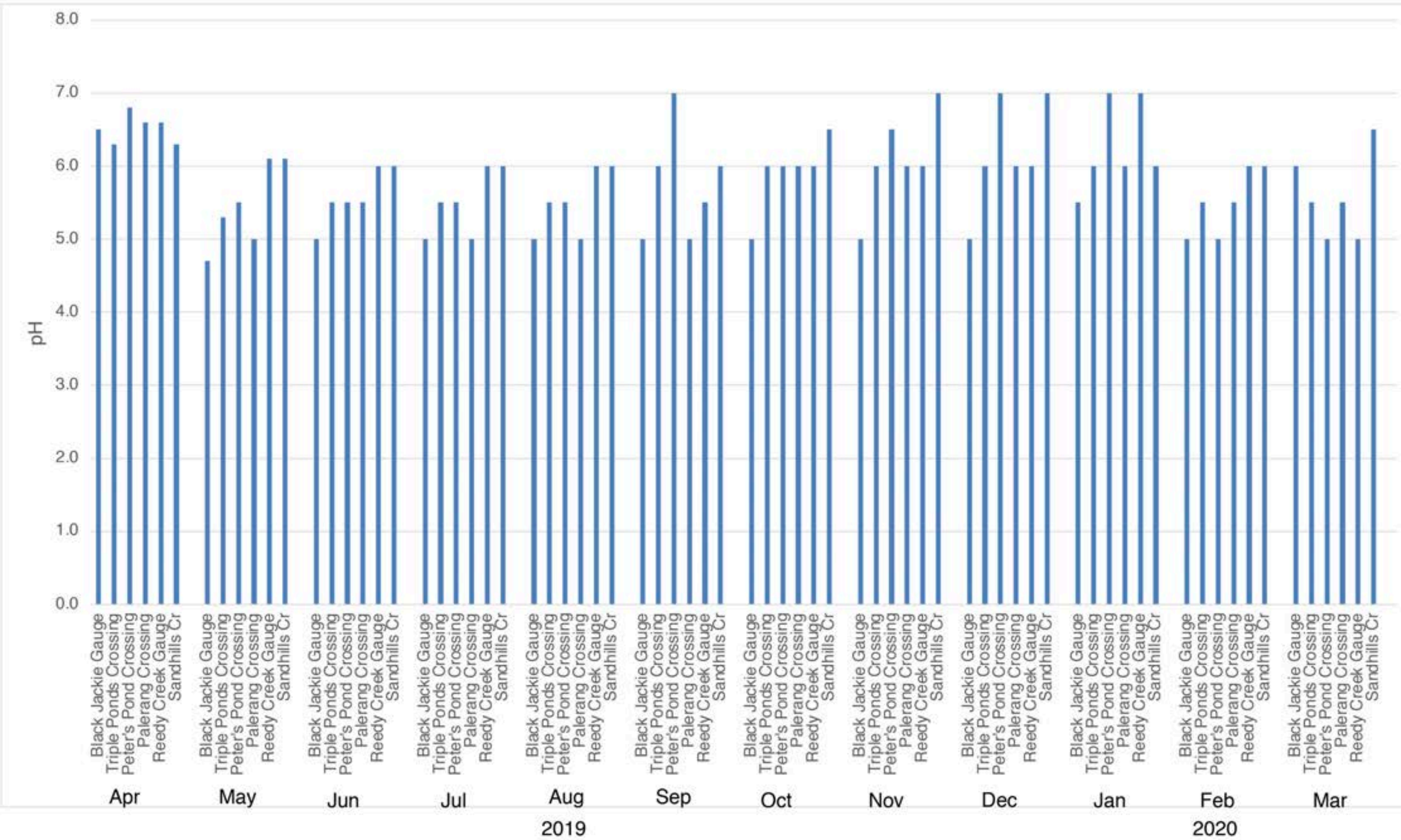


Figure 6. Rate of water flow (ms^{-1}) measured at the various sites during the study period. No flow occurred from April-January during the study.

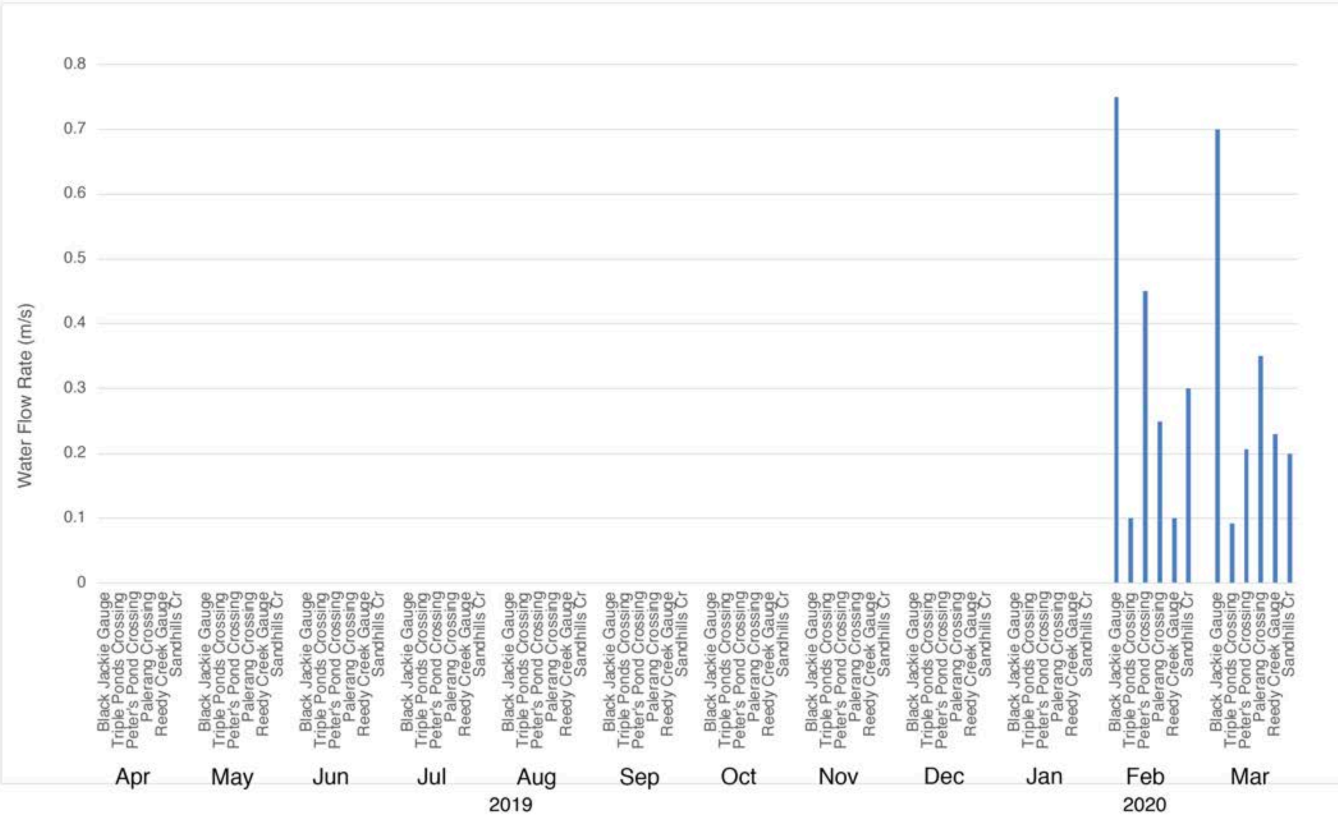


Figure 7. Maximum and minimum temperatures and rainfall taken from Mulloon Creek weather information courtesy of Tony Bernardi.

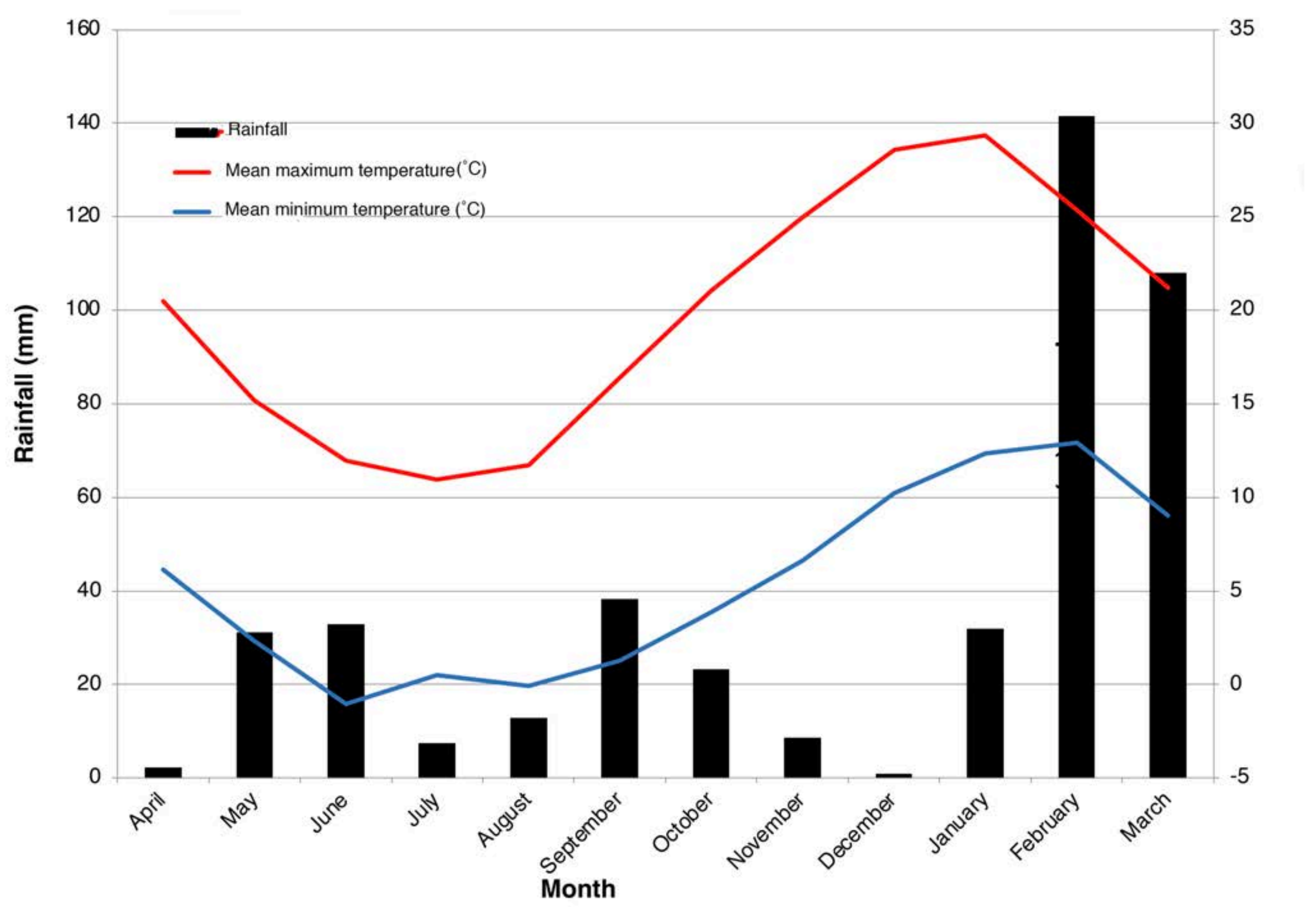


Figure 8. Principal component analysis of the physicochemical measurements shows how the individual parameters determine differences among the sites. The eigenvectors indicate that dissolved oxygen and conductivity are the major aspects differing among sites, but an important secondary effect is the rate of water flow, although that only occurred in February and March. Sandhills and Reedy Creeks are associated with conductivity, as they had higher values than other sites. (BJG=Black Jackie Gauge, TPC = Triple Ponds Crossing, PPC= Peter's Pond Crossing, PaC= Palerang Crossing, SHG= Sandhills Creek Gauge, RCG= Reedy Creek Gauge)

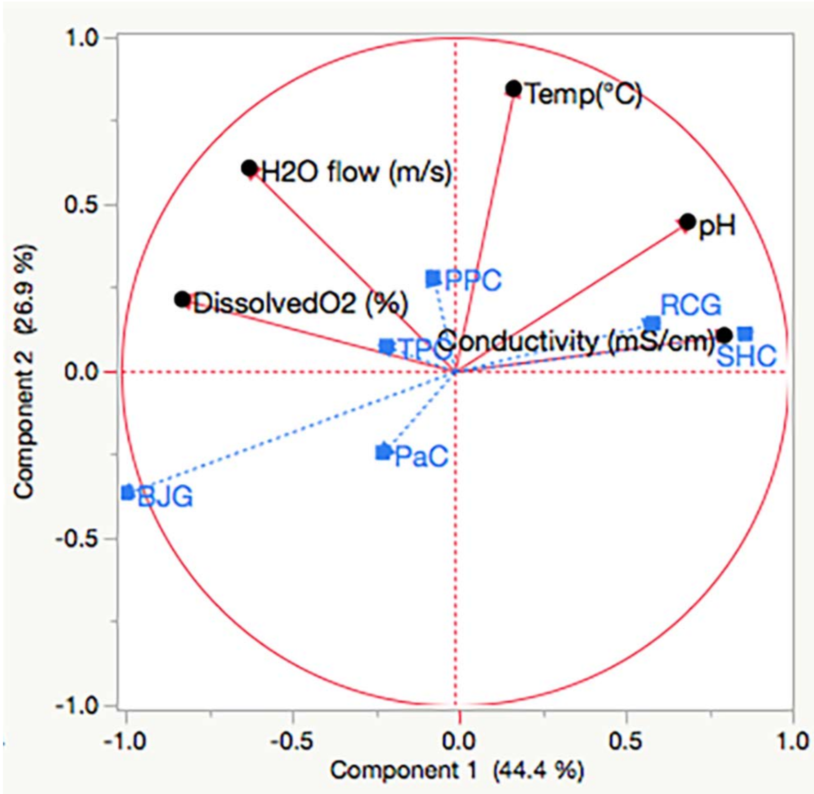


Figure 9. Distribution of total number and taxa of macroinvertebrates collected during the study. January had both the greatest number of invertebrates collected and the most number of taxa represented.

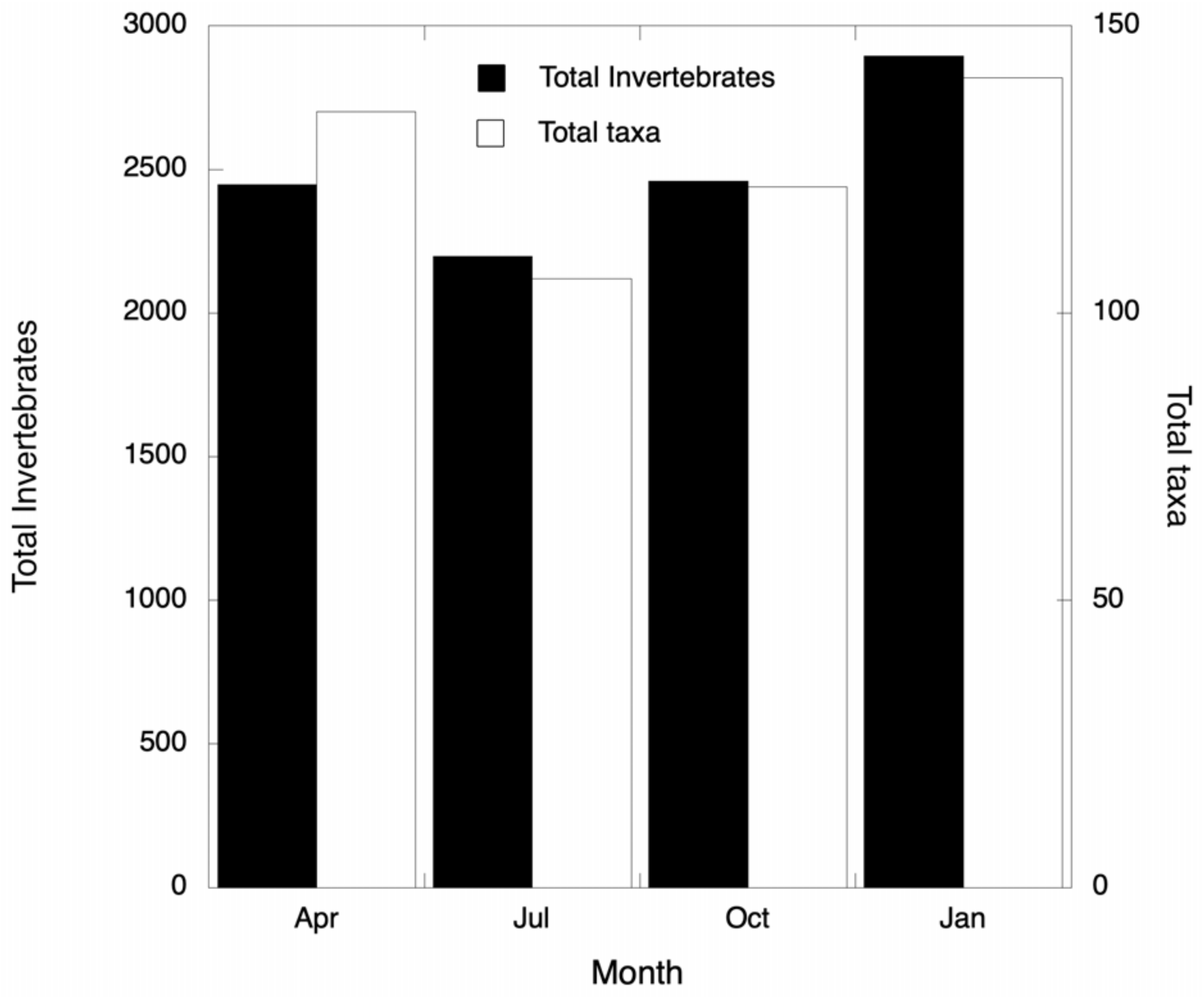


Figure 10. Monthly distribution of numbers of invertebrates and taxa collected at each site. Peter's Pond yielded the greatest number of individuals of all sites in July and January and Reedy Creek had the highest number of taxa in April, with a similar number collected in Triple Ponds in January.

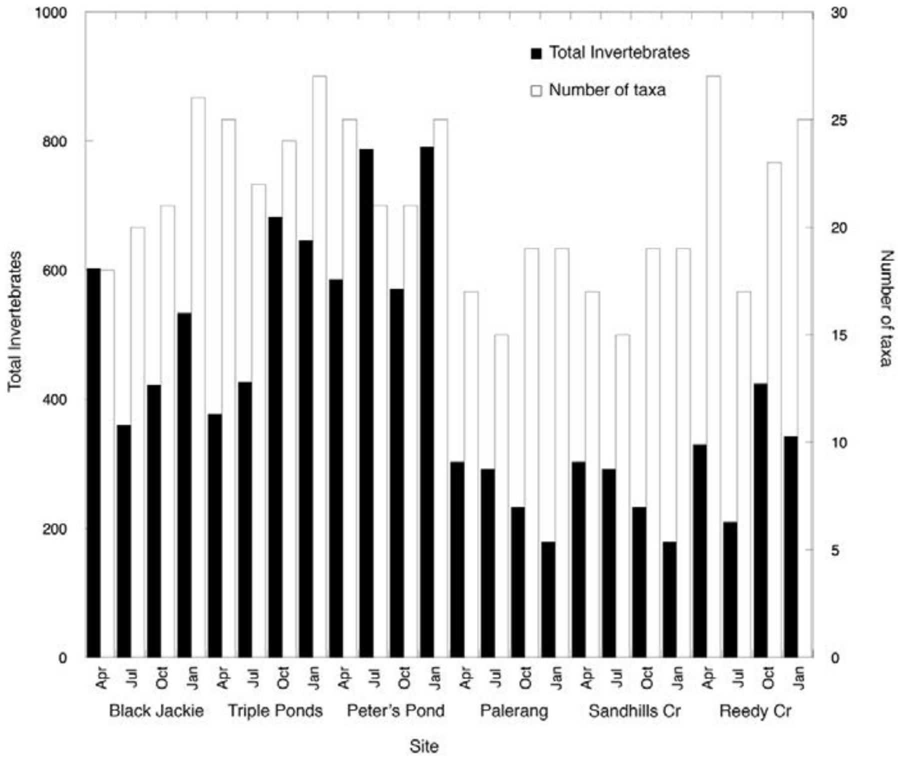


Figure 11. SIGNAL scores for 2006-08,2015-16 and 2019-20. Scores for 2015-16 are higher than the drought conditions in 2019-20, but Autumn values are very similar.

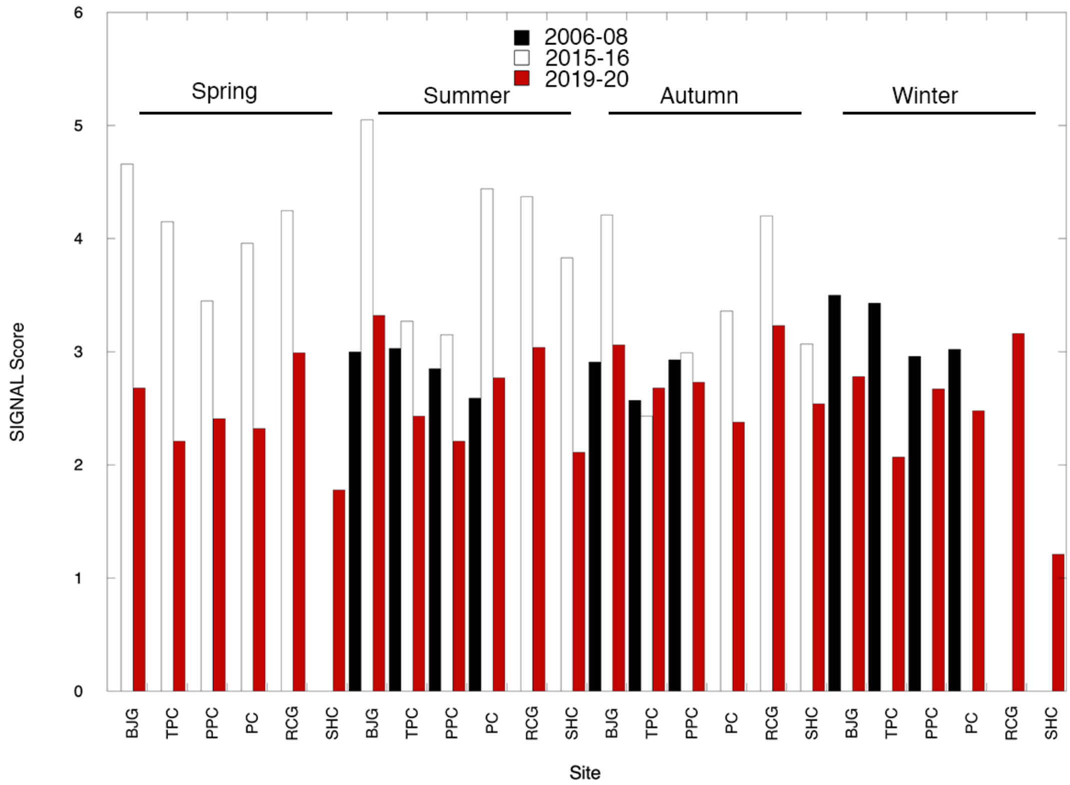


Figure 12. Site comparison across the seasons based upon the macroinvertebrate collection. Black Jackie, Triple Pond, Peter's Pond and Reedy Creek were similar and grouped together. Sandhills and Palarang Crossing were separated as a result of the differences in number of macroinvertebrates collected and possibly associated with some excavation that occurred at the Palarang site during the study. (BJG=Black Jackie Gauge, TPC = Triple Ponds Crossing, PPC= Peter's Pond Crossing, PaC= Palerang Crossing, SHC= Sandhills Creek Gauge, RC= Reedy Creek Gauge)

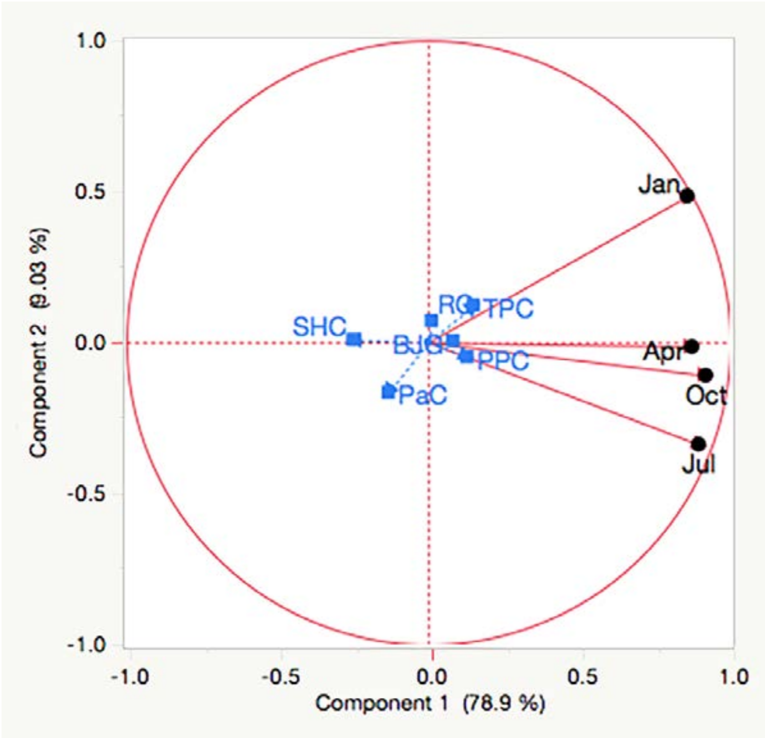
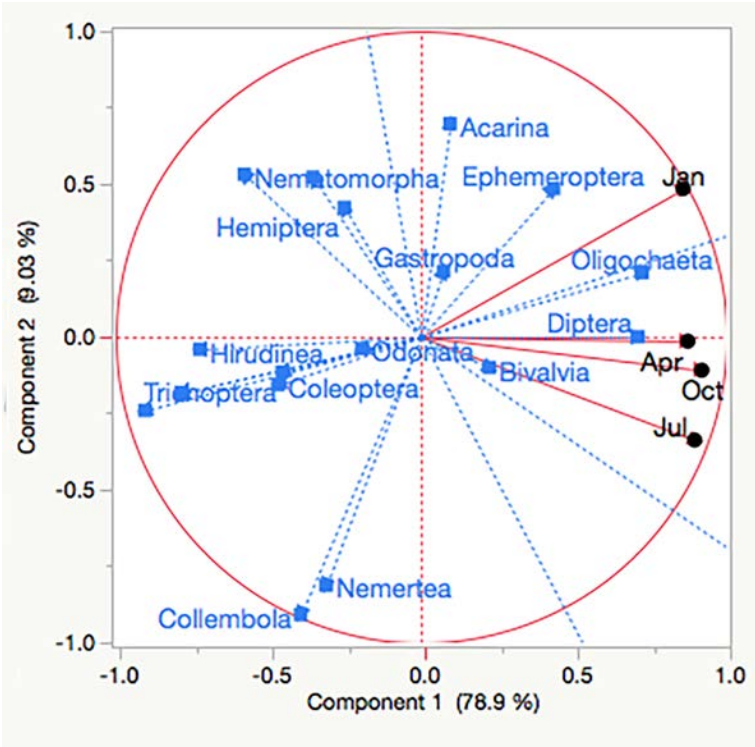


Figure 13. Principal component analysis to determine which taxa had the greatest effect on the variation among the sites. Examination of the vectors indicated that several taxa had equal weighting in the structure of the data and that 9 taxa had weighting in the positive direction and 12 in the negative direction along the Component 1 axis that explained nearly 79% of the variation with seasons and site. Comparison of the 9 PCA scores with SIGNAL grades for the same taxa indicated that no relationship was present. This PCA was much more uniform than the 2015-16 data, suggesting that the few taxa represented in this survey had similar changes for the various sites.



Appendix 1.
Description of sites

Black Jackie- Cool, shady location. Most of the collection time, only a single pool was present (Figure 14a and b)

Triple Pond – Shallow, reedy substrate below crossing. Pebbles were present at crossing, but rare elsewhere. No water at crossing, and only pools above crossing were sampled during the study period (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 – No water at crossing and increased vegetation apparent above crossing. Excavation above crossing created isolated ponds (Figure 17a and b)

Sandhills Creek – Mostly static pond with vegetation from overhanging trees. Sampling across pond, although size of pool decreased until January. Gauge not in water most of year (Figure 18).

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 19).

Figure 14a and b. Black Jackie sampling site in April (a) 2019 looking downstream towards Mulloon Farm and May (b) looking at pond that was sampled for most of year.

a)

b)

Figure 15. Triple Pond crossing, showing vegetation that was present within the water).

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 before (a) excavation and (b) after excavation, just above crossing.

a)

b)

Figure 18. Sandhills Creek gauge, although gauge was rarely in water during invertebrate sampling. Small pool that extended initially to Mulloon Creek, but became isolated from creek overtime as shown here for December 2019.

Figure 19. Reedy Creek sampling region above and below rocks, near reeds on left side, in April 2019.

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

Site	Taxon	Family	April	July	October	January
Black Jackie	Acarina	Acarina			1	
Black Jackie	Amphipoda	Ceinidae		11	12	4
Black Jackie	Bivalvia	Sphaeriidae				3
Black Jackie	Coleoptera	Dytiscidae	6	2	4	
Black Jackie	Coleoptera	Hygrobiidae				1
Black Jackie	Collembola	sp.		1		
Black Jackie	Crustacea	Cladocera	7	32	70	
Black Jackie	Crustacea	Copepoda	159	48	19	5
Black Jackie	Crustacea	Ostracoda	67	4	9	6
Black Jackie	Decapoda	Atyidae		3	18	16
Black Jackie	Decapoda	Parastacidae				3
Black Jackie	Diptera	Ceratopogonidae	35	2	3	3
Black Jackie	Diptera	Culicidae	5			
Black Jackie	Diptera	s-f Chironominae	82	57	78	135
Black Jackie	Diptera	s-f Orthocladiinae			3	19
Black Jackie	Diptera	s-f Tanypodinae	21	29	84	167
Black Jackie	Diptera	Sociomyzidae	1			
Black Jackie	Ephemeroptera	Baetidae	68	5		8
Black Jackie	Ephemeroptera	Caenidae	2		1	9
Black Jackie	Ephemeroptera	Leptophlebiidae	57	3	6	21
Black Jackie	Gastropoda	Lymnaeidae	40	12	31	13
Black Jackie	Gastropoda	Physidae	23			
Black Jackie	Hemiptera	Corixidae	1	4		
Black Jackie	Hemiptera	Notonectidae			18	71
Black Jackie	Hemiptera	Veliidae	2		3	
Black Jackie	Hemiptera	Nepidae				1
Black Jackie	Hydra	Hydra		6		4
Black Jackie	Nematomorpha		4			1
Black Jackie	Odonata	Aeshnidae				2
Black Jackie	Odonata	Coenagrionidae		20	4	6
Black Jackie	Odonata	Gomphidae				3
Black Jackie	Odonata	Lestidae		5	37	2
Black Jackie	Odonata	Synlestidae	23			
Black Jackie	Trichoptera	Calosidae		97	19	
Black Jackie	Trichoptera	Conosucidae			1	
Black Jackie	Trichoptera	Ecnomidae		6	1	
Black Jackie	Trichoptera	Hydroptilidae		13		19
Black Jackie	Trichoptera	Leptoceridae				5
Black Jackie	Turbellaria	Dugesiidae				7
Total number			603	360	422	534
Taxa			18	20	21	26

Triple Ponds	Acarina	Acarina	3	6	10	96
Triple Ponds	Amphipoda	Ceinidae	12	25	41	121
Triple Ponds	Bivalvia	Sphaeriidae	12			5
Triple Ponds	Coleoptera	Dytiscidae		3	2	1
Triple Ponds	Coleoptera	Hydrophilidae			1	
Triple Ponds	Coleoptera	Scirtidae	1			
Triple Ponds	Crustacea	Cladocera	2	65	74	6
Triple Ponds	Crustacea	Copepoda	15	67	4	16
Triple Ponds	Crustacea	Ostracoda	38	31	42	4
Triple Ponds	Decapoda	Atyidae	1			96
Triple Ponds	Diptera	Ceratopogonidae	76	32	70	28
Triple Ponds	Diptera	s-f Chironominae	44	96	63	52
Triple Ponds	Diptera	s-f Orthocladiinae	1	12	1	2
Triple Ponds	Diptera	s-f Tanypodinae	11	32	251	28
Triple Ponds	Ephemeroptera	Baetidae	15	4		2
Triple Ponds	Ephemeroptera	Caenidae			1	2
Triple Ponds	Ephemeroptera	Leptophlebiidae	5			8
Triple Ponds	Gastropoda	Lymnaeidae	10	5	17	32
Triple Ponds	Hemiptera	Belostomatidae			1	1
Triple Ponds	Hemiptera	Corixidae	14	3	4	5
Triple Ponds	Hemiptera	Gerridae				1
Triple Ponds	Hemiptera	Notonectidae	12	1	5	50
Triple Ponds	Hemiptera	Veliidae	2	2		
Triple Ponds	Hydra			6	2	
Triple Ponds	Nematomorpha					1
Triple Ponds	Nemertea			2	1	
Triple Ponds	Odonata	Aeshnidae	2		1	
Triple Ponds	Odonata	Coenagrionidae	89	23	82	18
Triple Ponds	Odonata	Gomphidae		2		
Triple Ponds	Odonata	Lestidae	3	2		22
Triple Ponds	Odonata	Libellulidae	4		1	
Triple Ponds	Oligochaeta	Oligochaeta	2	6		10
Triple Ponds	Trichoptera	Ecnomidae			1	5
Triple Ponds	Trichoptera	Hydroptilidae	2	2	4	12
Triple Ponds	Trichoptera	Leptoceridae	1			
Triple Ponds	Turbellaria	Dugesiidae				22
Triple Ponds	Turbellaria	Temnocephalidae			3	
Total number			377	427	682	646
Taxa			25	22	24	27
Peter's Pond	Acarina	Acarina		2	3	13
Peter's Pond	Amphipoda	Ceinidae	191	361	155	451
Peter's Pond	Bivalvia	Sphaeriidae	2			

Peter's Pond	Coleoptera	Dytiscidae	3	1	1	8
Peter's Pond	Coleoptera	Hygrobiidae				1
Peter's Pond	Crustacea	Cladocera	17	80	43	26
Peter's Pond	Crustacea	Copepoda	10	19	25	17
Peter's Pond	Crustacea	Ostracoda	22	28	20	10
Peter's Pond	Decapoda	Parastacidae				3
Peter's Pond	Diptera	Ceratopogonidae	100	13	24	19
Peter's Pond	Diptera	s-f Chironominae	44	85	46	68
Peter's Pond	Diptera	s-f Orthocladiinae	1	8	4	
Peter's Pond	Diptera	s-f Tanypodinae	59	57	110	65
Peter's Pond	Ephemeroptera	Baetidae	16	34	2	3
Peter's Pond	Ephemeroptera	Leptophlebiidae	9	8	16	11
Peter's Pond	Gastropoda	Lymnaeidae	1	10	25	21
Peter's Pond	Hemiptera	Belostomatidae	2			5
Peter's Pond	Hemiptera	Corixidae	6		1	2
Peter's Pond	Hemiptera	Notonectidae	2			14
Peter's Pond	Hirudinea	Glossiphoniidae				1
Peter's Pond	Hydra		2	9	3	
Peter's Pond	Lepidoptera	Pyrlidae	1			
Peter's Pond	Nemertea		1	1		1
Peter's Pond	Odonata	Aeshnidae	1	4	1	
Peter's Pond	Odonata	Coenagrionidae	85	58	80	29
Peter's Pond	Odonata	Gomphidae		2	6	
Peter's Pond	Odonata	Lestidae	1	2	3	4
Peter's Pond	Odonata	Libellulidae	7			2
Peter's Pond	Oligochaeta	Oligochaeta	2		1	10
Peter's Pond	Trichoptera	Hydroptilidae	1	3	2	2
Peter's Pond	Turbellaria	Dugesiidae		3		5
Total number			586	788	571	791
Taxa			25	21	21	25
Palerang	Acarina	Acarina			4	2
Palerang	Amphipoda	Ceinidae	3		6	20
Palerang	Bivalvia	Sphaeriidae	9	6	5	
Palerang	Coleoptera	Dytiscidae			2	3
Palerang	Crustacea	Cladocera	3	5	3	5
Palerang	Crustacea	Copepoda	19	63		7
Palerang	Crustacea	Ostracoda	19	43	61	
Palerang	Decapoda	Atyidae		1	9	1
Palerang	Diptera	Ceratopogonidae	59	45	35	8
Palerang	Diptera	s-f Chironominae	110	89	27	75
Palerang	Diptera	s-f Orthocladiinae	2	9	2	2
Palerang	Diptera	s-f Tanypodinae	5	17	40	24

Palerang	Ephemeroptera	Baetidae		1		5
Palerang	Ephemeroptera	Leptophlebiidae				4
Palerang	Gastropoda	Lymnaeidae	2	3	4	8
Palerang	Hemiptera	Belostomatidae			2	1
Palerang	Hemiptera	Corixidae	1		4	4
Palerang	Hydra			2	5	
Palerang	Nemertea		2	1	1	
Palerang	Nematomorpha					1
Palerang	Odonata	Coenagrionidae	24		12	7
Palerang	Odonata	Gomphidae	3			1
Palerang	Odonata	Lestidae			1	
Palerang	Oligochaeta	Oligochaeta	15	6	10	
Palerang	Trichoptera	Ecnomidae				1
Palerang	Trichoptera	Hydroptilidae	1	1		
Palerang	Turbellaria	Dugesiidae	26			
Total number			303	292	233	179
Taxa			17	15	19	19
Sandy Hills	Acarina	Acarina		1		
Sandy Hills	Amphipoda	Ceinidae	1	1		1
Sandy Hills	Coleoptera	Dytiscidae	6	1	6	2
Sandy Hills	Coleoptera	Hydrophilidae			1	
Sandy Hills	Coleoptera	Scirtidae	3			
Sandy Hills	Collembola	sp.	17	44		
Sandy Hills	Crustacea	Copepoda	5	28	20	3
Sandy Hills	Crustacea	Ostracoda	3	12	6	9
Sandy Hills	Decapoda	Atyidae	1			2
Sandy Hills	Diptera	Ceratopogonidae	4		1	
Sandy Hills	Diptera	s-f Chironominae	154	22	64	309
Sandy Hills	Diptera	s-f Orthocladiinae	3			2
Sandy Hills	Diptera	s-f Tanypodinae			4	4
Sandy Hills	Ephemeroptera	Baetidae	2		2	1
Sandy Hills	Ephemeroptera	Caenidae	7			
Sandy Hills	Gastropoda	Lymnaeidae	7	2	4	26
Sandy Hills	Hemiptera	Belostomatidae				1
Sandy Hills	Hemiptera	Corixidae	6			1
Sandy Hills	Hemiptera	Notonectidae	2		1	1
Sandy Hills	Hemiptera	Veliidae	2			
Sandy Hills	Hirudinea	Glossiphoniidae	1			
Sandy Hills	Hydra		4	7	3	18
Sandy Hills	Nematomorpha					1
Sandy Hills	Nemertea		1			
Sandy Hills	Odonata	Aeshnidae				1

Sandy Hills	Odonata	Coenagrionidae	4	1	1	3
Sandy Hills	Odonata	Gomphidae	2			
Sandy Hills	Oligochaeta	Oligochaeta	11	2	14	15
Sandy Hills	Trichoptera	Calosidae	2			
Sandy Hills	Trichoptera	Coenosuchidae				1
Sandy Hills	Trichoptera	Hydroptilidae			1	
Total number			248	121	128	401
Taxa			23	11	14	19
Reedy Creek	Amphipoda	Ceinidae	2	1	6	1
Reedy Creek	Bivalvia	Sphaeriidae	3	17	20	7
Reedy Creek	Coleoptera	Dytiscidae	6	11	21	1
Reedy Creek	Collembola	sp.	2			
Reedy Creek	Crustacea	Cladocera	22		1	5
Reedy Creek	Crustacea	Copepoda	43	31	23	70
Reedy Creek	Crustacea	Ostracoda	5	6	30	6
Reedy Creek	Decapoda	Atyidae	1		2	36
Reedy Creek	Diptera	Ceratopogonidae	23	1	13	11
Reedy Creek	Diptera	s-f Chironominae	115	81	90	56
Reedy Creek	Diptera	s-f Orthocladiinae	9		20	10
Reedy Creek	Diptera	s-f Tanypodinae	8	19	123	54
Reedy Creek	Ephemeroptera	Baetidae	12	3		2
Reedy Creek	Ephemeroptera	Caenidae	14	5	22	28
Reedy Creek	Ephemeroptera	Leptophlebiidae	9	4	5	4
Reedy Creek	Gastropoda	Lymnaeidae	5	1	14	12
Reedy Creek	Hemiptera	Notonectidae				5
Reedy Creek	Hemiptera	Veliidae	1			
Reedy Creek	Hydra		6	17	6	4
Reedy Creek	Nemertea		1	2		
Reedy Creek	Odonata	Gomphidae	3		3	2
Reedy Creek	Odonata	Coenagrionidae	4		2	8
Reedy Creek	Oligochaeta	Oligochaeta	7	5	9	3
Reedy Creek	Trichoptera	Calamoceratidae	1			
Reedy Creek	Trichoptera	Calosidae	6	5	2	4
Reedy Creek	Trichoptera	Ecnomidae	8	1	3	7
Reedy Creek	Trichoptera	Hydroptilidae	5		4	1
Reedy Creek	Trichoptera	Leptoceridae			4	
Reedy Creek	Trichoptera	Limnelphelidae				1
Reedy Creek	Turbellaria	Dugesiidae	9		1	5
Total number			330	210	424	343
Taxa			27	17	23	25

Appendix 3. 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 (**S**tream **I**nvertebrate **G**rade **N**umber – **A**verage **L**evel) 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 A3.1. 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 A3.2).

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 A3.2. 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