

The Effect of River Restoration on Aquatic Biodiversity

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ABSTRACT

The River Adur is a 32km stretch of river, located in West Sussex. The river restoration that took place on the Knepp Castle Estate was the largest on a UK River to date. There has been no published research on the effect of restoration on benthic macroinvertebrates and environmental parameters such as pH, temperature, conductivity, dissolved oxygen, phosphorus and nitrate levels, with their subsequent effects on water quality.

To investigate this, the study was conducted at ten sampling locations over three consecutive months (May - July 2017) within the Knepp Castle Estate, with macroinvertebrate individuals collected and physiochemical variables also measured to determine water quality.

In this study, six samples were analysed comparatively against six replicated sampling locations from 2015, and were further compared to pre-restoration 2011 data which consisted of three replica sites to establish whether river conditions had improved.

The comparative aspect of the study found that there were no significant differences in macroinvertebrate metrics and abiotic variables between 2011, 2015 and 2017. However, when comparing the two data sets conducted post-restoration (in 2015 and the current study), a statistically significant increase was seen in the two abiotic variables measured, phosphates and pH. Whilst macroinvertebrate metrics, ASPT and BMWP, did not show any significant changes, BMWP scores were higher at all replica sites (B,E,F,G and I), with the exception of site J, in 2017. ASPT scores were higher in 2017 at replica sites B,F,I and J, and lower at site G. Site E remained consistent in its score. This could be due to the short period of time since restoration was completed (4 years) which would have been a disruptive process in itself, meaning communities may need additional time for recolonisation. Additionally, four years may not be sufficient time to assess whether restorative works have had a positive impact on the River Adur.

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INTRODUCTION

Human activities have had a profound impact on river systems throughout the world. The impacts of catchment land use can dramatically alter water quality (Stein et al., 2002) and whilst these changes may not always be detrimental, the impacts are often synergistic and cumulative (Lake et al., 2000). As a result, river regulation has increased, with UK rivers managed primarily by the non-governmental Canal & River Trust, formed in 2012. The organisation, compliant with the EU Water Framework Directive 2000, seeks to establish a framework for community action in the field of water policy.

Lotic ecosystems contribute to global diversity greatly, and although freshwater streams, rivers, ponds, lakes and wetlands cover less than 1% of the Earth's surface, they can contain one-third of all vertebrates, and up to 10% of all animal species (Strayer & Dudgeon 2010). Understanding the processes which underpin distributions of aquatic diversity in these systems has been a core focus of ecological research for many years.

Benthic invertebrates are among the most diverse and complex communities found in riverine systems, with the abundance of macroinvertebrates utilised as an indicator of river quality and health (Brown et al., 2011). Macroinvertebrates play an important role in the trophic structure of aquatic ecosystems, as a key component of aquatic food webs serving both as decomposers of organic matter (e.g., leaf litter and detritus) and as food sources for other vertebrate and invertebrate species (Moulton et al., 2010). The tolerance level of particular invertebrate families can range from pollution sensitive to those that are pollution tolerant. For example, Plecoptera, the stonefly family, require a higher dissolved oxygen content and neutral pH, whereas Oligochaeta, a subclass adaptable to changes in pH, are able to tolerate reduced oxygen level and variable water temperatures. Studying the presence or absence of invertebrate groups with differing tolerances/sensitivities to organic pollution can be a biological indication tool in itself, whilst the number of different macroinvertebrates

found in a sample of river is also considered an important factor (Bressler et al., 2006).

Previous riverine research suggests that water of a better quality should contain an elevated number of ‘pollution intolerant’ species, which would be excluded from water samples where higher organic contaminants are present. Biomonitoring of macroinvertebrates is considered an effective tool in monitoring water quality. As mostly sedentary organisms, spending their entire life cycle in their habitat, they are able to reflect a long-term view of environmental stressors, both natural and anthropogenic, on water bodies. Biotic indices such as the BMWP apply numerical expressions, combining a quantitative measure of species diversity with qualitative information on the ecological sensitivity of individual taxa, among others, and is complimented by quantitative chemical analysis. Chemical sampling, whilst only indicative of current status of water quality, is pivotal in understanding the limits of a waterbody’s ability to assimilate some level of pollution without harming the water system and its aquatic inhabitants (Clesceri et al., 1999).

The impact of restorative river works based on geomorphological methods can be assessed through this combination of biological and chemical analysis. This seeks to eliminate any bias and provide a comprehensive and consistent characterisation of river and catchment area. Whilst there can be a multitude of reasons to undertake a river restoration, the current study centres on the River Adur, where restorative works sought to mitigate flooding, which had previously occurred as commonly as every decade, and to reduce any negative impacts on the landscape, hydrology and fishery potentials.

AIMS AND OBJECTIVES

This study aims to quantify the impacts of river restoration on aquatic health.

Objectives of the study:

1. To determine the impact of river restoration on environmental variables, related to water quality — pH, conductivity, total dissolved solids, dissolved oxygen temperature, nitrate and phosphate levels, using the ten sampling locations which move downstream of restorative work.
2. To determine whether the impact of river restoration on benthic macroinvertebrates affects presence and abundance, utilising the BMWP and ASPT systems.
3. To understand if there are continued increases in water quality and benthic macroinvertebrate populations , using comparative data from previous sampling on six sites conducted in 2015 post-restoration, and three sites during 2011, pre-restoration.
4. Provide a routine sampling protocol and act as a baseline study to be utilised in future research.

LITERATURE REVIEW

River restoration schemes have increased as a result of the Water Framework Directive 2000/60/EC (EPC 2000); implemented throughout Europe since 2013. With the directive stressing the need for environmental protection of rivers, stating that “member States shall protect, enhance and restore all bodies of surface water...for artificial and heavily modified bodies of water” (EPC 2000, article 4.1.a.ii). This has necessitated integrated approaches to provide rivers with good ecological potential have risen (Redondo, 2013.)

Reasons for River Restorations

While many motives drive restorative works, these usually consist of multiple objectives. Ecosystem regeneration, habitat restoration, aesthetic and water quality improvements, for both safety and visual appeal, riparian protection, sediment management

and flood and floodplain control are considered the eight most common motives (Wheaten, 2005). The Skjern River Restoration Project in Denmark, for example, aimed to address several objectives concurrently: (1) to improve conditions for local flora and fauna; (2) to restore meanders and natural dynamics; (3) to ensure a high water quality and (4) to improve the potential for outdoor leisure activities (Riber, 2000). Schiemer et al. (1999) argued that regardless of the driver behind a restoration, restoring processes (physical and biological) and functions (hydrological and geomorphological) should be the primary aims. At the Knepp Castle Estate, enhancement of the channel and floodplain habitat diversity was conducted through physical manipulation of the channel to reconnect the floodplain to the river channel (Janes et al. 2006). This was driven by the desire to mitigate flooding, which had previously occurred as commonly as every decade, to reduce adverse impacts on the landscape, hydrology and fishery potential. During this process the Shipley weir was removed, a technique adopted by 292 similar projects in the UK (RRC). Reasons behind this removal were to eradicate barriers to fish migration, control flow, benefit fish and macrophytes and increase the physiochemical dissolved oxygen and regulate phosphate levels. Weirs have been considered as obstacles to many species in rivers throughout the Europe. O'Connor (2017) has unfavourably compared the reduced *Lampetra fluviatilis* population in the Annocotty weir, located on the Lower River Shannon (designated Special Area of Conservation, SAC) in Limerick, Republic of Ireland, with that of the River Lamprey. Unable to pass through the weir, this presents a total barrier for *L. fluviatilis* migration can leave them vulnerable to heavy predation by Grey Herons, *Ardea cinerea*, and illegal fishing. While weir removal may not always be possible due to flood risks, physical modifications can also improve riparian habitat. In the River Neb, the Isle of Man, the removal and replacement of a

deteriorating weir with a rock-ramp, allowed free passage for Salmon, Sea and Brown Trout, and provided a habitat for over 1000 juvenile salmon (Janes, 2015).

Restoring Natural Meanders

Manually restoring the River Adur's historical meanders was a strategy also adopted in the River Rother, in Petworth, West Sussex. As with the site of the current study, the Rother was engineered for navigational purposes in the 18th century, bypassing the 'Shopham Loop,' a large meander and part of the natural course of the river. After navigation ceased, locked gates which originally blocked the meander were removed, causing a rapid deposition of sand, reduced flow velocity and a general decline in water quality. A restoration was undertaken to rectify this by diverting flow back through the meander loop. Two years post-construction, the newly created wetlands from the restoration has increased riparian vegetation and species diversity of the floodplain. Monitoring of the site for a consecutive seven years showed fish communities consistent with that of the wider Rother catchment, with populations of bullhead, chub and brown, grayling, barbel and sea trout higher than average for the catchment. However, though restoring meanders has shown improvements in fluvial geomorphology in the above study, documentation of ecological effects is fragmented. (Thodsen, Hasholt and Kjærsgaard, 2008).

Monitoring Protocols

In Europe, re-meandering projects in Denmark have shown initial increased erosion and transportation of sediment and nutrients, but concurrently a reduction in the number of taxa, with Thodsen et al., suggesting that recovery of rivers can differ substantially dependent on climate condition, restoration period and site specifics such as hydrology and geomorphology. Friberg et al., (1998) refute this, claiming that studies from 1989-1997 on the River Gelsa, Denmark, show no or very few short-term effects on biota. In this restoration

study, the river was monitored before the project in 1989, with a control area used as a comparative, and results showed that it was not until 1997-2008 that improvements were observed. This included increased ASPT scores, which peaked 18 years after the initial restoration, suggesting that long-term studies yield better results. The overall ecological diversity, including reduced nitrate levels, was also shown only to increase significantly nine years post-remeandering. Thodsen also suggested that monitoring should not start until after the first two years, and continue for a prolonged amount of time to allow for colonisation periods to occur. Ruben et al. (2009) also supported this view, stating that short-term increases of macroinvertebrate indicator species had been observed. This is reflected in Henderson et al. (2014) who report that continuous monitoring of geomorphological and ecological parameters should be intensified throughout restoration efforts. They further advise that 'restoration schemes should aim at restoring the natural physical structural complexity in the streams and at the same time enhance the possibility of regenerating the natural geomorphological processes sustaining the habitats in streams and rivers', as performed at the River Adur. In a recent publication, Clark and Montemarano (2017) addresses that channel reconfiguration is a common but debated method of restoration, as it can cause disturbance and produce subsequent negative impacts on biota. Clark and Montemarano find that that diversity is significantly lower in new channel sites in the Eagle Creek River, Ohio, post restoration work, compared to the upstream control site, concluding that in the short-term colonising communities were unable to recover to reflect upstream community composition within a short period of time (<5 years). However, Pedersen et al., (2015) dismiss these short-term negative impacts, stating that the chemical analysis of studies in restored Danish rivers, have shown nitrogen levels declining by 5% over the last 20 years.

Sampling Protocols

Sampling protocols used in assessing restorative works are also an important consideration. While biotic indices, such as the BMWP scoring system have been used prominently, the effect of sampling effort can be variable, with a prolonged sampling period of higher expertise, yielding a higher final score than one taken during a short time scale. To overcome this weakness, the calculation of the ASPT is commonly performed, ensuring a more reliable data set (Hawkes, 1997). Seasonal dependencies are another criticism associated with biotic indices based on macroinvertebrate tolerances, with Zamora-Munoz et al., (1994) recommending that biotic indices and physiochemical parameters should be taken consistently over multiple years to allow for annual variations.

Conclusions

Following the previous studies, it is suggestive that whilst a deferred gratification technique is preferential in long term river restoration, confounding variables such as seasonality and sampling method must also be taken into account, and a multilateral approach utilised to mitigate said variables.

METHODOLOGY

The study area

The River Adur, which spans 20 miles in the district of West Sussex, is managed through the Adur & Ouse River Trust, an original pilot collaboration initiative, instigated by the Environment Agency in 2001. The trust is responsible for both the Adur and Ouse River catchment areas, with a focus on maintaining and managing river systems whose geomorphology and habitats can help rebuild ecological networks.

As part of this process, the stretch of the river which runs through the Knepp Castle Estate, has been modified in a collaborative effort between the Environment Agency, Natural England and the Estate. The restoration sought to integrate biodiversity enhancement, improved landscape and flood risk management. The Knepp Castle estate is located to the south of Horsham, West Sussex (figure 1).

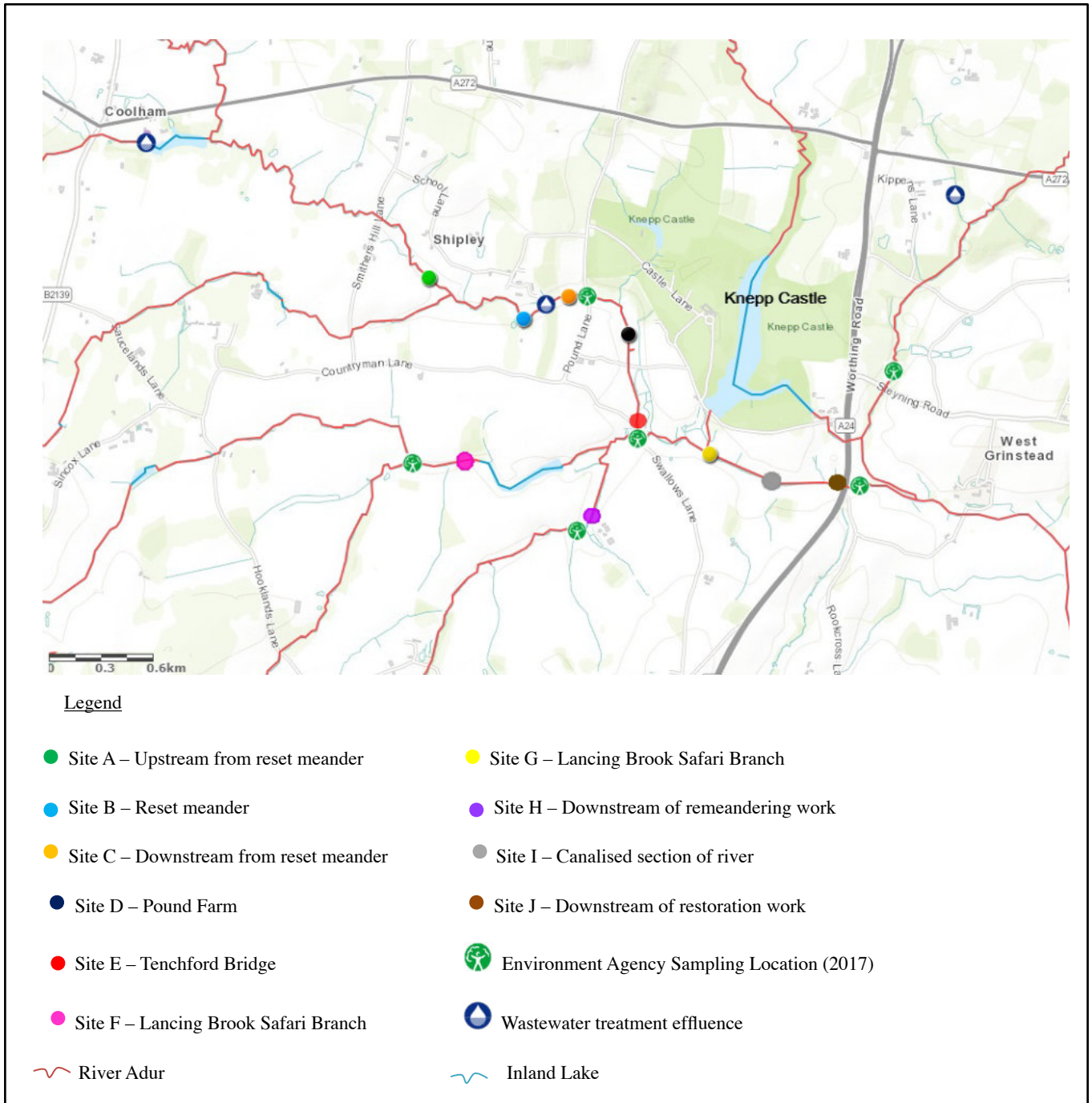


Figure 1. GIS Map of the Knepp Castle Estate, with sampling locations displayed.

Previously used for intensive farming, the estate was re-wilded in 2001. The Estate sought to achieve a ‘near-natural grazing’ system, adding small populations of Exmoor ponies, pigs and deer (Greenaway, 2007), with the goal of improving biodiversity across the site. With rewilding achieved through land modification, including the eradication of any pesticides or fertilisers and removal of all internal gates and fences, the concept of naturalising the river

system (which had been engineered and canalised two hundred years previously) materialised. Restoration work was carried out on the low gradient, clay-bed river between 2011-2013, in the ‘biggest proposed stretch of river to be naturalised in Britain’ (Dennis, n.d).

The restoration focused on returning the river to its natural, original, meandering course. The flow rate was slowed with the addition of woody debris and gravel berms, raising bed levels, installing pool-riffles, filling floodplain ditches and drains, planting riparian vegetation, and the creation of temporary floodplain wetlands (scrapes) throughout the site (as shown in figure 2). Downstream of the Lancing Brook tributary (see figure 1), less channel engineering was undertaken — the removal of the Shipley weir, channel modification to a single meander loop, and complimentary gravel berms added to increase sinuosity.

The restoration project was a finalist of the River Restoration Centre’s 2015 UK River Prize, and whilst the benefits of terrestrial land naturalisation can be observed through the bountiful local fauna present, quantification of the impacts that re-naturalisation of the river has had on both the riverine inhabitants, and on the water quality is necessary.



Figure 2. River Adur pre and post restoration works.

Sampling

In total, 10 sites were selected from the western branch of the River Adur catchment. Sampling was replicated three times between 01/05/2017 and 30/07/2017 – one replication per month. Invertebrates were collected from these sites. The sites included duplicate areas previously sampled during a 2015 environmental consultancy survey to assess whether the restoration work had affected river quality and biodiversity: On-site reset meander (Site B), Tenchford Bridge (E), Lancing Brook Hammer Branch (F), Lancing Brook Safari Branch (G) and a canalised section of the River Adur (I). The final replicated sampling area was located downstream of the previous sites, outside the border of the Knepp Castle Estate, at Bay Bridge (J). The purpose of these replications was to compare whether there was any trend in invertebrate communities between 2015 and 2017.

Sites A, C, D and H sampled areas where no prior research had been undertaken. These sites focused on the relationship between invertebrate communities, abundance and environmental variables 3 years post-restoration. As shown in Fig. 1, site A was located upstream from an area of 're-meandering,' whilst sites C and D were sample sites through the course of the restored meanders. The location of site H was downstream from the meanders. In comparing pre-restoration data, samples were taken across three replicated sites where spot sampling had occurred in 2011 (Sites J, E and I).

At each site, a sampling protocol was applied so that the results would be comparable in technique to that of previous study. Macroinvertebrates were collected through the standardised kick sampling method inline with "The Development and Testing of an Integrated Assessment System for the Ecological Quality of Streams and Rivers throughout Europe using Benthic Macroinvertebrates (AQEM)".

A single habitat kick net approach was performed to sample a 2m² composited area in front of the net for 3 minutes. The quadrangular 1 mm mesh hand net was used in 1-minute intervals to disturb and re-suspend the substrate, moving diagonally upstream. A further 1-minute was designated to hand searching any large stones or riparian vegetation where invertebrates may be found.

Samples were cleaned in situ with river water of lower turbidity collected prior to agitating the substrate. Duplicate water samples were stored in falcon tubes from each site for subsequent chemical analysis. Invertebrate identification occurred in situ where possible, with the use of a 30 X. Magnifying Eye Glass. Where identification in the field was not possible, samples were stored in a 70% IMS solution for further laboratory analysis. Taxa were classified to family level using different keys and assessed using the BMWP procedure for indication of water quality.

Physiochemical parameters

Temperature

Temperature is known to influence the solubility of oxygen, with solubility increasing with a decreasing temperature. It plays a crucial role in other parameters such as pH and conductivity, too (Awasthi and Tiwari, 2004). Water temperature was taken at each sampling site using the dual use Hach sensION 6 Dissolved Oxygen Meter. This was submerged to a depth of 7 cm until stabilisation was complete and accurate temperature could be recorded.

pH

The acidity or alkalinity of water is controlled by amount of free CO₂, Bicarbonate and Carbonate. pH of water samples was taken in-situ using a pre-calibrated Oakton EcoTestr pH 2. The probe was submerged and allowed to stabilise for a period of 2 minutes before an accurate reading was taken.

Conductivity

The measurement of water's ability to conduct electrical flow relates to the concentration of ions in the water, such as salts, chlorides, sulphides and carbonates. The greater the amount of ions present, the higher the water's conductivity. In this study conductivity was measured at all sites by the Oakton EcoTestr EC low 2 in-situ by submerging the probe in water, allowing a 2 minute stabilisation period before recording.

Dissolved Oxygen (DO)

Many of the chemical and biological processes that occur in riverine ecosystems rely on the presence of oxygen. It is essential in maintaining aquatic life, but also in balancing

populations of aquatic organisms (Enderlein, 1996). Oxygen content can fluctuate with seasonality, photosynthesis, respiration and decomposition. Hypoxic levels of DO have been associated with the addition of fertilisers and agricultural run-off causing eutrophication. This variable is not present at the study's location, where the concept of re-wilding is implemented. As the most significant parameter in the productivity of aquatic systems (Marker & Wetzel, 1985), DO readings were taken at every site with a pre-calibrated Hach sensION 6 Dissolved Oxygen Meter lowered 5 cm into the water and recorded when stabilisation was reached.

Phosphates

Phosphorus enters rivers through plant degradation, human and animal effluent and fertiliser run off. Phosphate stimulates the growth of aquatic plants and plankton, though an excessive amount will contribute to eutrophication, ultimately lowering oxygen levels and causing a reduction in aquatic invertebrate and fish communities. Measurements for phosphorus levels in the River Adur used the Hach Pocket Colorimeter, with a Silicon photodiode detector and Ascorbic Acid reagent. A stock solution of sodium phosphate was used to create a calibration curve. Instrument calibration such as this is an essential stage to ensure a linear relationship between the output of the measurement system (the instrument response) and the accepted values of the calibration standard (e.g., the amount of analyte present). Water was collected in secure falcon tubes at each sampling site and was analysed in the laboratory at room temperature soon after. Preservation was possible at or below 4°C for up to 48 hours if necessary. Reagent blank value was measured for each lot of new reagent before the addition of the ascorbic acid reagent in the separate sample cell. The reactive phosphorus was measured by spectrophotometry, with results digitally displayed on the Hach Colorimeter.

Nitrates

Nitrates are found in waterways through fertiliser runoff, sewage effluence and agricultural waste. Excess nitrogen can stimulate aquatic plant and algal growth, leading to eutrophication and reduction in oxygen content. To evaluate the level of nitrates present, samples were taken at each site, secured in falcon tubes and analysed in the laboratory using the Hach Nitrate Pocket Colorimeter. A calibration curve, with known stock solution of Sodium Nitrate was used to ensure instrument response and outputs were linear. The reagent blank value was taken for each sample before the addition of a Cadmium Reduction Method to a separate sample cell. Results of the spectrometry were digitally displayed after a 5-minute reaction wait time.

Total Dissolved Solids

Total dissolved solid readings were taken using the Extech EC400 to measure the total content of organic (industrial waste, sewage effluence, leaves) and inorganic particles (from rocks or air containing calcium bicarbonate, nitrogen and other minerals) dissolved in water. A high TDS level is associated with decreased photosynthesis, leading to an increase of water temperature. A low TDS is also detrimental to river health. The meter was lowered approximately 10 cm into the water and results recorded once stabilisation was established.

Invertebrates

Species richness was determined based on the presence/absence of different taxa collected during sampling. Assessment of the River Adur's integrity was based on the BMWP score system, Family Biotic Index (FBI), Ephemeroptera (mayflies) presence, Plecoptera (stoneflies) presence, Trichoptera (EPT Score) and Average Score Per Taxa (ASPT). The BMWP system assigns bio-indication values to different invertebrate families based on their tolerance to

various pollutants. Taxa were identified to family level and were assigned a score in accordance with the BMWP scoring system, with a higher BMWP score reflective of better water quality. The ASPT was calculated using the average scores of all taxa found within the sample.

$$ASPT = \frac{BMWP}{Total\ number\ of\ families}$$

Figure 3. Average score per taxa equation.

$$\frac{Total\ EPT\ Taxa}{Total\ Taxa\ Found} \times 100 = \% Abundance$$

Figure 4. EPT equation.

The FBI score of each sampling site (where further, intensive evaluation is required (Mackie, 2000)) was calculated as shown in figure 5 (Hilsenhoff, 1988).

$$FBI = \frac{\sum [(TV_j)(n_j)]}{N}$$

Figure 5. Family Biotic Index equation.

$$H' = - \sum_{i=1}^S p_i \ln p_i$$

Figure 5a. Shannon Wiener Diversity Index equation.

$$E = \frac{H'}{H_{max}}$$

Figure 5b. Shannon evenness equation

The BMWP and ASPT scores calculated from the River Adur were interpreted with the threshold values stated in table 1, inline with the monitoring values used by the Ouse & Adur River Trust. The FBI values were compared with the criteria shown in table 2.

The Shannon Diversity Index was utilised to determine species diversity within a given community. By incorporating abundance measurements into the calculations, this statistical procedure aims to provide a more inclusive understanding of zonal populace dynamics.

Table 1. BMWP and ASPT Score Interpretations.

	BMWP	ASPT	
BMWP Score	Interpretation	ASPT	Interpretation
>130	Very good - Unpolluted	> 6.9	Very good
81-130	Good – Clean but slightly impacted	6.0 – 6.9	Good
51-80	Fair – Moderately impacted	5.0 – 5.9	Fair
11-50	Poor – Polluted or impacted	4.0 – 4.9	Poor
0-10	Very poor – Heavily polluted	3.9 or less	Very poor

Table 2. Evaluation of water quality using the family level biotic index (Hilsenhoff, 1988)

Family Biotic Index	Water Quality	Degree of Organic Pollution
0.000 – 3.75	Excellent	Organic pollution unlikely
3.76 – 4.25	Very good	Possible slight organic pollution
4.26 – 5.00	Good	Some organic pollution probable
5.01 – 5.75	Fair	Fairly substantial pollution likely
5.76 – 6.50	Fairly poor	Substantial pollution likely
6.51 – 7.25	Poor	Very substantial pollution likely
7.26 – 10.00	Very poor	Severe organic pollution likely

Table 3. Example of EPT index ranges and their corresponding water quality ratings. Modified from Watershed Science Institute Watershed Condition Series Technical Note 3 The EPT Index (2000).

Rating	Excellent	Good	Good-Fair	Fair	Poor
EPT Score	>27	21-27	14-20	7-13	<6

DATA ANALYSIS

To establish significance of data, statistical testing was required. Initially, Kolmogorov-Smirnov and Shapiro-Wilk tests were run on all data as a goodness of fit test, comparing observed data to quantiles of their normal distribution. Once normality of distribution had been established, suitable tests were undertaken.

Data Analysis on Comparative Data

Paired sample t-tests were conducted to identify any significant differences in phosphate levels and pH between 2015-2017 at replica sample sites (B, E, F, G, I, J). Additionally, paired t-tests were also conducted on ASPT and BMWP scores across the replica sites. A one-way ANOVA was used on the three available comparative datasets (Site J, E and I) from 2011, 2015 and the current study, to establish whether there were any statistically significant differences between years.

Analysis of 2017 Data

Principal component analysis (PCA) was performed to further understand the relationship between (i) water quality physio-chemical parameters, (ii) benthic macroinvertebrate families and (iii) characteristics of habitat/what restorative works had occurred. Any correlation between components was further investigated. Community similarity was accounted for by conducting Cluster Analysis with the Jaccard similarity index to compare communities between sites. Multidimensional scaling was implemented to provide quantitative estimates of similarity amongst the macroinvertebrate communities at sampling locations. Additionally, to address whether there was any seasonal heteroscedasticity across the 2017 sampling months (May, June, July), paired samples Kruskal-Wallis tests were performed. This was conducted on ASPT because of its accuracy (Zamora-Muñoz et al., 1995), D02, as this

measure is said to influence the most variables (Mackie, 2004), nitrates due to their seasonal variation (Deek, 2010) and number of families to gain a comprehensive view of sample conditions.

RESULTS

BENTHIC MACROINVERTEBRATES

The study ended with a cumulative collection of 39 macroinvertebrate families, as indicated in table 4. The table presents the presence and absence of families found during the 2011 pre-restoration spot-sampling, conducted on three sites, the 2-years post restoration 2015 spot-sampling on six sites and the data collected during the current 2017 study, across all ten sampling sites, as shown in Figure 1.

Table 4. Presence/Absence of families found during 2011 three-location spot sampling pre-restorative works, 2015 six-location spot sampling 2-years post-restoration and the current study: 2017, across all ten sampling sites.

Taxon name	2011	2015	2017
Mollusca - Limpets & mussels			
Sphaeriidae	●	●	●
Unionidae	●	●	●
Ancylidae	●		
Amphipoda - Crustaceans			
Asellidae	●	●	●
Gammaridae	●	●	●
- Snails			
Planorbidae		●	
Lymnaeidae		●	●
Viviparidae		●	●
Hydrobiidae		●	●
Tricladida - Flat worms			
Planariidae	●		
Dendrocoelidae			
Leeches			
Piscicolidae		●	●
Glossiphoniidae	●	●	●
Erpobdellidae	●	●	●
Megaloptera - Alderflies			
Sialidae	●	●	●
Ephemeroptera - Mayflies			
Baetidae	●	●	●

Taxon name	2011	2015	2017
Caenidae	●	●	●
Ephemeraidae	●	●	●
Siphonuridae		●	●
Leptophlebiidae		●	●
Heptageniidae			●
Trichoptera - Caddis flies			
Goeridae		●	●
Leptoceridae	●	●	●
Polycentropodidae	●	●	●
Sericostomatidae	●	●	●
Philopotamidae		●	●
Psychomyiidae		●	●
Limnephilidae	●	●	●
Hydropsychidae	●	●	●
Diptera - True flies			
Simuliidae	●	●	●
Chironomidae	●	●	●
Odonata - Damselflies			
Coenagrionidae	●	●	●
Calopterygidae	●	●	●
Lestidae		●	●
Coleoptera - Beetles			
Dytiscidae		●	●
Gyrinidae		●	●
Hydrophilidae		●	●
Platynemidae	●		
Hemiptera - Bugs			
Pleidae			●
Notonectidae		●	●
Corixidae		●	●
Mesoveliidae		●	●
Nepidae		●	●
Annelida - Worms			
Oligochaeta	●	●	●

Table 4. Displays 23 benthic macroinvertebrate families collected during the spot-sample in 2011, which had increased to 37 in the 2015 spot-sample, though this was conducted at three more sites than the 2011 study.

The 2017 data contained a further two benthic macroinvertebrate families, including *Pleidae* of the Hemiptera order and *Heptageniidae* - a mayfly.

Having shown a clear increase of invertebrate numbers based on the spot-sampling studies, the data was analysed further, as shown in table 5.

Table 5. Macroinvertebrate Metrics Table

Site	A	B	C	D	E	F	G	H	I	J
EPT Score (%) 2017	22	20.5	34.7	28.6	21.3	18.6	24.7	18.8	16.3	14.4
EPT Quality 2017	Good	Good - Fair	Excellent	Excellent	Good	Fair	Good	Fair	Fair	Fair
ASPT Score 2017	5.57	5.2	5.35	5.71	6.16	5.62	5.55	6.66	5.34	5.64
ASPT Quality 2017	Fair	Fair	Fair	Fair	Good	Fair	Fair	Good	Fair	Fair
ASPT Score 2015	x	4.42	x	x	6.15	4.81	5.25	x	4.5	5.33
ASPT Quality 2015	x	Poor	x	x	Good	Poor	Fair	x	Poor	Fair
ASPT Score 2011	x	4.42	x	x	6.15	4.81	x	x	x	x
ASPT Quality 2011	x	Poor	x	x	Good	Poor	x	x	x	x
BMWP Score 2017	80	89.3	96.3	101	96	96	94.3	121.3	81	97.6
BMWP Quality 2017	Fair	Good	Good	Good	Good	Good	Good	Good	Good	Good
BMWP Score 2015	x	53	x	x	80	77	84	54	x	112
BMWP Quality 2015	x	Fair	x	x	Fair	Fair	Good	Fair	x	Good
BMWP Score 2011	x	x	x	x	80	x	x	62	x	90

BMWP Quality 2011	x	x	x	x	Fair	x	x	Fair	x	Good
FBI Score 2017	5.56	5.18	6.29	5.55	5.48	5.98	6.26	5.72	5.92	5.07
FBI Quality 2017	Fair	Fair	Fairly poor	Fair	Fair	Fairly poor	Fairly poor	Fair	Fairly poor	Fair
Shannon Diversity Index 2017	2.508	2.794	2.415	2.523	2.477	2.784	2.659	2.542	2.397	2.010
Shannon Diversity Evenness 2017	0.68	0.76	0.65	1.00	0.67	0.75	0.72	1.00	0.65	0.54

Macroinvertebrate metrics (displayed in table 5), showed a consistency or increase of ASPT and BMWP quality from 2011 to 2015, as displayed in figure 5a. The BMWP quality across all sites was described as good, with the exception of site A. The FBI index showed an overall quality of fair, with fairly poor conditions observed in site C, F, G and I.

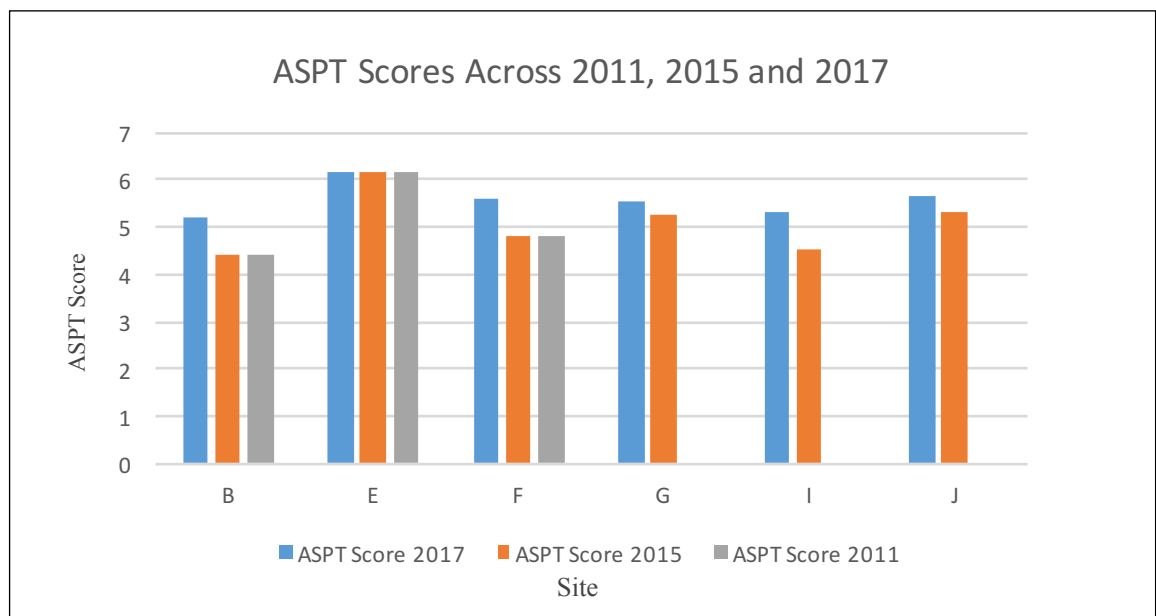


Figure 5a. ASPT scores across six sampling locations for 2015 and 2017 data. Scores displayed across three sites (B, E & F) for 2011 data.

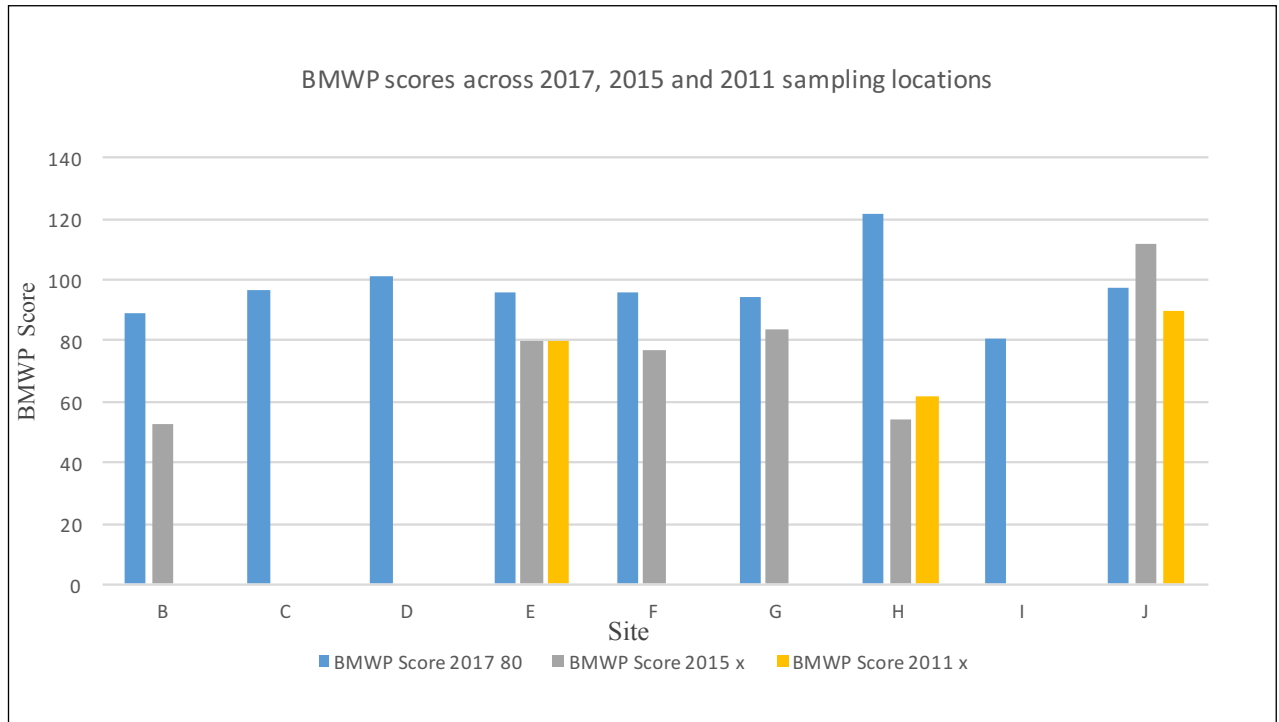


Figure 5b. BMWP scores across six 2017 and 2015 sampling location. Scores displayed across three sites from 2011 data.

The Shannon Diversity Index is indicated in table 5, with the lowest indicated score found furthest downstream from restorative works. Furthermore, the lowest evenness also occurred at the furthest site, sample location J.

The EPT score indicated conditions ranging from excellent at sites C and D, to good to fair at site B, good at sites A, E and G and fair at site F, remaining consistent further downstream at sites H I J. The pie-chart shown in figure 6a, depicts the EPT percentage across all sampling locations, revealing *Baetidae* was the most commonly distributed of the order Ephemeroptera. In contrast, *Heptageniidae* was the least observed. The highest percentage of Trichoptera was *Philopotamidae*, whilst no macroinvertebrates of the order Plectoptera (stoneflies) were found. Site specific EPT scores (see fig. 6) showed the highest site was C, with the lowest observed at furthest downstream sampling location, J.

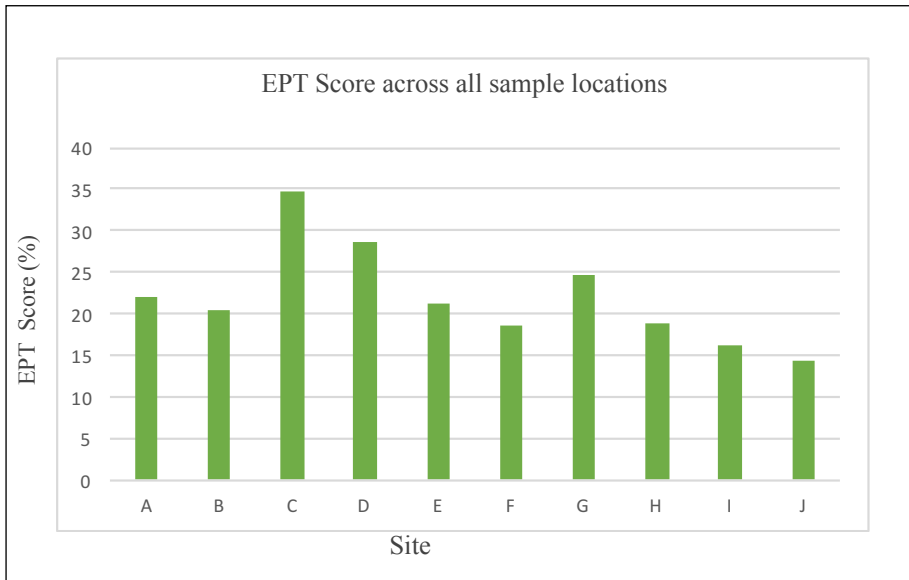


Figure 6. Histogram of EPT scores derived from sample locations A-J in the current 2017 study.

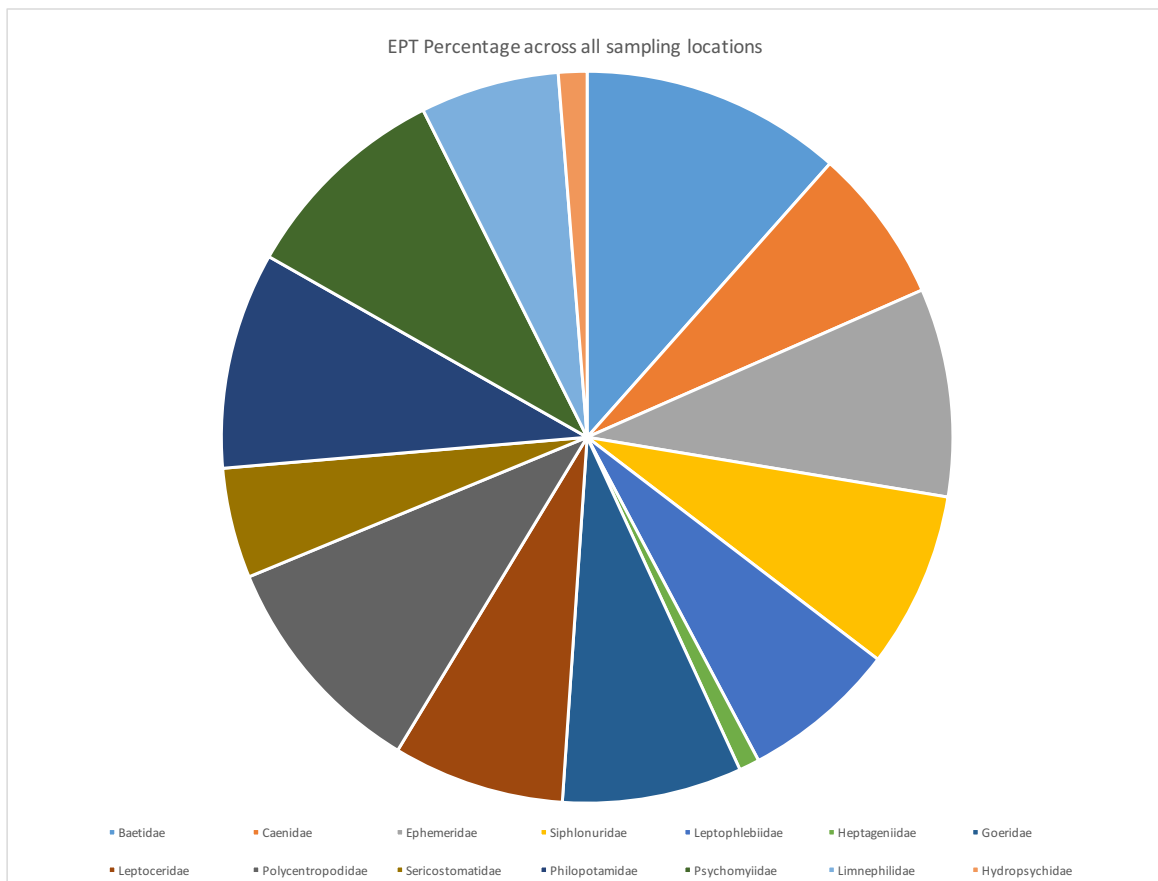


Figure 6a. EPT percentages, calculated using the equation shown in fig.4, and distribution shown across all ten sample locations.

The pie chart shown in figure 7, displays the highest prevalence of Amphipoda - amphipods (malacostracan crustaceans with no carapace), with Trichoptera - caddisfly larvae, the second most observed invertebrate, followed by Mollusca - molluscs (aquatic snails in the current study). The least commonly occurring macroinvertebrate order was that of the Annelida - segmented worms.

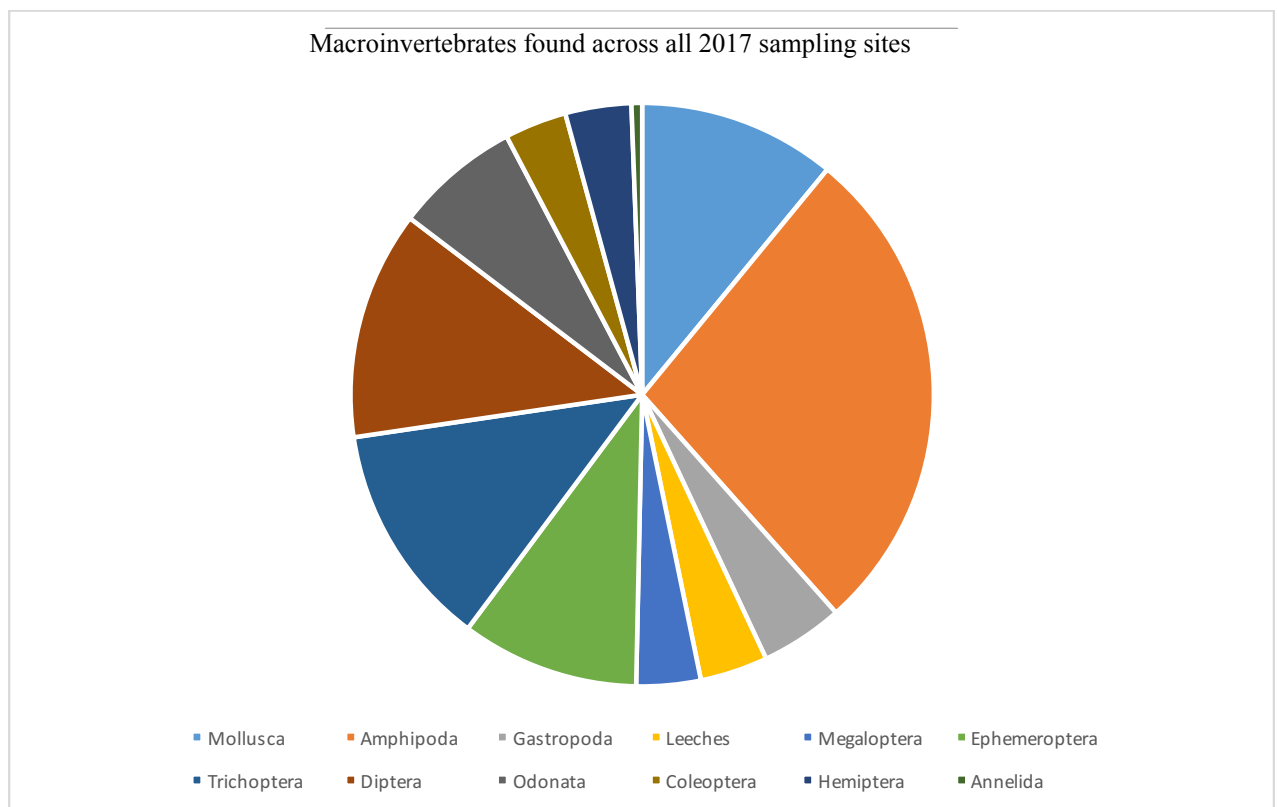


Figure 7. Macroinvertebrate percentages across all 2017 sampling sites.

ENVIRONMENTAL VARIABLES

Table 6 shows the environmental variables measured across all ten sampling locations in 2017, with comparison against previous available 2015 pH and phosphate data from their six sampling locations - the only environmental variables measured during this prior study.

pH values show very little change, whilst the phosphate data range was found to be higher in the current study, with a mean increase of 0.33 mg/l. Within the current 2017 study across ten sites, nitrates were found at a mean 3.87 mg/l, dissolved oxygen 11.88 mg/l, conductivity 647.33 U/s and total dissolved solids 442.10 ppm.

Table 6. Environmental Variable Descriptive Statistics

Parameter	Mean	Data Range	Standard Deviation	Confidence Interval	
				Lower	Upper
pH value 2017	6.80	6.40 - 7.06	0.35	5.92	7.67
pH value 2015	6.68	6.50 - 6.80	0.13	6.54	6.82
Phosphates 2017(mg/l)	0.53	0.07 - 0.92	0.20	0.45	0.60
Phosphates 2015 (mg/l)	0.20	0.02 - 0.40	0.15	0.04	0.36
Nitrates 2017(mg/l)	3.87	3.81 - 3.91	0.56	3.74	4.01
DO 2017(mg/l)	11.88	11.53 - 12.41	0.47	10.72	13.04
Conductivity 2017 (U/s)	647	638 - 652	8.08	627	667
TDS 2017 (ppm)	442.10	436.20 - 451.10	7.92	422.43	461.77

STATISTICAL ANALYSIS

COMPARISON BETWEEN 2015 DATA AND 2017 DATA ACROSS SIX SAMPLING LOCATIONS

The above data, of both macroinvertebrate and environmental, was subjected to further statistical analyses, with results shown below.

BENTHIC MACROINVERTEBRATES

BMWP

The BMWP results indicated equal variances assumed by Levene's Test for Equality of Variances ($p=0.097$). The paired samples t-test and group statistics showed the difference between BMWP score in 2015 ((Mean))(M) = 76.67, SD =21.85) and 2017 (M = 92.37, SD = 6.26). Though the histogram shown in figure 8 displays a higher BMWP at each sampling site in 2017, with the exception of 'J,' this was a non-significant result, CI [-36.38, 4.98]; $t(10) = -1.692$, $p = 0.122$.

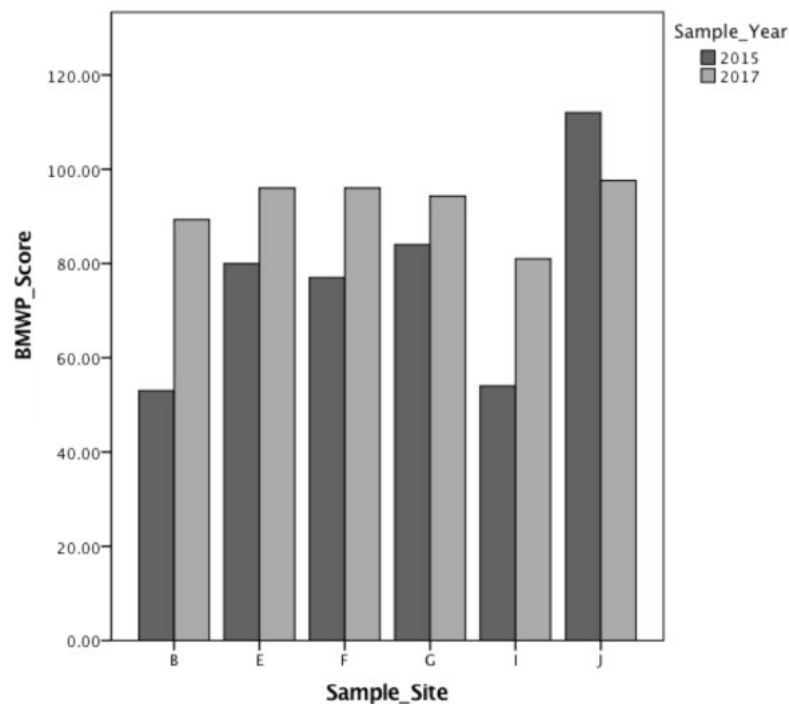


Figure 8. BMWP score across six replica site locations from 2015 spot-sample and 2017 3-sample study.

To understand whether there is an increase without site J, a paired samples t-test was run, exempting site J, which, as shown in figure 8, was the only occurrence of a higher BMWP score in 2015 than 2017. With n=5, the results of this showed for the 2015 spot-sample (M = 75.92 SD =18.05) and 2017 (M = 91.32, SD = 6.39). This result showed no significant differences CI [-25.15, 14.35]; $t(8) = -0.631$, $p = 0.167$, between the BMWP score derived through the 2015 spot-sample and the 3-month 2017 study.

ASPT

The ASPT had confirmed homogeneity throughout Levene’s Test for Equality of Variances ($p = 0.20$). With normal distribution confirmed, a paired samples t-test revealed the ASPT from 2015 (M = 5.127, SD = 0.672) and 2017 (M=5.532, SD = 0.358). The test showed a non-significant difference, $M = -0.405$, CI [-1.096, 0.286]; $t(10) = -1.306$, $p = 0.221$. Despite the non-significant result of the statistical test, figure 9 demonstrates that ASPT scores were higher across 4 sites (B,F,I and J), in 2017 compared to 2015, with site E remaining constant and G revealing a higher score in 2015.

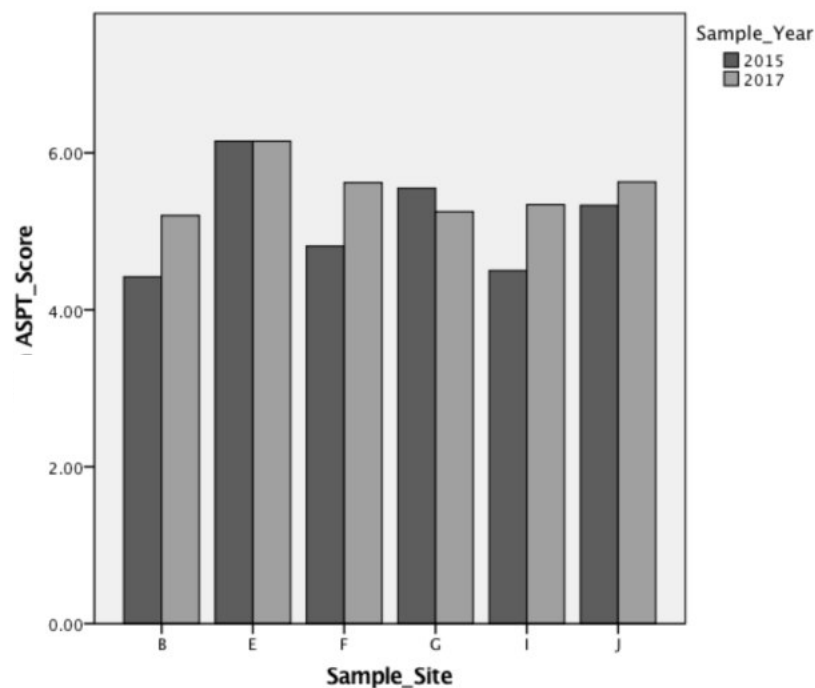


Figure 9. Histogram of site specific ASPT scores from 2015 and 2017 sampling.

ENVIRONMENTAL VARIABLES

With two parameters available to compare the current study's data - pH and phosphates, these were subjected to paired samples t-tests to determine if there were any significant differences from the 2015 and 2017 sampling results of six replica sites.

To ensure validity in the study, normal distribution was established through the Shapiro-Wilk Test of Normality ($p > 0.05$). No outliers in any data from 2015 or 2017 were found through examination of box plots and the null hypothesis H_0 was rejected – the data follow a normal distribution.

Phosphates

Homogeneity of variances was confirmed by Levene's Test for Equality of Variances ($p=0.126$), rejecting the null hypothesis and concluding that equal variance in phosphate level between 2015 and 2017 is assumed. The phosphate values between the two sampling years showed phosphate levels were higher in 2017 ($M= 0.5383$, $SD= 0.21$) than in 2015 ($M=0.0833$, $SD = 0.11$). This was a statistically significant difference, $M=-0.46$, 95% CI [-0.67, -0.24], $t(10)=-4.69$, $p = 0.001$.

The box plot displayed in figure 10 displays that phosphate levels in the 2017 study were significantly higher than those from 2015.

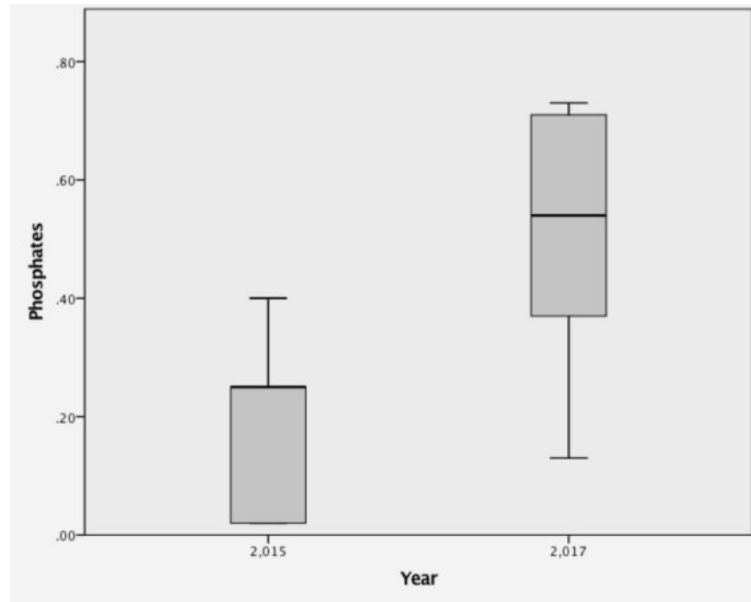


Figure 10. Box plot of phosphate levels from 2015 spot-sampling and the 3 month study conducted in 2017.

Additionally, a histogram is included in figure 11 to show site specific phosphate levels. With the exception of site B, where the phosphate reading recorded during the 2015 spot-sample was higher than that of the current 2017 research, all sites had a significantly larger amount of phosphates than in 2015.

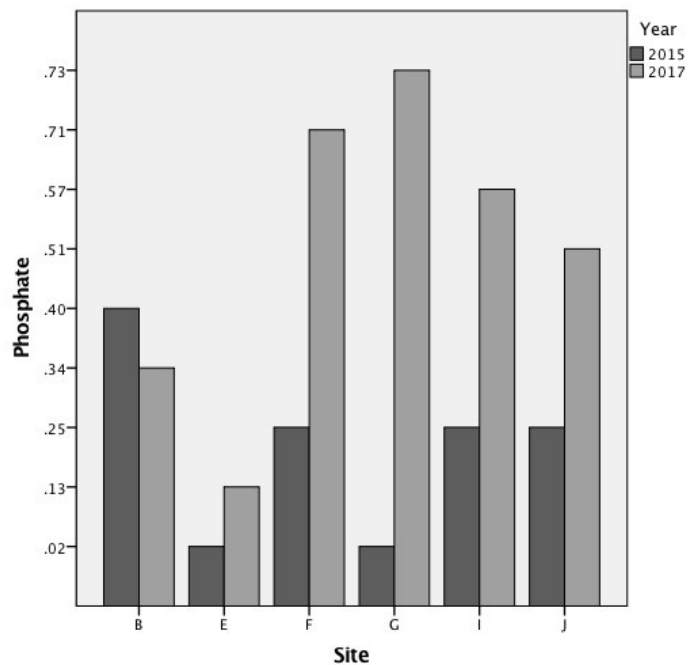


Figure 11. Histogram of site specific phosphate levels from the 2015 spot-sample and the 3-month 2017 current study. The figure shows the increase of phosphates at all sites in the 2017 sampling, except for site B.

pH

Levene's Test for Equality of Variances confirmed homogeneity ($p = 0.298$). The pH results assumed equal variance. The t-test revealed a difference in pH values between the 2015 sampling ($M = 6.750$, $SD = 0.12$) and 2017 study ($M = 7.177$, $SD = 0.171$). This was a statistically significant difference, $CI [-0.62, -0.24]$, $t(10) = -4.97$, $p = 0.001$.

The box plot displayed in figure 13 shows a significant rise in pH from 2015 and 2017. The histogram shown in figure 12 also displays the pH values at each sampling site across 2015 and 2017, revealing that all pH values were higher during the 2017 study.

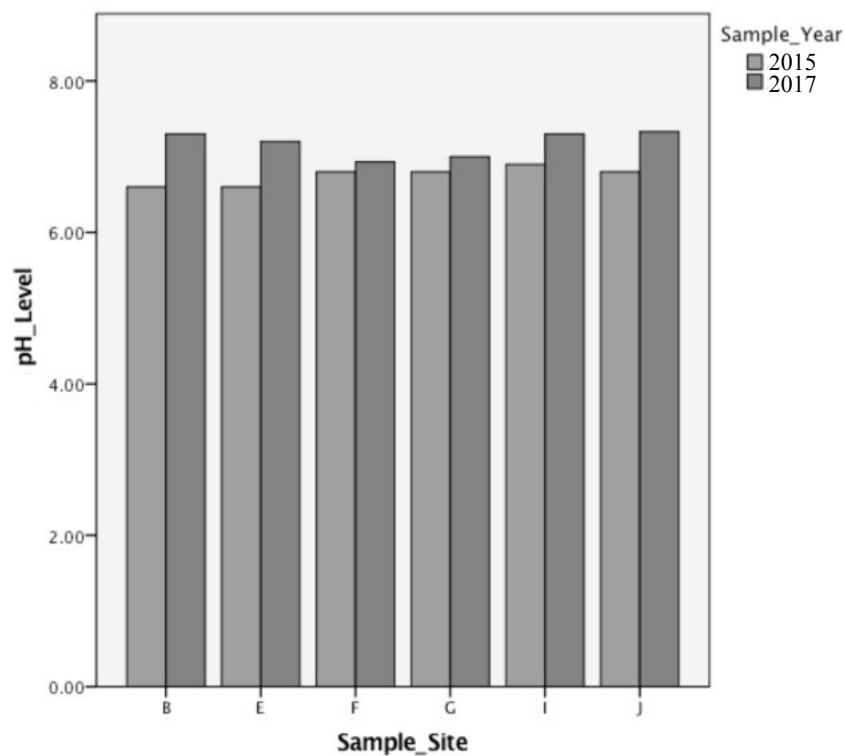


Figure 12. pH level across 6 replicate sample sites, from 2015 and 2017 sampling.

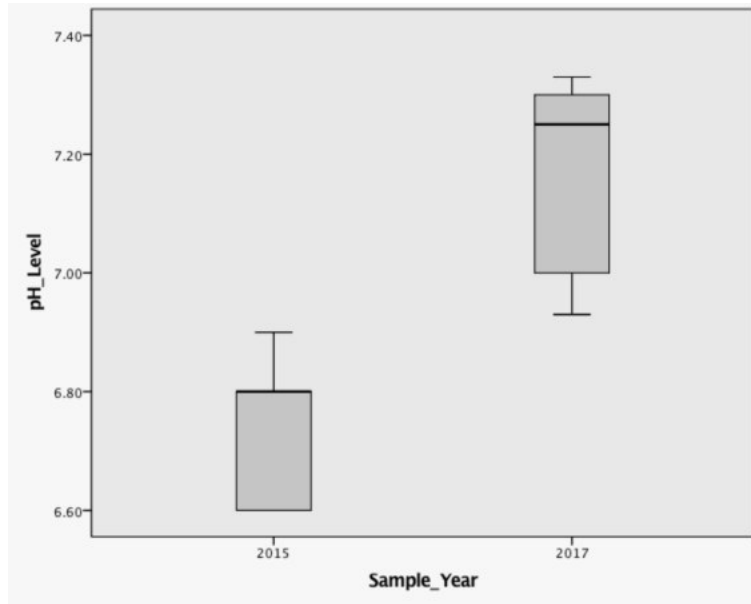


Figure 13. Box plot of pH level comparison between 2015 and 2017 sampling.

COMPARISON BETWEEN 2011, 2015 DATA AND 2017 DATA ACROSS THREE REPLICASAMPLING LOCATIONS

With two sets of previous benthic macroinvertebrate data available for three replica sites from the 2011 spot-sample pre-restoration and the 2015 spot-sample, this was compared to the data derived from the current three-month study. This was conducted on sites E, I and J, which are all located in the main channel. Analysis of data through the Shapiro-Wilk test reported $p > 0.05$ for all data, rejecting the H_0 and concluding that the data follows a normal distribution.

ASPT

Figure 14 displays a box plot of ASPT scores across three replicated sites of available data from 2011, (M = 77.33, SD = 14.19), 2015 (M = 82.00, SD = 29.05) and 2017 (M= 91.53, SD = 9.16). There was not a statistically significant difference between groups, determined

by a one-way ANOVA ($f = 2,6 = 0.418$, $p = 2.017$, $p = 0.676$). The box plot shows the greatest amount of variance in data in the 2015 spot-sample, indicated by the largest distance between the lower and upper quartile groups, with the 3 sections of the box plot uneven in size, suggesting a higher level of variance between the sampling years.

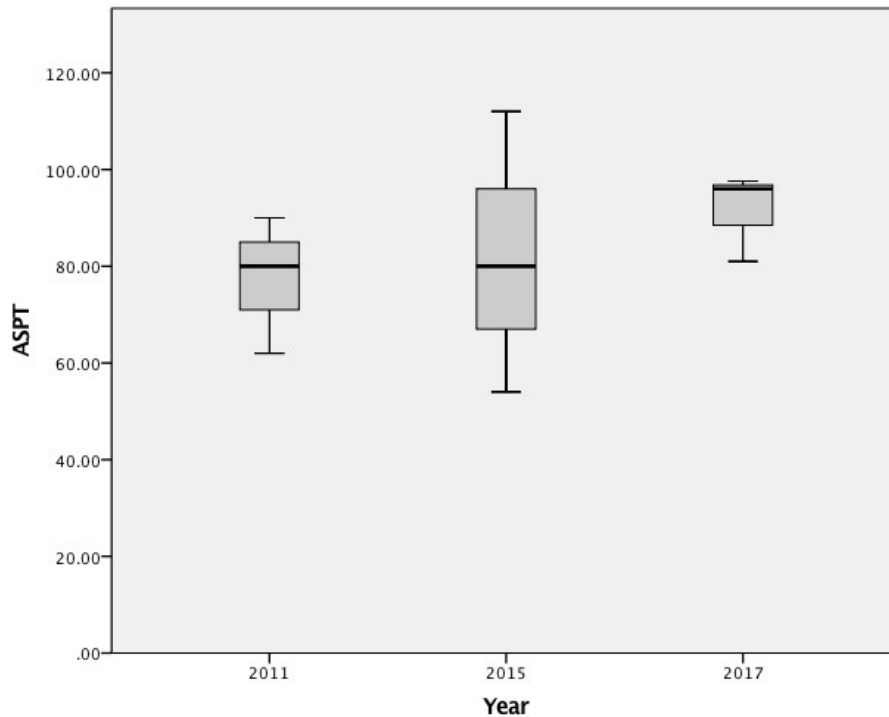


Figure 14. Box Plot of ASPT scores across sampling years 2011, 2015 and 2017.

BMWP

A one-way ANOVA test showed BMWP from 2011 ($M = 77.33$, $SD = 14.19$), 2015 ($M=82.00$, $SD = 29.05$) and 2017 ($M=104.97$, $SD = 14.17$). There was no statistically significant difference between the three groups ($F = 2, 6 = 1.580$, $p = 0.281$). However, the box plot shown in figure 15 identifies that there are different distributions among the box plots, with the highest median shown in 2017, and the lowest in 2015. The greatest range of

data is found in the 2015 spot-sample, demonstrated by the distance between the upper and lower-quartiles and the extended whiskers, representing scores outside of the middle 50% of data.

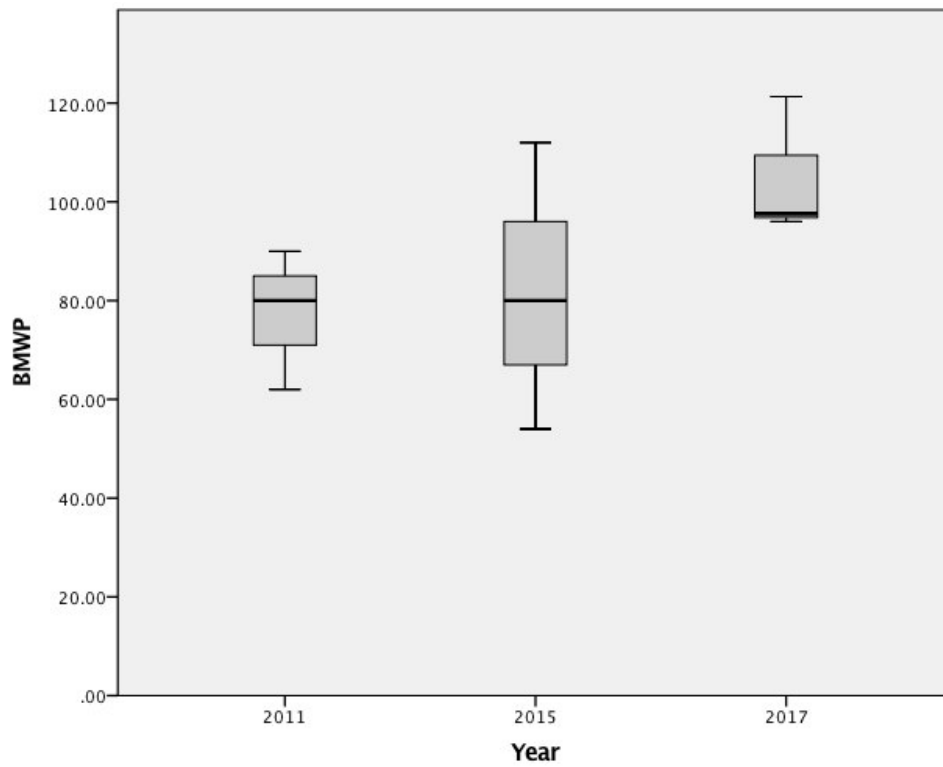


Figure 15. BMWP across sampling years 2011, 2015 and 2017.

2017 CURRENT STUDY

SEASONALITY

To assess for seasonality and any changes during the three sampling times in May, June and July, the results of the current 2017 dataset across 10 sampling locations were subjected to further statistical tests.

To address normality of data the Shapiro-Wilk Test was undertaken. Homogeneity was not confirmed for number of families and ASPT. Without normal distribution established, the H_0 was accepted, concluding that the data do not follow normal distribution. The D02 and nitrate samples had confirmed homogeneity, with the H_0 rejected.

BENTHIC MACROINVERTEBRATES

Number of families

A non-parametric Wilcoxon matched pairs test showed that there was no significant difference in number of families across the sampling months. The scatter plot shown in figure 16 displays the number of families collected during sampling against the month of collection. Despite the plot showing a negative trend in families found, the statistical test proved there was no effect of seasonality on data.

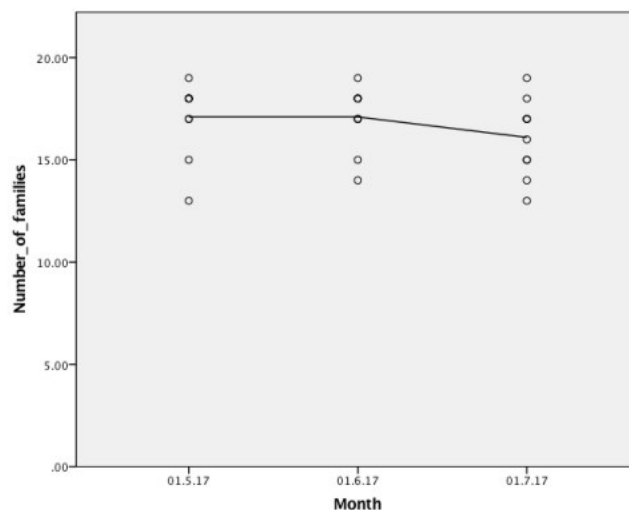


Figure 16. The scatter plot shows the results of the Wilcoxon matched pairs test for seasonality across the three sampling months of the current study.

ASPT

A Wilcoxon matched pairs test revealed statistical significance between ASPT and seasonality ($Z=-4.78$, $p<0.01$). Figure 17 displays a box plot of the ASPT scores against sampling months, the medians are seen to decrease with month, with the elongated whiskers shown for the April sampling, suggest a greater data variance in this month.

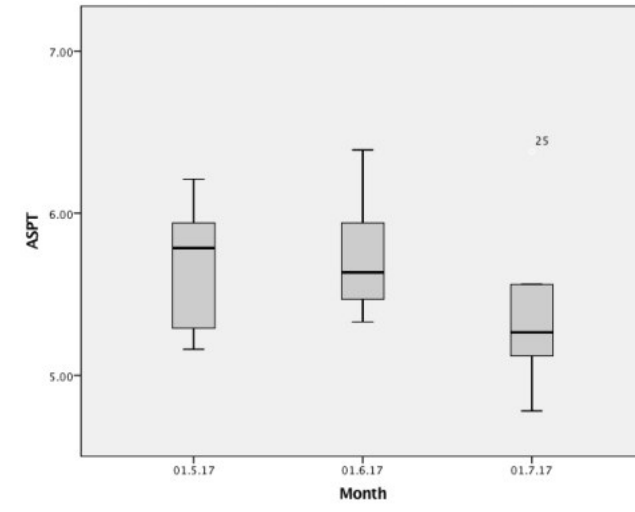


Figure 17. shows the results of the Wilcoxon matched pairs test for seasonality across the three sampling months of the current study.

ENVIRONMENTAL VARIABLES

With benthic macroinvertebrate data assessed for variance between sampling months, two environmental variables which are known to be influenced by seasonality, were subjected to further statistical analysis.

Nitrates

Nitrates were subjected to a Pearson correlation, the results of this showed showed a weak positive relationship between nitrate level and seasonality, though this was a non-significant result ($r=0.86$, $n=30$, $p=0.651$, $R^2=0.007$). These results are displayed in the correlation

plot in Figure 18. The plot identifies that site J has the highest level of nitrates in both May and June, and is the second highest level in July.

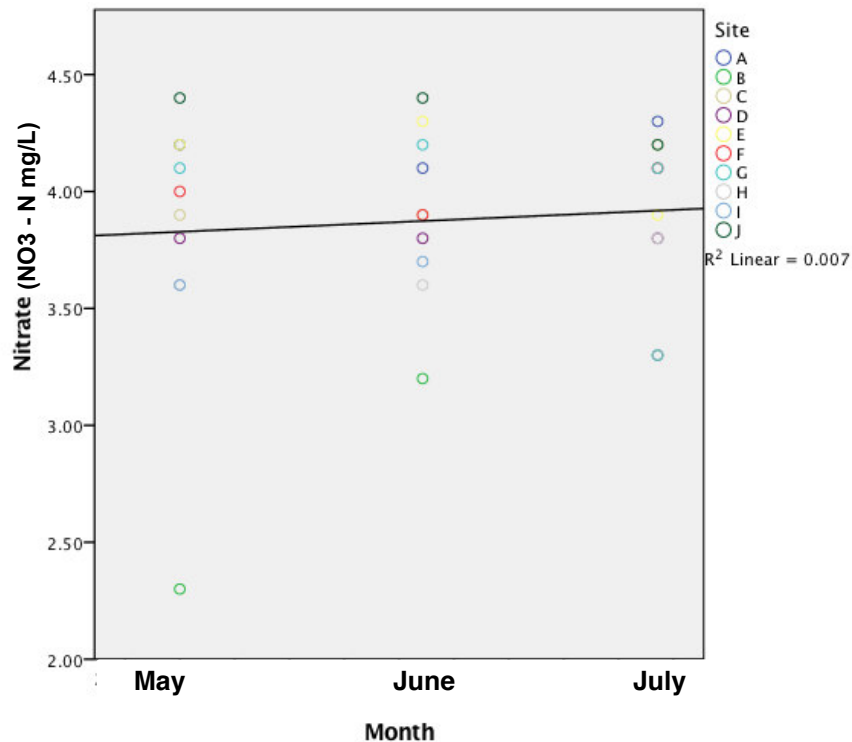


Figure 18. Pearson’s correlation shown in the plot, comparing nitrate levels across the three sampling months.

Dissolved Oxygen

Dissolved oxygen was also subjected to Pearson’s correlation, the results of this showed a slightly insignificant result between seasonality and nitrate levels ($r = 0.358$, $n = 30$, $p = 0.052$). Figure 26 displays there is a weak correlation between the two variables, though still statistically insignificant ($R^2 = 0.128$). The correlation plotted in figure 18 shows sites J and G are consistently the lowest dissolved oxygen levels, across all three sampling time points in May, June and July.

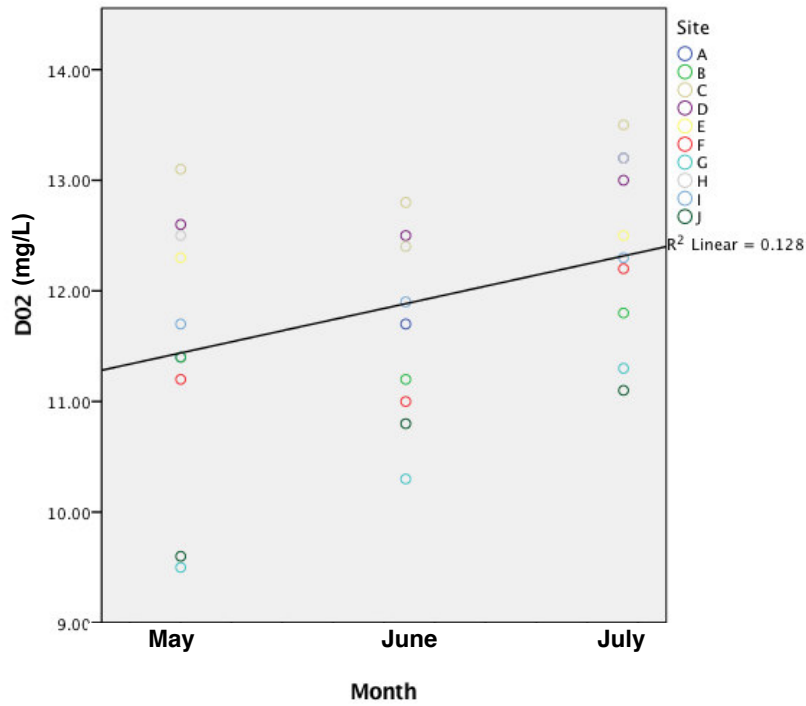


Figure 19. Pearson’s correlation shown in the plot, displaying dissolved oxygen levels compared to sampling months.

PRINCIPAL COMPONENT ANALYSIS

SAMPLING ACROSS 10 SITES AT THREE TIME POINTS IN 2017

Principal component analysis (PCA) was conducted on all 10 sites to assess any correlations in data across the 3 sample points. In total, PCA was performed on 6 environmental variables (see table 9) and 39 benthic macroinvertebrates across 10 sites (A-J). Scree plots were used to identify the number of extracted components. Kaiser’s criterion was used to retain factors that had eigenvalues <1 , this is to ensure that unless a factor extracts at least as much as the equivalent of one original variable, it is omitted, thus yielding the most precise and comprehensive results (Kaiser, 1960). Varimax rotation was used to simplify the expression of a sub-space by showing just a few major factors in each.

ENVIRONMENTAL VARIABLES

The PCA was performed on 6 environmental variables (as show in table 9), results showed 2 principal components, with 35.70% of variance explained by the 1st component. An additional 25.38% of variance was explained by the 2nd component. With commonalities after extraction >0.7 , all factors with Eigen values above 1 were retained, shown in figure 20, (as per Kaiser's criterion). After rotation, the first component accounted for 32.57% of total variance, with component two accounting for 28.51% of the variance.

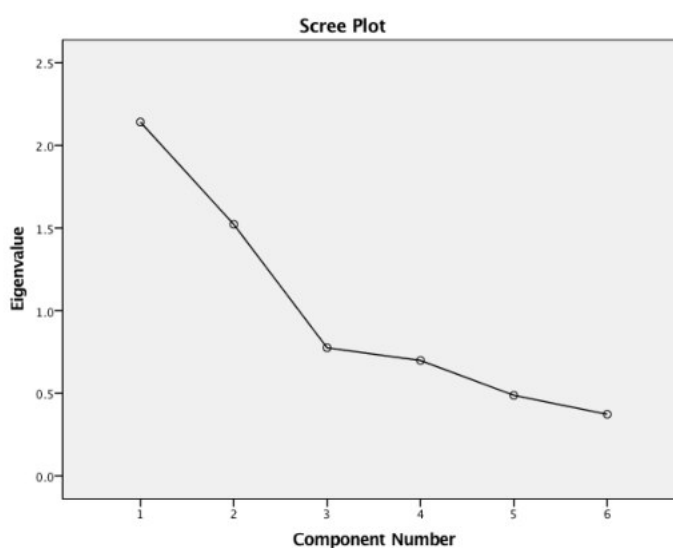


Figure 19. Scree plot of PCA on environmental variables showing after the first two components, differences between the eigenvalues decline and are less than 1.0.

Table 7. Rotated Component Matrix, with Varimax and Kaiser Normalization, for environmental variables across 10 sampling locations. Correlations above 0.5 are considered of importance.

	PC1	PC2
Phosphates	0.769	
pH	-0.706	
Conductivity	0.677	
Nitrates	0.575	0.505
TDS		0.878
Oxygen		-0.821

Figure 20 show all loadings for each component and demonstrates how closely related phosphates, conductivity and nitrates are as variables. The loadings show strong correlations between phosphates, pH, and conductivity to component 1, whereas nitrates are equally correlated to both component 1 and 2. Additionally oxygen is strongly negatively correlated to component 2.

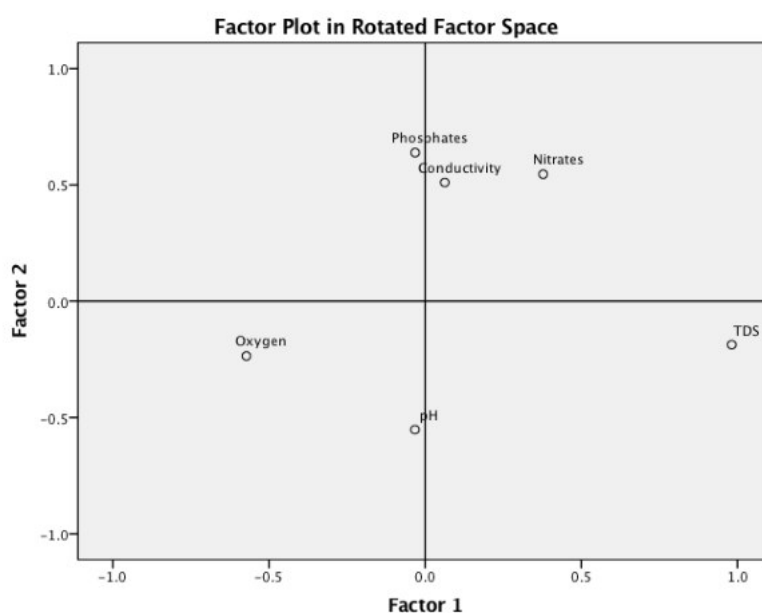


Figure 20. Component plot in rotated space for environmental variables, derived through PCA on 2017 environmental data.

BENTHIC MACROINVERTEBRATES

The PCA was performed on data of 39 found macroinvertebrates (as shown in table 4) and 10 site numbers, results showed 2 principal components, with the 1st component accounting for 75.74% of the variance, with component 2 accounting for 9.50%. All communalities after extraction were >0.7 , (with the exception of Site F = 0.082).

The scree plot shown in figure 21 demonstrates that after the first two components, differences between the eigenvalues decline and are <1.0 , which supports the two-component solution.

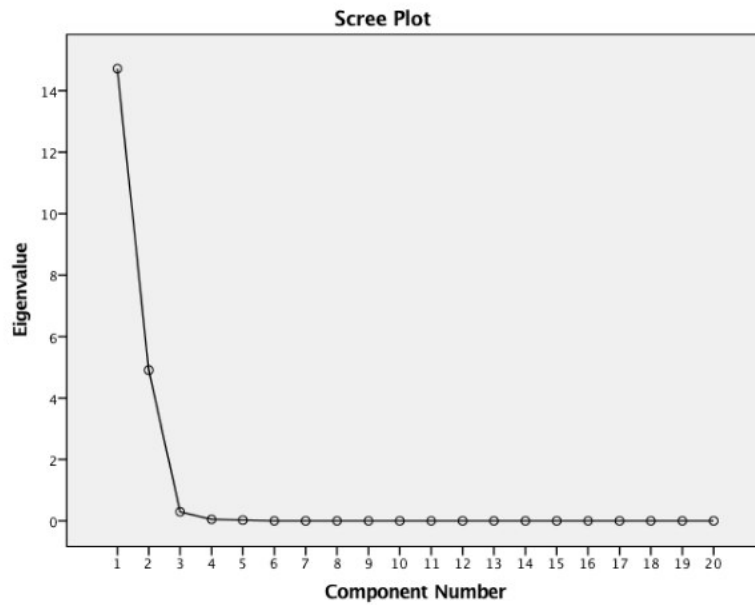


Figure 21. Scree plot on benthic macroinvertebrate data derived from PCA, across all 2017 sites.

The component plot in rotated space (see figure 22) reveals that all sites are loaded highly by the strong correlation with component one, with the exemption of site F which is strongly correlated to component two. To a lesser extent, site A showed a weak correlation to component two. The rotated loadings of these two components in displayed in table 7.

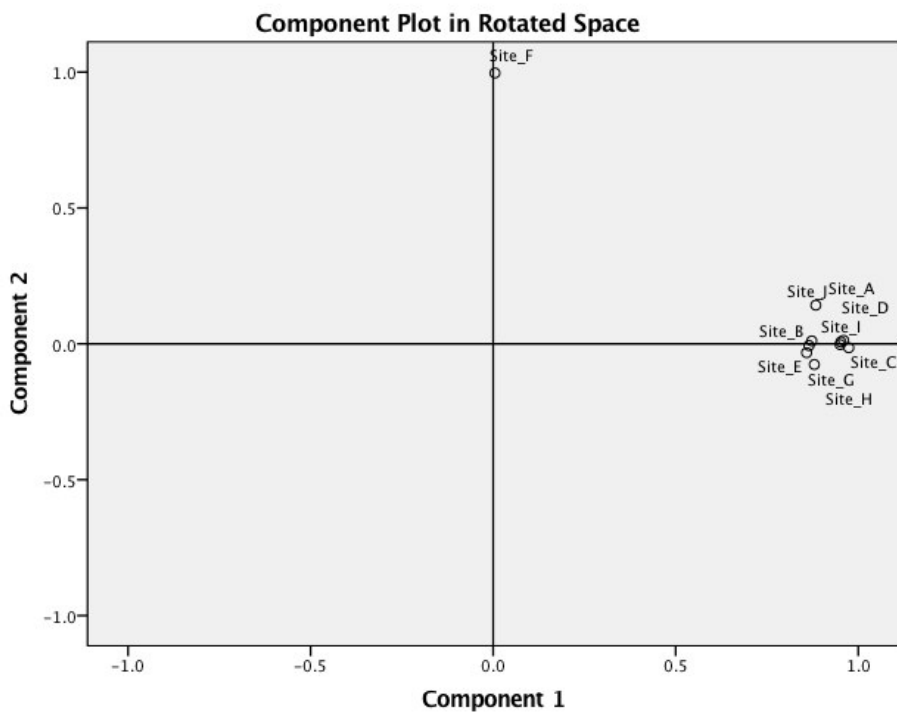


Figure 22. Component plot in rotated space for invertebrate data derived from ten 2017 sampling sites.

Table 7. Rotated loadings for invertebrate PCA across 10 sampling sites. The table displays coefficients over the absolute value of 0.3. Those coefficients below were suppressed and present as ‘-’.

	PC1	PC2
Site H	0.976	-
Site D	0.966	-
Site J	0.951	-
Site C	0.950	-
Site A	0.911	-
Site I	0.891	-
Site B	0.880	-
Site G	0.877	-
Site E	0.876	-
Site F	-	0.997

With Site F presenting as highly loaded to the second component, further investigation was necessary to establish the reasons behind this. A histogram was created to represent benthic macroinvertebrate abundance at the site. As shown in figure 23, this shows that the highest set invertebrate abundance at site F was that of *Gammaridae*. It also displays the second highest abundance is *Oligochaeta* - segmented worms, whose tolerance value are 1 (BMWP). Additionally, a high abundance of *Philopotamidae* was also observed. Site F contained the third highest abundance of *Leptophlebiidae*, and the presence of *Siphonuridae* was not found at any other sample location. The absence of *Coenagrionidae* at site F is also noted, as there was no other absence of this invertebrate during sampling.

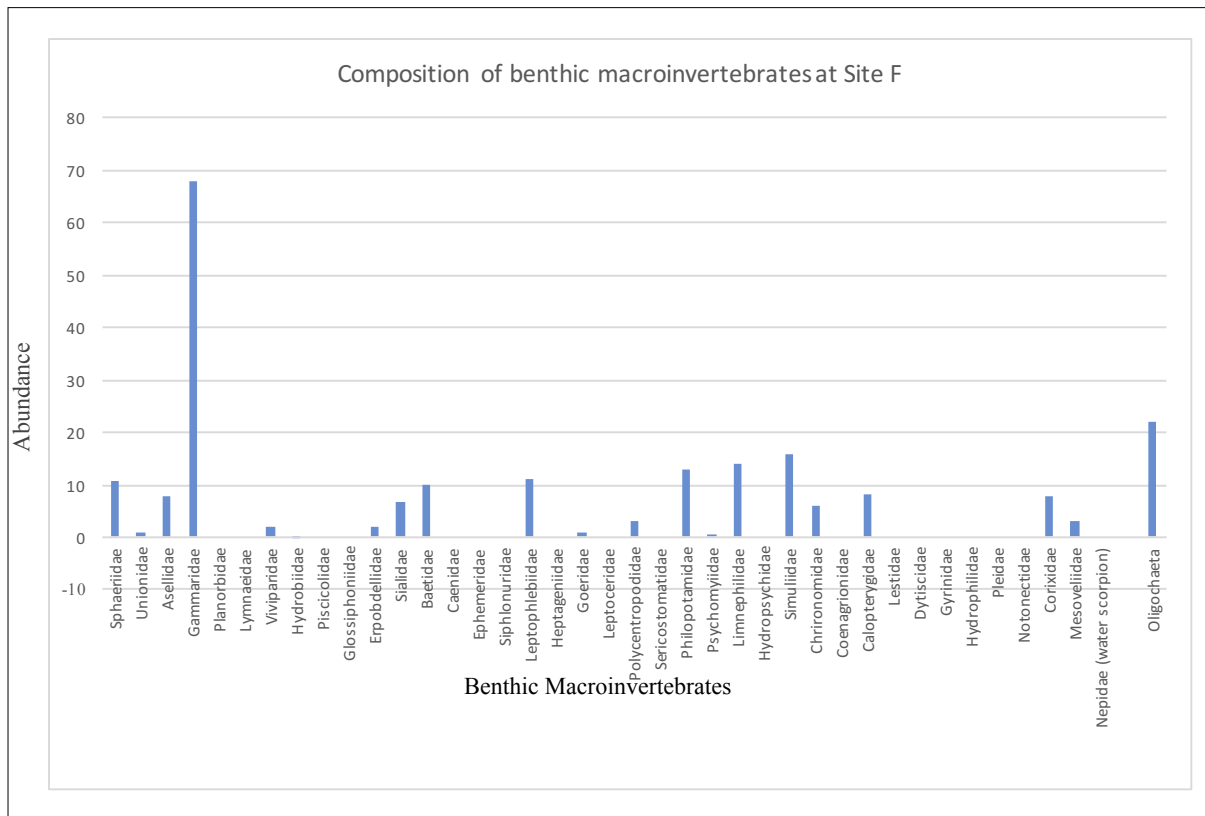


Figure 23. Histogram of benthic macroinvertebrate abundance at site F, 2017.

Further benthic macroinvertebrate data was evaluated through histogram plots to compare whether the abundance shown in figure 23 was similar across the other nine sample sites.

Distribution of macroinvertebrates of particular interest, as highlighted above, were plotted against all sample locations, to evaluate whether there are any observational trends and further analyse what separates site F from the others (see figure 24).

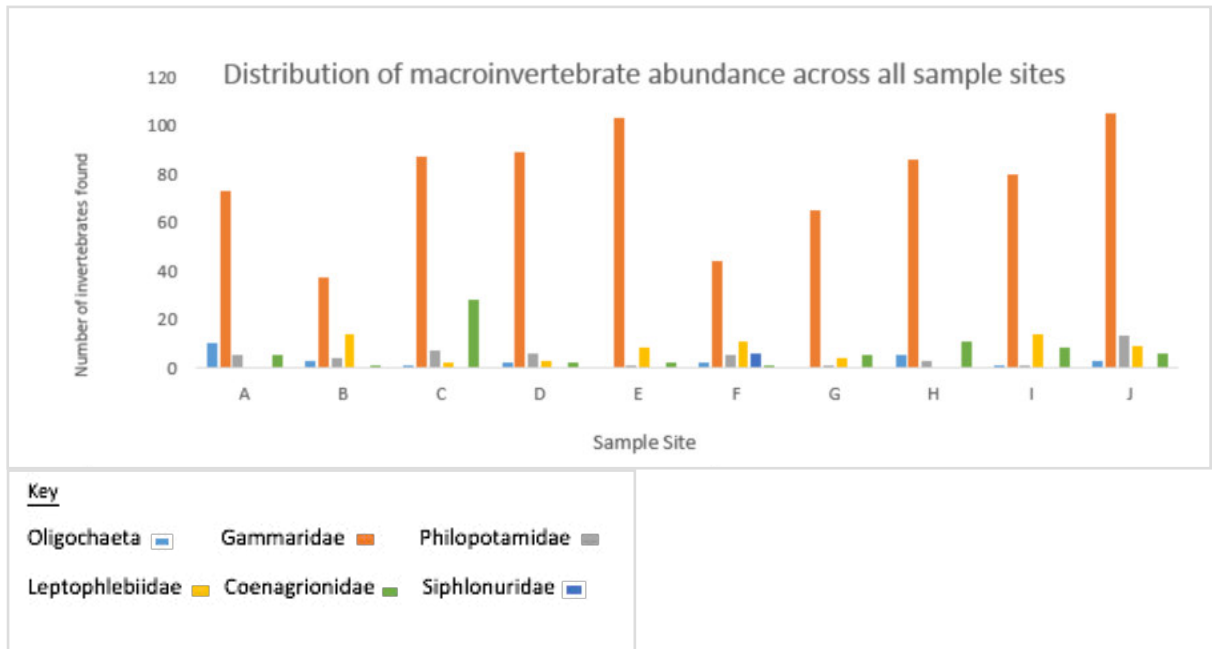


Figure 24. Histogram of benthic macroinvertebrate distribution across all sites (A-J).

Analysis of pollution tolerant invertebrates was performed to find whether there was a higher number of these present at site F. The distribution of pollution tolerant benthic macroinvertebrates (see fig. 25) does not show that site F contains a high density of these families, with the exception of *Asellidae*, which occurs at its highest abundance at Site F and Site D equally.

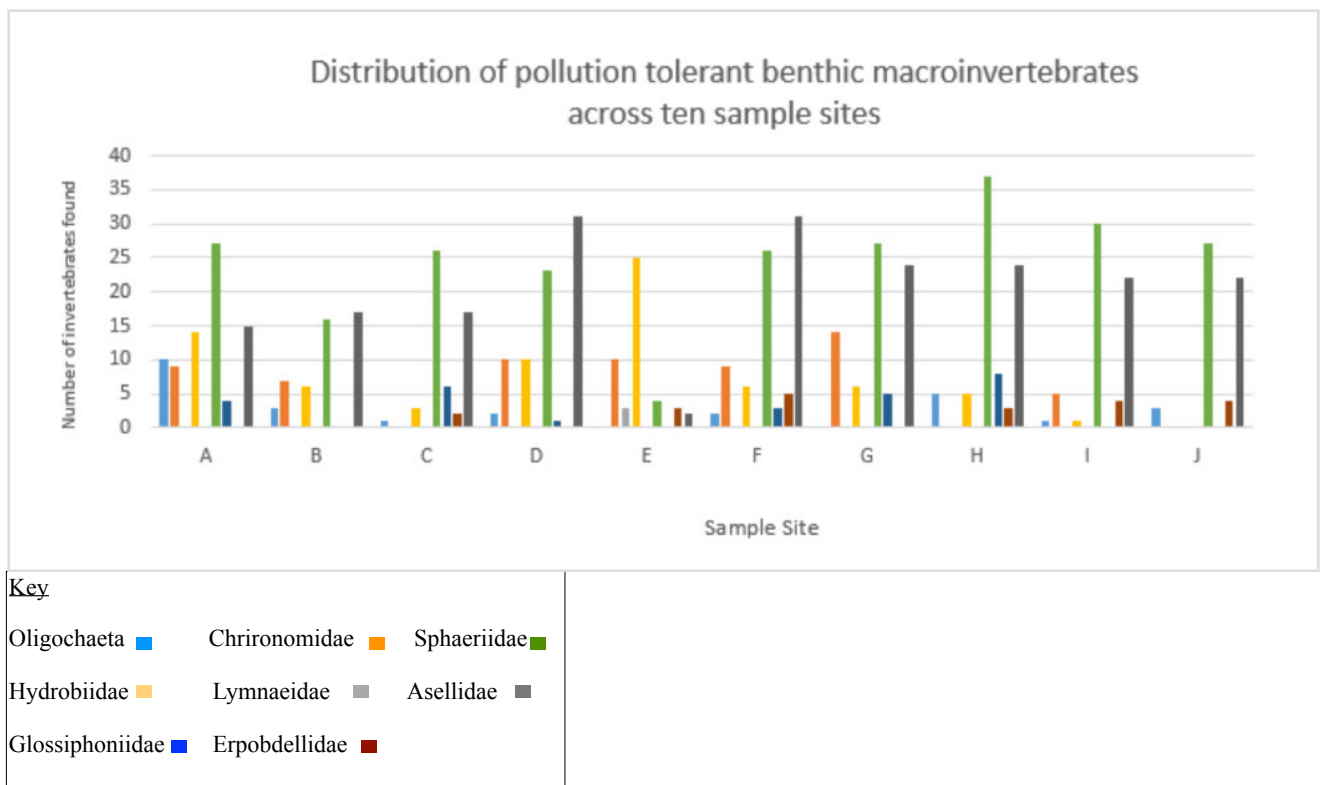


Figure 25. Histogram displaying pollution tolerant (BMWP <3) macroinvertebrate distribution, across all ten sampling sites.

Pollution intolerant macroinvertebrates, with BMWP > 8, were plotted against sampling sites, shown in figure 26. The histogram shows there is no observational trend that site F contains fewer high BMWP scoring invertebrates. It in fact contains four macroinvertebrates with low tolerance.

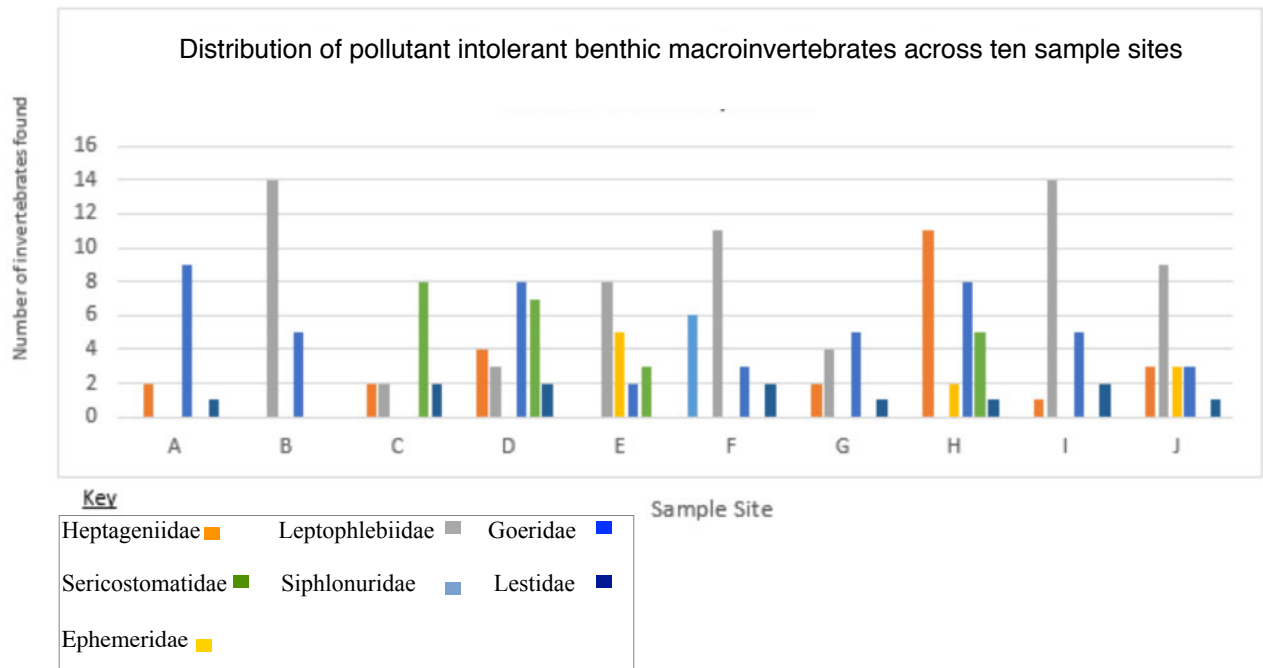


Figure 26. Histogram showing the distribution of shows pollution intolerant macroinvertebrates (BMWP score >8).

Further analysis was required to understand whether physiochemical parameters had an impact on benthic macroinvertebrates at site F. The following histograms display the sites in sequence of occurrence as the river moves downstream from site A-J, with sampling sites F and G located on separate tributaries downstream. This revealed that site F had the highest amount of total dissolved solids, as shown in figure 27. Whilst pH (figure 28), nitrogen (figure 29) and conductivity (figure 32) did not show any trends by observation, figure 30 showed site F had the second lowest phosphate reading of 0.34mg/l, compared to the next site, further downstream, located in the main channel, site G, which had 0.73mg/l.

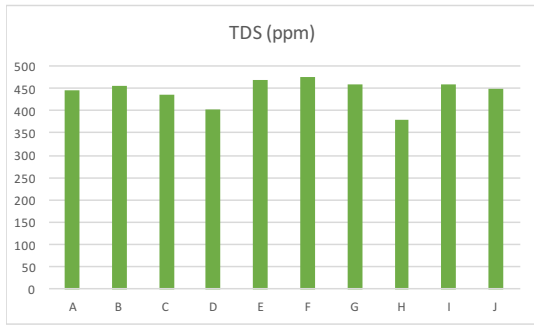


Figure 27. TDS results across all ten sites.

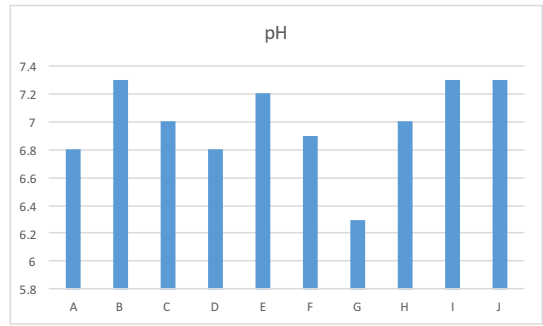


Figure 28. pH results across all ten sites.

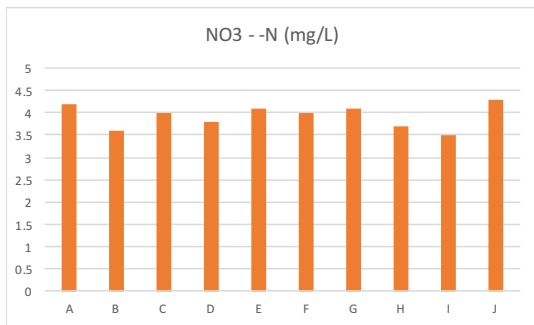


Figure 29. Nitrate results across all ten sites.

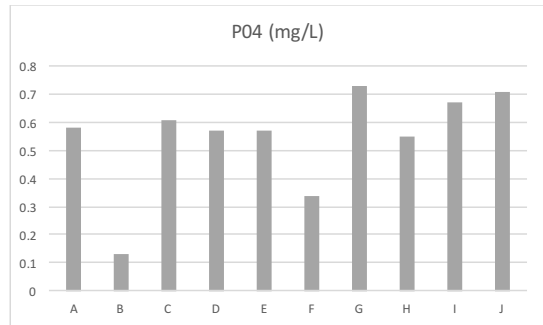


Figure 30. Phosphate measurements across all ten sites.

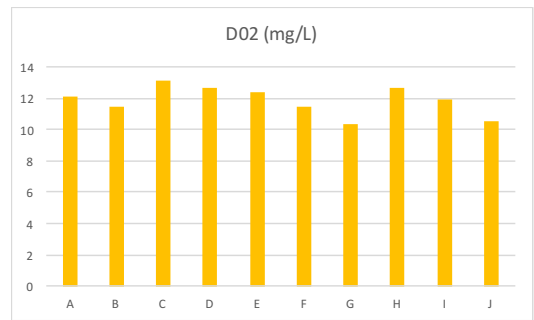


Figure 31. Dissolved oxygen levels across ten sites

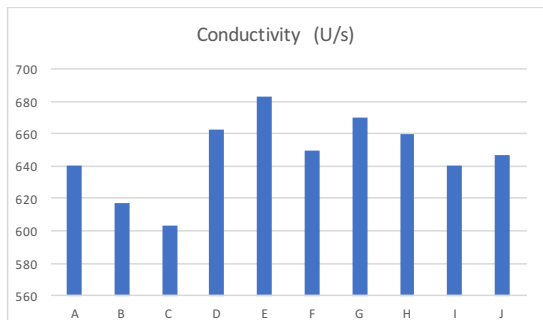


Figure 32. Conductivity measurements across all ten sites.

ENVIRONMENTAL AND MACROINVERTEBRATE METRIC CORRELATIONS

The PCA revealed three principal components, extracted from 8 different variables, with eigenvalues <0.5, as shown by the scree plot (Figure 33). Communalities showed that the variables were well represented (at least 0.65). The components accounted for 83.81% of total variance of variables in the study, with component one accounting for 41.73%, component two 23.70% and component three 18.40%. Table 9 displays the three components extracted by the PCA.

Table 9. Rotated loadings for 3 extracted components derived from 2017 sampling across 10 sampling locations. Absolute values above the value of 0.3 are shown. Those coefficients below were suppressed and present as “-”. Coefficients above the 0.5 value are considered of importance and highlighted in bold.

<u>Variables</u>	PC1	PC2	PC3
<u>Water quality parameters</u>	-	-	-
pH	-	0.896	-
Conductivity	0.706	-	0.354
D02	-	-0.848	-
TDS	-0.602	0.667	-
Nitrates	-	-	0.984
Phosphates	-	-	0.919
<u>Macroinvertebrate metrics</u>	-	-	-
ASPT	0.933	-0.885	-
BMWP	0.870	-	-

The PCA results showed that for benthic macroinvertebrate metric data there was a strong correlation between BMWP and ASPT, and there were further correlations between the macroinvertebrate metrics BMWP, ASPT and the environmental variable conductivity. Additionally, with environmental variables, nitrates correlated with conductivity, pH levels were found to correlate with TDS, whilst phosphates correlated strongly with nitrate levels.

With four of the original variables correlating with the first principal component, this suggests they vary together. The first component can be seen as a measure of conductivity, TDS, ASPT and BMWP. The second principle component increases only with TDS, with the no increases for the third component.

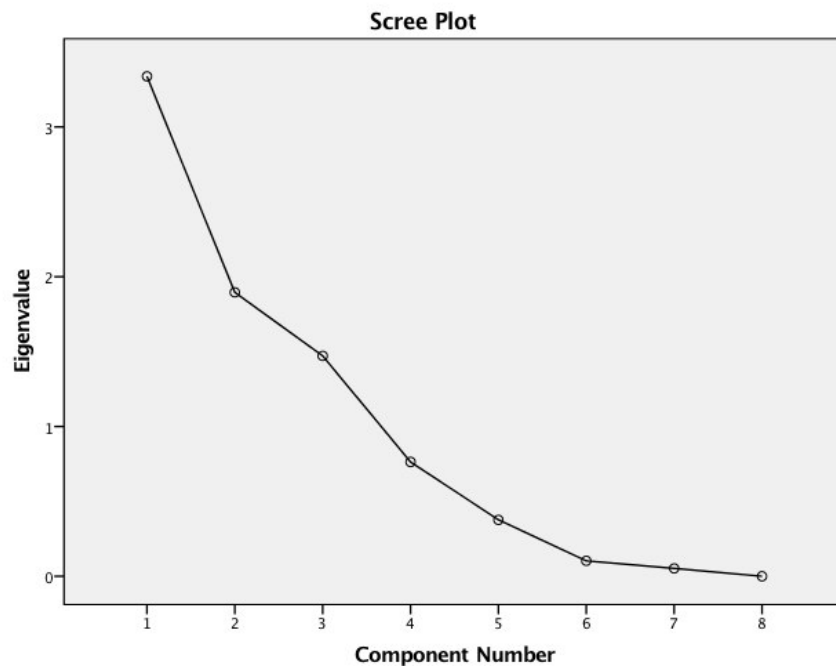


Figure 33. Scree plot of 2017 PCA data. The scree plot displays that each factor's eigenvalue may be compared to 1 to see how much more (or less) variance it represents than does a single variable.

Figure 34 displays pH as the strongest component 2 factor (0.896), followed by TDS (0.667). It also shows conductivity, ASPT and BMWP are clustered to component 1, with nitrates and phosphates clustered by component 3.

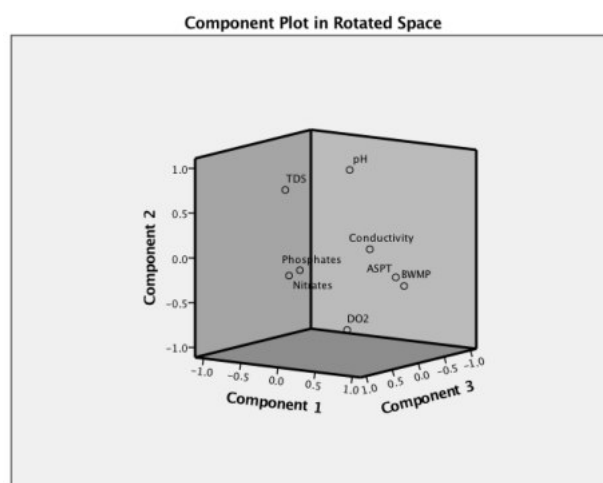


Figure 34. Component Plot in Rotated Space of environmental variable PCA.

PCA ANALYSIS ON BENTHIC MACROINVERTEBRATE AND ENVIRONMENTAL VARIABLES

Principal component analysis was performed on all benthic macroinvertebrate and environmental variables across all ten sampling locations. The analysis yielded 9 components when using eigenvalues >1 with Kaiser normalisation, figure 35 represents these 9 factors, with components 1 and 2 account for 44% of data variation. The remaining 66% were spread throughout the remaining 7 components. Two components were then forced to simplify the component plot in figure 36. This involves extracting only the first two components which account for large amounts of data variation in themselves and can provide a more comprehensive view of correlations, without the noise that is associated with a high number of components displayed in a component plot.

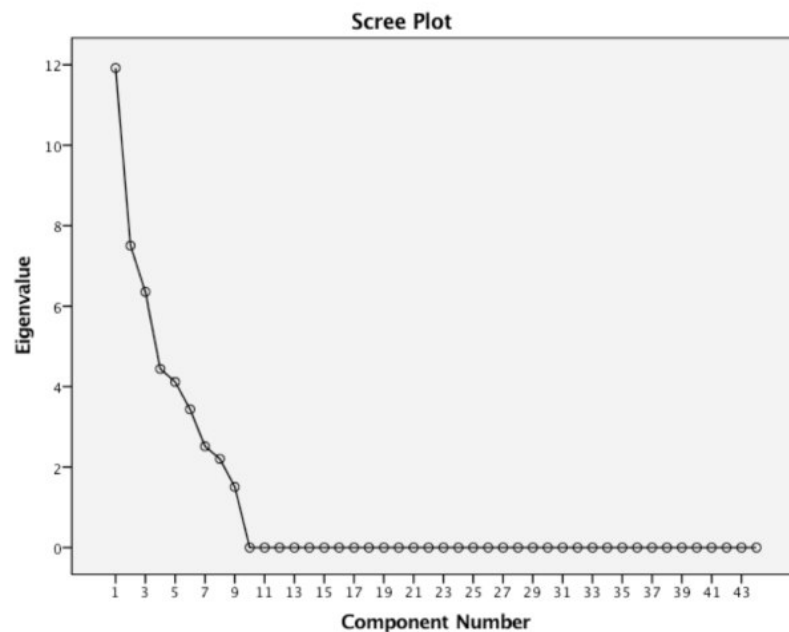


Figure 35. Scree plot of PCA on macroinvertebrate and environmental variables, measured across all ten sampling locations.

36 overall correlations were established, with 28 variables correlated with component 1 (, whilst 17 correlated with component 2. Additionally, the component 1 correlations were stronger, with the highest 0.977, showing Leptoceridae, Hydropsychidae and Pleidae, with these variables almost completely represented by component 1. Based on this, component 1 could be described as primarily a measure of these three macroinvertebrates. Correlations above 0.5 are considered of importance, with these figures in bold in table 10. The first principal component, shown in table 10, is strongly correlated with 18 of the original components, with the highest TDS negatively correlated (-0.948) with component 1. The greatest correlation between the original components and component 2 was that of Sialidae at 0.962. The first principle component increases 14 of the original benthic macroinvertebrate components, and just one environmental original component, TDS, This suggesting that these fifteen criteria vary together. The second principal component increases with only two of the values, increasing with Piscicolidae and Corixidae .

Table 10. Rotated loadings for 2 extracted components derived from 2017 sampling across 10 sampling locations. Absolute values above the value of 0.3 are shown. Those coefficients below were suppressed and present as “-”. Coefficients above the 0.5 value are considered of importance and highlighted in bold.

	PC1	PC2
TDS	-0.948	
Simuliidae	0.858	
Ephemeroidea	0.834	
Gammaridae	0.834	
Polycentropodidae	0.794	
Goeridae	0.790	0.430
Hydropsychidae	0.782	
Leptoceridae	0.782	
Pleidae	0.782	

Unionidae	0.730	
Sericostomatidae	0.718	
Dytiscidae	0.711	
Glossiphoniidae	0.682	
Sphaeriidae	0.668	
Asellidae	0.589	
Psychomyiidae	0.418	0.381
Oxygen	0.376	
Lymnaeidae	-0.371	
Chironomidae	-0.366	
Heptageniidae	0.362	-0.338
Siphonuridae	-0.336	
Oligochaeta	-0.330	
Hydrobiidae		
Gyrinidae		
Calopterygidae		
pH		
Caenidae		
Sialidae		0.962
Phosphates		-0.771
Viviparidae		0.763
Notonectidae		0.742
Piscicolidae	0.562	0.719
Corixidae	0.512	0.708
Baetidae	0.491	0.679
Leptophlebiidae	-0.502	0.655
Limnephilidae		-0.653
Erpobdellidae		-0.635
Nitrates		-0.581
Lestidae	0.442	-0.552
Coenagrionidae		-0.440
Mesoveliidae		-0.328
Philopotamidae		
Hydrophilidae		
Conductivity		

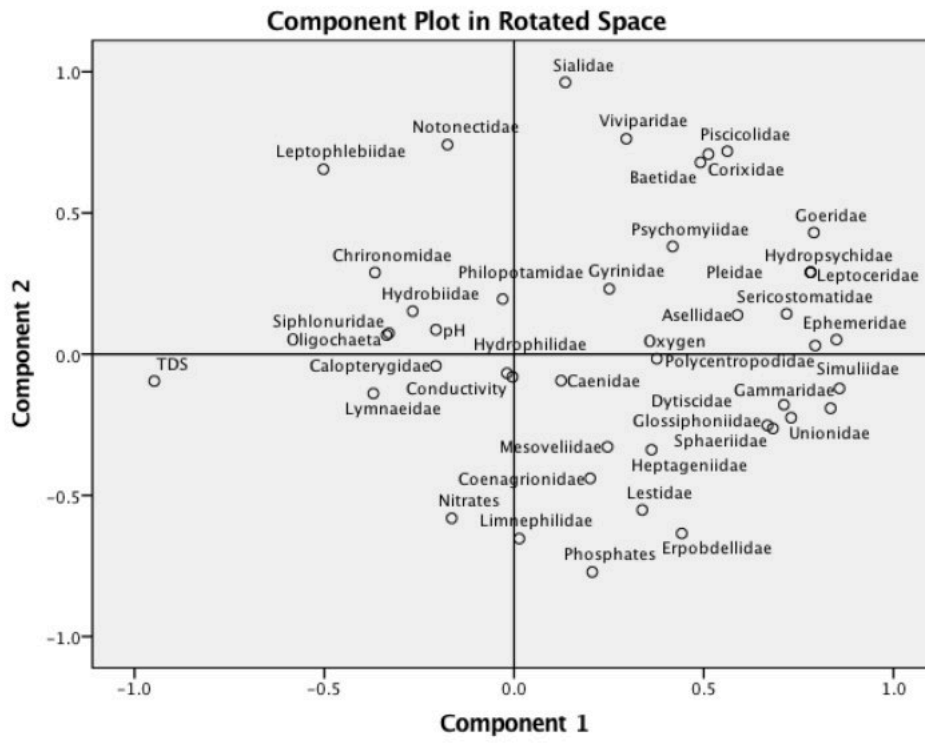


Figure 36. Component plot in rotated space showing all macroinvertebrates and environmental variables.

COMPARISONS BETWEEN THE INVERTEBRATE COMMUNITIES OF DIFFERENT SITES, USING THE JACCARD SIMILARITY COEFFICIENT

Hierarchical cluster analysis was performed on all benthic macroinvertebrate variables to compare the similarity and diversity of invertebrates across the ten sample sites. A visual representation of the Jaccard distance at which clusters are combined is displayed in the dendrogram, Figure 33, where vertical lines show joined clusters.

At Stage 1, Case 1 is clustered with Case 2. The Jaccard similarity between these two cases is 0.774. Neither variable has been previously clustered, with the next stage of cluster 1 combining with a case at Stage 4. As we move horizontally along the dendrogram, the compound clusters get bigger and differences between the compound clusters increases. Highly correlated clusters (with a correlation value close to 1) are highlighted in the dendrogram in green, which show the most similarity. The dendrogram indicates that, after the two clusters highlighted, a distance of at least 10.0 is required to move between compounds, increasing up to 25.00. This shows there is variety in individual abundance behaviour and primarily dissimilarity between invertebrate communities across the ten sample sites.

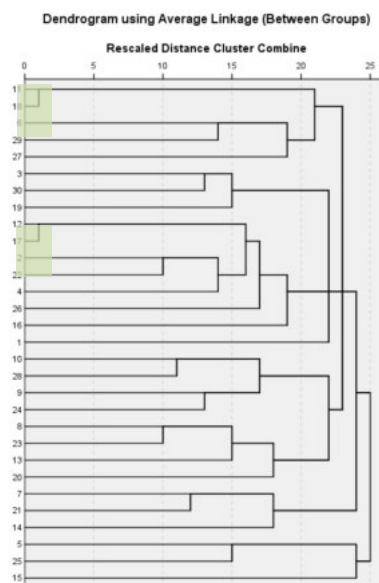


Figure 37. Dendrogram showing jaccard similarity coefficients between invertebrate communities across sites A-J.

**MULTIDIMENSIONAL SCALING ON MACROINVERTEBRATE COMMUNITIES
ACROSS THE 10 SAMPLE SITES**

To reduce the complexity of the dataset and to obtain a quantitative estimate of similarity amongst the benthic macroinvertebrate communities, multidimensional scaling (PROXSCAL) was undertaken. The statistical test was run containing all macroinvertebrate family data, across all ten sampling locations. Figure 38. Shows the dissimilarity of Site F, as it is isolated between 1.5 and 2.0 of Dimension 1, further confirming the previous result of the PCA in Figure 22. The plot also shows the clumping of the remaining 10 sampling sites and their macroinvertebrate communities pulled towards Dimension 2.

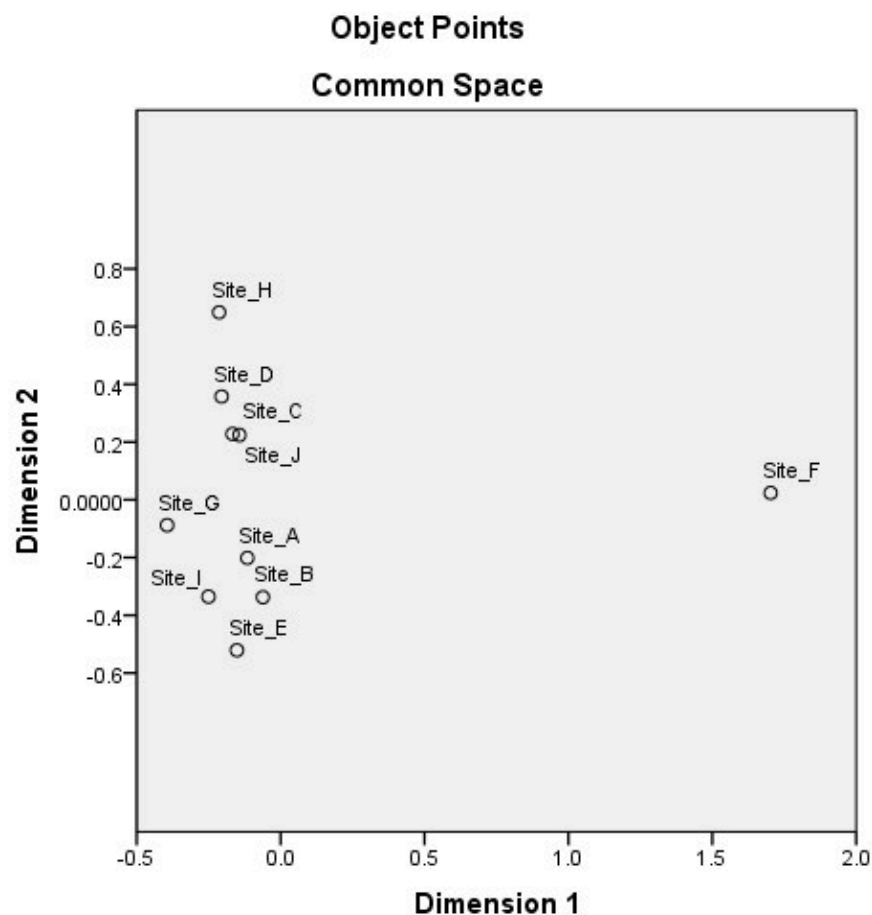


Figure 38. Multidimensional scaling plot showing site F as a point of interest, grouped between 1.5-2.0, compared to the other sites which cluster between -0.5 and 0.0.

CANONICAL CORRESPONDENCE ANALYSIS (CCA) ON MACROINVERTEBRATE COMMUNITIES AND ENVIRONMENTAL VARIABLES ACROSS TEN SAMPLING LOCATIONS

The permutation test, with a p value of 0.437 means that the null hypothesis should be accepted. This states that the species data is not linearly related to the environmental variables, or to the sites. It should be noted however, that the constrained Canonical Correspondence Analysis corresponds to only 25.5% of the inertia, suggesting that site/species correlations should not be analysed in depth through CCA.

The CCA produced map, shown in figure 39, allows for the clear delineation of variables whilst showing possible variable interrelation. From this map, *lymnaeidae*, *leptophlebiidae*, *pleidae*, *hydropsychidae*, *dytiscidae* and *piscicolidae* are clear outliers. It shows that *hydrobiidae* and *lestidae* abundance is influenced by conductivity. It also demonstrates *ephemeridae*, *mesoveliidae* and *caenidae* abundance could be influenced by nitrate and dissolved oxygen content. *Sphaeriidae*, *philopotamidae*, *oligochaeta*, *unionidae* and *gammaridae* all seem to be most sensitive to pH change. There seems to be no significant relationships between species abundance and TDS or phosphate.

DISCUSSION

The current study acted as a comparative analysis of environmental factors (pH and phosphate were the only available datasets) and benthic macroinvertebrates from 2015.

This study also assessed a further ten sampling locations that can be used as baseline data for further research. Additionally, benthic macroinvertebrate data was available from three sites spot-sampled in 2011 (pre-restoration work). Comparisons were made across these three sites from 2011 and 2015 data, and replicated in the current study to assess if there are any general trends or improvements in river water quality.

Comparative Analysis across three sites in 2011, 2015 and 2017

Benthic macroinvertebrates

The results of the study showed an increase of benthic macroinvertebrates from 2011 – 2017. An additional 16 families were found since pre-restoration sampling in 2011, which suggests a general improvement in river health. The presence of *Pleidae* (BMWP tolerance value 5) in this study - a family absent in 2011 and 2015, indicates that water flow has slowed since previous samplings, as this invertebrate prefers slow moving or still water. Their affinity for riparian vegetation could also suggest intensified plant growth, in line with Ecke et al., (2016) who stated that restorative works increase aquatic vegetation prevalence.

The mayfly family *Heptageniidae* (tolerance value 4) was also collected during sampling. This family is commonly found living under pebbles or branches in the currents of streams (clingers). Its presence in this study implicates the addition of large woody debris (an in-stream restorative technique) as a possible success story in habitat fabrication. The order Hemiptera (to which *Heptageniidae* belongs) was not present in any sample from 2011, further suggesting restorative work incited colonisation.

Benthic macroinvertebrate metrics

Sites E, G and J retained their ASPT values. Though scores remained consistent for sites G and J, it is worth noting that these sites are located in a different channel, in separate tributaries.

BMWP scores across the three 2011 sampling locations and six 2015 locations were replicated in the current study and showed improved scores across all sites except G and J, which remained classified as 'good'. Hensen (2000) suggests tributary exclusion because sampling results can be uncharacteristic of primary channels, meaning that the data is not entirely representative. It is advised that any further study omits these locations if assessing general channel health.

Abiotic variables

Abiotic variable comparisons between 2015 data and the current study showed a significant increase in pH. Point source pollution is a common cause of lowered pH values (Awasthi & Tiwari, 2004). The adoption of a 're-wilding concept' at the sampling sites halted agricultural runoff (which may have lowered pH previously), allowing for restabilization.

Since 2015, phosphate levels have increased by 0.33mg/l (over double that of 2015). The increased phosphate levels could be due to grazing pressures or faecal runoff from free-ranging livestock and deer present on the Knepp Castle Estate, or from bank erosion through entering/exiting the river. Increased phosphate levels may benefit the Adur, as phosphates are essential for the growth of aquatic organisms, and low levels of phosphorus limit the production of freshwater systems (Ricklefs, 1993).

To ensure that phosphate levels remain at a desirable level, they should be monitored. Excessive phosphate levels contribute to algae growth, culminating in eutrophication and

organismal death (hypoxia). Appropriate action should be taken if future levels rise significantly (i.e. utilising internal fencing).

Current study – Analysis across ten sampling locations

Percent EPT

The current 2017 study found EPT scores ranging from fair to excellent. Clark and Montemarano (2017) explain that short-term colonies can take multiple years to recover after the disturbance of restorative work, which may explain the observed results. Additionally, the length of time before accurate observations of river quality can be made may extend beyond the four years that have passed since the restorations.

Biotic Indices

Biotic indices are based on the idea that pollution tolerance for various benthic organisms is different (Resh, et al., 1996). The FBI quality showed rankings from fairly poor to fair. Whilst this is indicative of poor river quality, the index can require adjustments for different world areas, something not undertaken during this study, but something to be considered for future research.

Examining patterns of taxon richness, diversity and evenness

Diversity

The Shannon Diversity Index indicated that in this study, site B was most diverse. Figures between all locations showed little disparity, though, with the range between site B and Site J (the least diverse site) being 0.784. The change in diversity could be a product of shifting water composition from site to site. For example, higher levels of riverine phosphate have previously been implicated as an indicator of poor water quality, and in turn, a harsher environment for invertebrate species to thrive in (Takhelmayum et al., 2013). Site J had a

mean phosphate level of 0.71 over the three sampling time points conducted in May, June and July, whereas site B – the most diverse — had a mean phosphate level of 0.26, supporting the aforementioned claim. The second most diverse site — F, also had a similarly low phosphate level to that of site B. Nitrates, too, could explain the diversity inconsistencies. It is well documented that nitrates are a primary cause of eutrophication, which in turn attenuates the dissolved oxygen content of the water, creating an anoxic and inhospitable environment (McIsaac et al., 2001, Howden et al., 2013). The mean nitrate level of site B was 3.27, whereas in site J it was 4.33 – this coupled with the dissolved oxygen content at the two locations (Site B – 11.47, Site J – 10.5) further supports the notion of eutrophication inciting hypoxia. It should be noted that this is a baseline data set, and further research examining water composition in years to come will aid in extricating whether this is the primary cause of diversity fluctuation along the River Adur.

Evenness

The species evenness follows relative suit, except in sites D & H. Site J had the lowest evenness score (0.545), and site B the third highest (0.758) after locations D and H which both had an evenness score of 1, suggesting complete group frequency equity. Interestingly, sites D and H both follow large meanders in the river. There is evidence suggesting that riverine structural complexity and channel dynamics (e.g., sediment flux) play important roles in local diversity regulation, as well as the creation and preservation of heterogeneous habitat conditions. It is therefore not far-fetched to assume that the meandering is a causative factor in the elevated evenness scores seen at these sites (Downes et al., 1998, Harrison et al., 2011).

Principal Component Analysis

Benthic macroinvertebrates

The PCA showed a number of potential macroinvertebrates that could be used in the future to monitor water quality in the Knepp Castle Estate (those with values >0.5). These included *Simuliidae*, *Ephemeridae*, *Gammaridae*, *Polycentropodidae*, *Goeridae*, *Hydropsychidae*, *Leptoceridae*, *Pleidae*, *Unionidae*, *Sericostomatidae*, *Dytiscidae*, *Glossiphoniidae*, *Sphaeriidae*, and *Asellidae*. The BMWP tolerance values of these families range from 3-10. However, 4 of these 14 invertebrates have a tolerance value of 10, suggesting that the families of *Ephemeridae*, *Goeridae*, *Leptoceridae* and *Sericostomatidae* can be used to confirm good water quality, as their numbers and species diversity decrease as pollution increases (Plafkin et al. 1989). Also, the use of the most tolerant taxa in PCA - *Glossiphoniidae*, *Sphaeriidae*, and *Asellidae* can be used to detect pollution increases.

The order of Trichoptera (caddisfly larvae) was most prevalent. Although the range of pollution tolerance is wide within this order, generally high densities of caddisflies suggest that water is relatively unpolluted (Bonada, 2006). Their presence is also advantageous to overall river health, with collecting-gathering and filtering caddisflies breaking down particulate nutrients, allowing other invertebrates/vertebrates in the habitat to benefit. It is suggested by Buss and Borges (2008) that if abiotic chemical sampling is not feasible, this order can be used to detect the presence of pollution.

Additionally, *Piscicolidae* and *Corrixidae* both showed importance (>0.5 values) in both components 1 and 2, indicating their importance. The BMWP scores for these species are 4 and 5 respectively, both moderately tolerant to pollution, these families were found in their highest abundance downstream of re-meandered areas. The families of *Sialidae*, *Viviparidae*, *Notonectidae*, *Baetidae*, *Leptophlebiidae*, *Limnephilidae*, *Erpobdellidae* and *Lestidae*,

showed significant importance (values >0.7) representative of component 2. The *Erpobdellidae* family is very tolerant to pollution and low dissolved oxygen (Pliūraitė and Mickėnienė 2009; Paisley et al. 2003; Muñoz and Prat 1996). Marques et al. (1999), stated that *Erpobdellidae* does not have specific habitat requirements but tends to favour high organic loading and low oxygen levels (Miserendino et al. 2008). However, the presence of the damselfly *Lestidae* (tolerance value 10) contradicts interpretations of a low water quality. Additionally, this family can be used to assess not only pollution but also the water flow, as females tend to breed in slow moving or still waters.

Site F irregularities

PCA revealed site F as a point of importance, through its outlying position on the rotation plot (see fig. 22). Further analysis was undertaken to evaluate this. The Shannon Diversity Index revealed that site F is the second most diverse site in the study. With high abundance of both pollution tolerant invertebrates (*Oligochaeta* - BMWP score of 1), and intolerant (*Leptophlebiidae* - BMWP score of 10), this contradiction of invertebrate communities could have highlighted site F. Additionally, the absence *Coenagrionidae*, which did not occur at any other sample site, could suggest that Site F could contain a higher level of pollution. Patrick et al., (1994) suggest that bodies of water where *Coenagrionidae* are found are less likely influenced by human impact or outside disturbances, suggesting this is not applicable at Site F. The location of the site is downstream of re-meandering works, on a separate tributary, reiterating Hensen's (2000) view that locations outside of the main channel should be excluded to retain validity and consistency in the dataset.

Significant Abiotic variables

TDS showing a -0.948 correlation with component one, is in line with the view of Samborn (2008) - that TDS is the most common parameter for water quality testing, with low levels

detrimental to aquatic life, owing to the flow of these dissolved solids in and out of an organism's cells. During future monitoring, TDS levels should be tested. Nitrate levels were also found, to a lesser extent, to be deemed important through PCA, and future testing of this variable is also suggested.

Limitations and recommendations

Seasonality

Seasonality was addressed to ensure a reliable and valid dataset that could be used as a baseline for future studies. There was a significant difference in ASPT score across the sampling months of May, June and July, to mitigate this, a study with longer duration is recommended, as suggested by Arslan (2016). No significant differences were noted between the sampling months and number of families, Nitrogen and dissolved Oxygen levels. Pearson's correlation showed only a slightly insignificant result between seasonality and dissolved oxygen levels (see Fig. 25), which is understandable as the solubility of oxygen decreases as temperature increases (Romanescu and Stoleriu, 2013). Dissolved oxygen can also affect the solubility and availability of nutrients, which are released from sediments under conditions of low dissolved oxygen causing an influx of total dissolved solids available in the water (Melp et al. 1998). This suggests that in future studies, as echoed by Zamora-Munoz et al., (1994); a study of greater longevity would be beneficial.

Sampling method

The reliability of data must be addressed when using comparative datasets. The sampling methodology, though comparative in its protocol (kick-net sampling as described in methodology), did not reflect the time points utilised in the previous 2011 and 2015 study. Both these studies incorporated spot-sampling as their technique, whereas the current study used continuous monitoring over a 3-month period. The latter gathers a larger dataset, which

can be used in statistical analyses that would otherwise be skewed by small sample sizes. It also represents the river quality with more validity than spot-sampling, however, to compare these two techniques in terms of their results is a key limitation of the study. The continuous sampling methodology should be implemented where possible, over the same time frame, with samples taken in May, June and July in any future studies.

Biotic Indices

The ability of some indices to perform better than others, dependent on sampling location, is something that must be taken into account. Where stated in the current study, adaptations may be necessary for the indices to be directly applicable. When viewing the results of the FBI in particular within this study, it is recommended that this is considered.

Additionally, the use of the Proportion of Sediment-Sensitive Invertebrates (PSI) should be implemented in further studies. This would give a complete overview of the river system, and would assess whether the addition of different substrates (i.e. gravel) would be advantageous in any further river restoration at the Knepp Castle Estate. Similarly, calculation of the Community Conservation Index (CCI) would empirically summarise the rarity and richness of observed species with conservation initiatives in mind (Chad and Extance, 2004).

A multi-taxa approach

The current study assessed water quality with benthic macroinvertebrates as indicators of pollution. However, indication based on a single taxon can have limitations. Conversely, by applying a multi-taxa approach, a more robust reflection of freshwater health may be provided (Hawkes, 1997). It has been proposed that birds could be used to provide further monitoring, as well as amphibians, reptiles and fish (Welsh & Ollivier, 1998, Dallas, 2007). Additionally, mammals are extremely well represented in ecological research, and their

interactions with rivers could be studied to provide linkage between riverine and terrestrial environments (Veron et al., 2008).

Conclusions

As discussed by Friberg et al., (1998), the time-scale before changes associated with river restoration can be observed may exceed 10 years. To gather data that can be reliably compared, annual sampling of the sites visited during this study is suggested. By continuously monitoring these sites, and ensuring the same data collection protocols, this study can be used as a baseline and comparative dataset for assessing the ecological health of the River Adur at the Knepp Castle Estate in years to come.

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APPENDIX I

Table of data used in analysis for site specific conditions

	A	B	C	D	E	F	G	H	I	J
Total BMWP Score	87	91	90	111	101	95	101	118	75	105
Number of families	15	18	17	18	17	18	18	19	13	18
Average score per taxon (ASPT)	5.8	5.16	5.29	6.17	5.94	5.28	5.61	6.21	5.77	5.83
pH	6.8	7.4	6.9	6.6	7.2	6.9	5.9	7	7.2	7.5
NO3- (mg/L)	18.48	10.12	17.16	16.72	18.48	17.6	18.04	15.84	15.84	19.36
NO3 - N (mg/L)	4.2	2.3	3.9	3.8	4.2	4	4.1	3.6	3.6	4.4
P04 (mg/L)	0.63	0.07	0.66	0.57	0.5	0.4	0.92	0.53	0.16	0.76
D02 (mg/L)	11.4	11.4	13.1	12.6	12.3	11.2	9.5	12.5	11.7	9.6
Conductivity (U/s)	620	600	540	640	700	650	670	580	630	750
TDS (ppm)	430	441	422	411	463	480	462	387	450	444
Total BMWP Score	80	96	98	100	104	115	93	112	82	101
Number of families	14	18	18	18	17	18	17	19	15	17
Average score per taxon (ASPT)	5.71	5.33	5.44	5.56	6.12	6.39	5.47	5.89	5.47	5.94
pH	6.9	7.3	6.9	6.8	7.2	7	6.7	7.1	7.2	7.5
NO3- (mg/L)	18.04	14.08	17.16	16.72	18.92	17.16	18.48	15.84	16.28	19.36
NO3 - N (mg/L)	4.1	3.2	3.9	3.8	4.3	3.9	4.2	3.6	3.7	4.4
P04 (mg/L)	0.6	0.12	0.61	0.58	0.62	0.31	0.66	0.55	0.65	0.72
D02 (mg/L)	11.7	11.2	12.8	12.5	12.4	11	10.3	12.4	11.9	10.8
Conductivity (U/s)	650	620	630	680	680	660	670	710	640	580
TDS (ppm)	446	451	410	380	465	486	452	364	460	448
Total BMWP Score	73	81	101	92	83	78	89	134	86	87
Number of families	14	15	19	17	13	15	16	17	18	17
Average score per taxon (ASPT)	5.21	5.12	5.32	5.41	6.38	5.2	5.56	7.88	4.78	5.12
pH	6.8	7.2	7.1	6.9	7.2	6.9	0.4	7	7.5	7
NO3- (mg/L)	18.92	14.52	18.48	16.72	17.16	18.04	18.04	16.72	14.52	18.48
NO3 - N (mg/L)	4.3	3.3	4.2	3.8	3.9	4.1	4.1	3.8	3.3	4.2
P04 (mg/L)	0.5	0.21	0.56	0.57	0.58	0.3	0.62	0.58	0.71	0.65
D02 (mg/L)	13.2	11.8	13.5	13	12.5	12.2	11.3	13.2	12.3	11.1
Conductivity (U/s)	650	630	640	670	670	640	670	690	650	610
TDS (ppm)	459	474	472	420	479	460	463	393	468	453

APPENDIX II

BMWP Average Scores for each site

Site	A	B	C	D	E	F	G	H	I	J
BMWP	80	89.3	96.3	101	96	96	94.3	121.3	81	97.6
BMWP Score [May 2015]	x	53	x	x	80	77	84	54	x	112
BMWP Score [May 2011]	x	x	x	x	80	x	x	62	x	90

ASPT Average Scores for each site

Site	A	B	C	D	E	F	G	H	I	J
ASPT 2017	5.57	5.2	5.35	5.71	6.15	5.62	5.55	6.66	5.34	5.63
ASPT 2015	x	4.42	x	x	6.15	4.81	5.25	x	4.5	5.33

APPENDIX III

Current study descriptive statistics

May Sampling

Variable	N	Minimum	Maximum	Mean	Std. Deviation
BMWP	10	75.00	118.00	97.4000	12.49178
Number of families	10	12.00	19.00	17.0000	2.05480
ASPT	10	5.16	6.21	5.7060	.36731
pH	10	5.90	7.50	6.9400	.45753
N03	10	2.30	4.40	3.8100	.59151
P04	10	.07	.92	.5200	.25794
Conductivity	10	540.00	750.00	638.0000	59.96295
D02	10	9.50	13.10	11.5300	1.20927
TDS	10	387.00	480.00	439.0000	27.47524

June Sampling

Variable	N	Minimum	Maximum	Mean	Std. Deviation
BMWP	10	80.00	115.00	98.1000	11.26893
Number_of_families	10	14.00	19.00	17.1000	1.52388
ASPT	10	5.33	6.39	5.7320	.34415
pH	10	6.70	7.50	7.0600	.24585
N03	10	3.20	4.40	3.9100	.36040
P04	10	.12	.72	.5420	.18402
Conductivity	10	580.00	710.00	652.0000	36.75746
D02	10	10.30	12.80	11.7000	.84196
TDS	10	45.00	486.00	395.6000	129.08757

July Sampling

Variable	N	Minimum	Maximum	Mean	Std. Deviation
BMWP	10	73.00	134.00	90.4000	17.15420
Number_of_families	10	13.00	19.00	16.1000	1.85293
ASPT	10	4.78	7.88	5.5980	.90481
pH	10	.40	7.50	6.4000	2.11765
N03	10	3.30	4.30	3.9000	.35901
P04	10	.21	.71	.5280	.15583
Conductivity	10	610.00	690.00	652.0000	23.47576
D02	10	11.10	13.50	12.4100	.82792
TDS	10	393.00	4533.00	862.1000	1290.10546

APPENDIX V

Data from 2015 Ecosulis Survey

Knepp Adur Inverts Paper	Family	Family Value	1 to 4		5 and 6		7 to 9	
			OSRS	Tenchford Bridge	OSRS	Tenchford Bridge	OSRS	Bay Bridge
MOLLUSCA								
75. <i>Pisidium</i> sp.	Sphaeriidae		3	3	3	3	3	3
76. <i>Anodonta anatina</i> L.	Unionidae		6					6
GASTROPODA								
77. <i>Ancylus fluviatilis</i> Müller	Planorbidae		3	3	3	3	3	
78. <i>Lymnaea peregra</i> (O.F.M.)	Lymnaeidae		3	3	3	3		
79. <i>Bithynia leachi</i> (Sheppard)	Bithynia		3	3	3	3		
80. <i>Planorbis (Tropidiscus) planorbis</i> L.	Planorbidae		3	3	3	3	3	3
81. <i>Physa fontinalis</i> (L.)	Physidae		3	3	3	3	3	3
82. <i>Hydrobia ulvae</i> (Pennant)	Hydrobiidae		3	3	3	3	3	3
AMPHIPODA								
111. <i>Gammarus pulex</i> (L.)	Gammaridae		6	6	6	6	6	6
112. <i>Asellus aquaticus</i> Odenwall	Asellidae		3	3	3	3	3	3
113. <i>Asellus meridanius</i> Racovitz	Asellidae							
MEGALOPTERA								
116. <i>Sialis lutaria</i> (L.)	Sialidae		4	4	4	4	4	4
EPEMEROPTERA								
117. <i>Proclleon pennulatum</i> (Eaton)	Baetidae		4	4	4	4	4	4
118. <i>Proclleon</i> sp.	Baetidae							
119. <i>Proclleon</i> cf. <i>bifidum</i>	Baetidae							
120. <i>Cloeon dipterum</i> (L.)	Baetidae							
121. <i>Caenis horaria</i> (Linne)	Caenidae		7	7	7	7	7	7
122. <i>Caenis rivulorum</i> Eaton	Caenidae							
123. <i>Ephemera vulgata</i> L.	Ephemerae		10	10	10	10	10	10
TRICHOPTERA								
162. <i>Polycentropus flavomaculatus</i> (Pictet)	Polycentropodidae		7	7	7	7	7	7
163. <i>Cheumatopsyche lepida</i> (Pictet)	Hydropsychidae		5	5	5	5	5	5
164. <i>Notidobia ciliaris</i> (L.)	Sericostomatidae		10	10	10	10	10	10
165. <i>Limnephilus griseus</i> (Kolenat)	Limnephilidae		7	7	7	7	7	7
166. <i>L. subcentralis</i> Brauer	Limnephilidae							
167. <i>L. flavicornis</i> (Fabricius)	Limnephilidae							
168. <i>Cyrtus trimaculatus</i> (Curtis)	Polycentropodidae		10	10	10	10	10	10
169. <i>Atripodes bilineatus</i> (L.)	Leptoceridae							
170. <i>Hydropsyche angustipennis</i> Pictet	Hydropsychidae							
CHIRONOMIDAE	Chironomidae		2	2	2	2	2	2
SIMULIIDAE	Simuliidae		5	5	5	5	5	5
158. <i>Simulium reptans</i> L.	Simuliidae							
ODONATA								
124. <i>Platycnemis pennipes</i> (Pallas)	Platycnemidae		6	6	6	6	6	6
125. <i>Ischnura elegans</i> (Vander Linden)	Coenagrionidae		6	6	6	6	6	6
126. <i>Calopteryx splendens</i> (Harris)	Calopterygidae		8	8	8	8	8	8
COLEOPTERA								
127. <i>Platambus maculatus</i> L.	Dytiscidae		5	5	5	5	5	5
128. <i>Oulimnius</i> sp.	Emidae		5	5	5	5	5	5
129. <i>Elmis aenea</i> (Müller)	Emidae							
BMWP SCORE			134	62	62	62	90	
BMWP quality			Very Good	Fair	Fair	Fair	Good	
IBXB			25	13	13	12	17	
ASPT			5.36	4.77	4.77	5.17	5.29	
ASPT Quality			Fair	Poor	Poor	Fair	Fair	

APPENDIX VI

Shannon Diversity Index Calculations

	TEST/EQUIT	PI	Site F	PI	PI*(P/PI)	PI*(P/PI)
MOLLUSCA - Limpets & mussels	Sphaeriidae	1	-0.092221986	28	0.098939929	-0.228872038
	Unionidae	1	-0.092221986	15	0.067137809	-0.181139754
AMPHIPODA - Crustaceans	Aeolidae	1	-0.092221986	25	0.102793988	-0.231460109
	Gammaridae	1	-0.092221986	54	0.190821721	-0.316074184
GASTROPODA - Snails	Planorbidae	1	-0.092221986			
	Lymnaeidae	1	-0.092221986			
	Viviparidae	1	-0.092221986	2	0.007697138	-0.044998585
	Hydrobiidae	1	-0.092221986	14	0.049469965	-0.148525986
LEECHES	Piscicolidae	1	-0.092221986			
	Glossiphoniidae	1	-0.092221986	7	0.032349482	-0.011507976
	Epiplatidae	1	-0.092221986	3	0.01600707	-0.044819966
MEGALOPTERA - Alderflies	Stelidae	1	-0.092221986	11	0.038869258	-0.126229222
EPHEMEROPTERA - Mayflies	Baetidae	1	-0.092221986	15	0.067379809	-0.181139754
	Caenidae	1	-0.092221986			
	Ephemeridae	1	-0.092221986			
	Siphonuridae	1	-0.092221986			
	Leptophlebiidae	1	-0.092221986	11	0.038869258	-0.126229222
	Heptageniidae	1	-0.092221986			
TRICHOPTERA - Caddis flies	Goeridae	1	-0.092221986	3	0.01600707	-0.044819966
	Leptoceridae	1	-0.092221986			
	Polycentropodidae	1	-0.092221986	3	0.01600707	-0.044819966
	Sericostomatidae	1	-0.092221986			
	Philopotamidae	1	-0.092221986	5	0.017467845	-0.071307579
	Psychomyiidae	1	-0.092221986	8	0.02818851	-0.00805805
	Limnephilidae	1	-0.092221986	3	0.01600707	-0.044819966
	Hydropsychidae	1	-0.092221986	7	0.032349482	-0.011507976
DIPTERA - True flies	Simuliidae	1	-0.092221986	21	0.074208947	-0.193001462
	Chironomidae	1	-0.092221986	2	0.007697138	-0.044998585
ODONATA - Damselflies	Coenagrionidae	1	-0.092221986			
	Calopterygidae	1	-0.092221986	4	0.014134276	-0.000200036
	Lestidae	1	-0.092221986			
Coleoptera - Beetles	Dytiscidae	1	-0.092221986			
	Gyrinidae	1	-0.092221986			
	Hydrophilidae	1	-0.092221986	7	0.02734982	-0.011507976
HEMIPTERA - Bugs	Pseidae	1	-0.092221986			
	Notonectidae	1	-0.092221986			
	Corixidae	1	-0.092221986	12	0.042402827	-0.134015541
	Mesovellidae	1	-0.092221986	2	0.007697138	-0.044998585
	Nepidae (water scorpion)	1	-0.092221986			
Annelida - Worms	Oligochaeta	1	-0.092221986	9	0.031807312	-0.105460781
		SW Dvmax	3.68839454	SW Dv	2.78517495	
				even		0.75454654

APPENDIX VI CONT'D


Site B	PI	P1(NMPP)	Site J	PI	P1(NMPP)	Site G	PI	P1(NMPP)	PI	P1(NMPP)
	1E	0.0752072	-0.1855593	2C	0.02915459	-0.1036582	3E	0.1364159		-0.2729428
				3	0.0433378	-0.0237562	3	0.0386955		-0.04545876
	17	0.0765767	-0.13674976	1E	0.023332615	-0.08765684	42	0.13213913		-0.28657281
	37	0.1666667	-0.29362578	100	0.14577295	-0.28971571	6E	0.23913435		-0.344214843
	1C	0.0465045	-0.13664377	5	0.0728663	-0.03587052	5	0.03115942		-0.07262272
	6	0.0227027	-0.09793376				11	0.03855072		-0.12643304
	1C	0.0465045	-0.13664377	2	0.00291452	-0.01701942	6	0.0217913		-0.08231335
				2	0.00291452	-0.01701942	5	0.01811594		-0.07746272
				2	0.00291452	-0.01701942	3	0.01086955		-0.049145876
	1E	0.041081	-0.20770456	15	0.02186889	-0.08269521	4	0.01449754		-0.041363867
	17	0.0765767	-0.13674976	14	0.00408163	-0.07942484	13	0.04710149		-0.14931404
				14	0.00408163	-0.07942484	4	0.014442754		-0.041363862
							1	0.00623188		-0.029363771
	14	0.0503063	-0.17426246	13	0.01892047	-0.07515675	2	0.00724677		-0.035704737
	5	0.0252323	-0.08543331	1	0.01447726	-0.0092023	5	0.01811594		-0.07262272
	11	0.0495485	-0.1488856	2	0.00291452	-0.01701942	5	0.01811594		-0.07262272
				3	0.00437378	-0.0237562	5	0.01811594		-0.07262272
	4	0.04080818	-0.07232782	8	0.01166188	-0.051911791	1	0.002623188		-0.029363771
	3	0.01533514	-0.04816042	17	0.024781341	-0.09163388	3	0.01086955		-0.049145876
	1	0.0054045	-0.02432835	14	0.02042163	-0.07942484	6	0.0217913		-0.049145876
	1E	0.041081	-0.20770456	15	0.02796793	-0.09933249	2	0.00724677		-0.035704737
	7	0.0131332	-0.0899745	6	0.00746356	-0.04440013	12	0.04478261		-0.136325835
	1	0.0064045	-0.02432835	3E	0.052478134	-0.15471885	9	0.03268695		-0.11425314
	7	0.0153352	-0.0899745	1C	0.01457259	-0.04636017	6	0.01317913		-0.081331335
				1	0.01457259	-0.04636017	1	0.00623188		-0.029363771
	3	0.01533514	-0.04816042	5	0.00728663	-0.03597052	4	0.014442754		-0.041363862
							14	0.03974638		-0.13212751
	1	0.0064045	-0.02432835	13	0.01892047	-0.07515675	4	0.014442754		-0.041363862
	12	0.0505054	-0.13771737	4	0.00383004	-0.02999297	1	0.00623188		-0.029363771
	1	0.0064045	-0.02432835							
	3	0.01533514	-0.04816042	343	0.5	-0.4665789				
	SW DW	2.9468978		SW DW	2.0107715				SW DW	2.6027489
	Even	0.7572078		Even	0.5462824				Even	0.7281842

APPENDIX VI CONT'D

Site E	W	PT(NP9)	Site I	PI	P*(INP)	Site A	Site C	Site D	Site H	Site H				
4	0.0428514	-0.0000789	0.0774879	16	0.1323032	-0.7876219	0.1741805	-0.3498703	17	0.11523466	-0.34931171	3E	0.2802078	-0.15518
4	0.0428514	-0.0000789	0.0774879	4	0.0739732	-0.0712390	0.03488721	-0.1706224	6	0.0286055	-0.08300399	11		
2	0.0774879	-0.0327446	0.1544878	36	0.0564257	-0.0608393	0.0697942	-0.1877614	17	0.05451525	-0.3790167	36	0.0907078	-0.15518
10	0.1876743	-0.1071944	0.3094704	65	0.2812488	-0.1877614	0.1381393	-0.1877614	95	0.1779389	-0.3547129	116	0.1328395	-0.1662
3	0.0774879	0.0486013		2	0.0080329	-0.0378444			2	0.072022	0.0062922	6	0.0082939	-0.0304
2E	0.0838714	-0.2170687	0.0513282	4	0.0564257	-0.0608393	0.0382707	-0.0379716	3	0.044403	0.08119428	6	0.0323806	-0.0401
1	0.044403	0.044403	0.044403	11	0.044403	-0.1178178	0.03155814	-0.0379716	6	0.0286055	-0.2073425	1C	0.0347816	-0.0793
3	0.0774879	0.0486013	0.0774879	3	0.0774879	-0.0774879	0.0486013	-0.1706224	6	0.0382707	-0.0774879	2	0.0347816	-0.0793
1E	0.044403	-0.1421827	0.0241225	5	0.0241225	-0.0500815			4			11	0.0310387	-0.0582
1E	0.0774879	0.0774879	0.0774879	5	0.0774879	-0.0774879	0.0774879	-0.1706224	8	0.0512078	-0.0774879	25	0.0774879	-0.081
8	0.0838714	-0.2170687	0.0513282	2	0.008661	-0.0323845	0.03488721	-0.1706224	6	0.044403	0.08119428	1C	0.0323806	-0.0401
8	0.0838714	-0.2170687	0.0513282	15	0.0774879	-0.0608393	0.03155814	-0.0379716	2	0.0512078	-0.08119428	5	0.0082939	-0.0304
2	0.0774879	0.0486013	0.0774879	3	0.03488721	-0.1178178	0.03155814	-0.0379716	2	0.03155814	-0.0379716	8	0.0774879	-0.081
5	0.0774879	0.0486013	0.0774879	3	0.03488721	-0.1178178	0.03155814	-0.0379716	12	0.0486013	-0.1178178	1E	0.03488721	-0.1178178
1	0.044403	0.044403	0.044403	7	0.044403	-0.044403	0.044403	-0.044403	3	0.044403	-0.044403	6	0.044403	-0.044403
3	0.0774879	0.0486013	0.0774879	7	0.044403	-0.044403	0.044403	-0.044403	7	0.044403	-0.044403	6	0.044403	-0.044403
2	0.0774879	0.0486013	0.0774879	12	0.0381169	-0.3725295	0.0381169	-0.0877915	16	0.0381169	-0.0877915	2	0.0381169	-0.0877915
1	0.0377419	-0.2013429		3	0.0377419	-0.0377419	0.0377419	-0.0377419	2	0.0377419	-0.0377419	8	0.0377419	-0.0377419
14	0.0774879	-0.0774879	0.0774879	14	0.0774879	-0.0774879	0.0774879	-0.0774879	44	0.15884305	-0.20214707	65	0.1877679	-0.2382
1C	0.0774879	-0.1000704	0.1000704	21	0.0417004	-0.2214954	0.0417004	-0.1877614	3				0.0323806	-0.0401
2	0.0774879	0.0327446	0.0774879	6	0.0080329	-0.0378444	0.0382707	-0.0379716	9	0.0382707	-0.1113842	1C	0.0347816	-0.0793
1E	0.0428514	-0.1742488	0.0428514	3	0.0428514	-0.0428514	0.0428514	-0.0428514	7	0.0327446	-0.08300399	8	0.0347816	-0.0793
4	0.0428514	0.0000789	0.0428514	1	0.0000789	-0.0000789	0.0000789	-0.0000789	7	0.0000789	-0.0000789	6	0.0000789	-0.0000789
1E	0.0377419	-0.2679851		1E	0.0377419	-0.0377419	0.0377419	-0.0377419	8	0.0377419	-0.0377419	1	0.0377419	-0.0377419
9	0.0314837	-0.1104912		2	0.0314837	-0.0314837	0.0314837	-0.0314837	9	0.0314837	-0.0314837	14	0.0314837	-0.0314837
SW Div	2.1879761	2.1879761	2.1879761	SW Div	2.1879761	2.1879761	2.1879761	2.1879761	SW Div	2.1879761	2.1879761	SW Div	2.1879761	2.1879761
Even	0.67937951	0.67937951	0.67937951	Even	0.67937951	0.67937951	0.67937951	0.67937951	Even	0.67937951	0.67937951	Even	0.67937951	0.67937951

APPENDIX VII

CCA

XLSTAT 2017.4.46344 - Canonical Correspondence Analysis (CCA) - Start time: 29/08/2017 at 13:28:52							
Sites/Objects data: Workbook = DataAnalysis.xlsx / Sheet = Sheet2 / Range = Sheet2!\$A\$1:\$AM\$31 / 30 rw and 39 clms							
Sites/Variables data / Quantitative: Workbook = DataAnalysis.xlsx / Sheet = Sheet2 / Range = Sheet2!\$AO\$1:\$AU\$31 / 3							
Method: CCA							
Run again:							
Summary statistics:							
Variable	Observations	with missing	without missing	Minimum	Maximum	Mean	Std. deviation
sphaeriidae	30	0	30	1.000	16.000	8.100	3.863
unionidae	30	0	30	0.000	9.000	1.667	2.294
asellidae	30	0	30	0.000	17.000	6.833	5.072
gammaridae	30	0	30	4.000	49.000	25.633	10.223
planorbidae	30	0	30	0.000	0.000	0.000	0.000
lymnaeidae	30	0	30	0.000	3.000	0.100	0.548
viviparidae	30	0	30	0.000	6.000	1.267	1.461
hydrobiidae	30	0	30	0.000	13.000	2.533	3.550
piscicolidae	30	0	30	0.000	7.000	1.433	2.315
glossiphoniidae	30	0	30	0.000	6.000	0.900	1.729
erpobdellidae	30	0	30	0.000	3.000	0.700	1.055
sialidae	30	0	30	0.000	8.000	3.000	2.573
baetidae	30	0	30	0.000	12.000	4.133	3.491
caenidae	30	0	30	0.000	7.000	0.667	1.900
ephemeridae	30	0	30	0.000	4.000	1.033	1.542
siphonuridae	30	0	30	0.000	6.000	0.200	1.095
leptophlebiidae	30	0	30	0.000	13.000	2.167	3.281
heptageniidae	30	0	30	0.000	7.000	0.833	1.533
goeridae	30	0	30	0.000	6.000	1.600	2.127
leptoceridae	30	0	30	0.000	2.000	0.333	0.711
polycentropodidae	30	0	30	0.000	7.000	2.333	2.202
sericostomatidae	30	0	30	0.000	8.000	0.767	2.112
philopotamidae	30	0	30	0.000	6.000	1.533	1.814
psychomyiidae	30	0	30	0.000	8.000	1.200	1.990
limnephilidae	30	0	30	0.000	11.000	2.000	2.573
hydropsychidae	30	0	30	0.000	5.000	0.500	1.225
simuliidae	30	0	30	0.000	27.000	8.733	8.271
chironomidae	30	0	30	0.000	10.000	2.133	3.037
coenagrionidae	30	0	30	0.000	21.000	2.300	4.036
calopterygidae	30	0	30	0.000	11.000	2.167	2.627
lestidae	30	0	30	0.000	2.000	0.400	0.675
dytiscidae	30	0	30	0.000	3.000	0.567	1.073
gyrinidae	30	0	30	0.000	4.000	1.100	1.398
hydrophilidae	30	0	30	0.000	9.000	1.200	2.413
pleidae	30	0	30	0.000	1.000	0.033	0.183
notonectidae	30	0	30	0.000	1.000	0.033	0.183
corixidae	30	0	30	0.000	9.000	2.600	2.191
mesoveliidae	30	0	30	0.000	3.000	0.400	0.770
oligochaeta	30	0	30	0.000	7.000	0.900	1.539
month	30	0	30	1.000	3.000	2.000	0.830
ph	30	0	30	0.400	7.500	6.800	1.249
N	30	0	30	2.300	4.400	3.873	0.437
P	30	0	30	0.070	0.920	0.530	0.197
DO	30	0	30	9.500	13.500	11.880	1.018
cond	30	0	30	540.000	750.000	647.333	41.848
TDS	30	0	30	364.000	486.000	443.100	31.541

APPENDIX VII cont'd

Principal coordinates (Variables):							
	F1	F2	F3	F4	F5	F6	F7
month	0.045	0.056	-0.216	0.005	0.021	-0.015	0.00
ph	0.050	0.011	0.020	0.009	-0.045	-0.007	-0.0
N	0.041	0.133	0.025	0.113	-0.020	-0.032	0.0
P	0.156	0.133	0.031	0.033	-0.062	0.026	0.0
DO	0.089	0.107	-0.007	-0.068	0.094	-0.029	-0.0
cond	-0.160	0.130	-0.033	-0.021	-0.079	-0.038	0.0
TDS	-0.155	0.040	-0.078	0.086	0.013	0.064	-0.0
Standard coordinates (Variables):							
	F1	F2	F3	F4	F5	F6	F7
month	0.152	0.211	-0.944	0.032	0.149	-0.118	0.0
ph	0.168	0.041	0.089	0.056	-0.314	-0.058	-0.9
N	0.137	0.496	0.107	0.742	-0.143	-0.252	0.2
P	0.528	0.497	0.137	0.219	-0.432	0.199	0.4
DO	0.302	0.400	-0.031	-0.450	0.656	-0.228	-0.2
cond	-0.542	0.486	-0.143	-0.141	-0.551	-0.300	0.1
TDS	-0.524	0.149	-0.343	0.566	0.092	0.498	-0.0
Regression coefficients:							
	F1	F2	F3	F4	F5	F6	F7
month	0.388	-0.200	-1.023	0.072	-0.142	-0.287	0.0
ph	0.239	0.101	-0.059	0.145	-0.435	-0.065	-0.8
N	0.028	0.081	0.226	0.959	0.288	-0.973	0.0
P	0.585	0.584	0.036	-0.340	-0.441	0.972	0.2
DO	-0.041	0.894	0.264	-0.365	0.718	0.115	-0.2
cond	-0.628	0.561	-0.024	-0.442	-0.429	-0.255	0.0
TDS	-0.497	0.519	-0.044	0.222	0.307	0.950	-0.2

APPENDIX VII cont'd

Squared cosines (Objects):							
	F1	F2	F3	F4	F5	F6	F7
sphaeriidae	0.191	0.092	0.439	0.148	0.058	0.066	0.00
unionidae	0.451	0.095	0.027	0.029	0.106	0.043	0.24
asellidae	0.010	0.613	0.104	0.066	0.002	0.005	0.21
gammaridae	0.032	0.851	0.045	0.000	0.005	0.045	0.07
lymnaeidae	0.358	0.344	0.228	0.006	0.018	0.007	0.03
viviparidae	0.054	0.258	0.549	0.011	0.005	0.121	0.00
hydrobiidae	0.376	0.479	0.003	0.000	0.044	0.098	0.00
piscicolidae	0.039	0.820	0.004	0.004	0.099	0.026	0.00
glossiphoniidae	0.150	0.002	0.605	0.004	0.004	0.180	0.09
erpoobdellidae	0.062	0.283	0.363	0.001	0.130	0.012	0.14
sialidae	0.540	0.345	0.003	0.037	0.008	0.035	0.03
baetidae	0.585	0.301	0.034	0.001	0.011	0.065	0.00
caenidae	0.009	0.217	0.012	0.091	0.657	0.012	0.00
ephemeridae	0.028	0.395	0.002	0.245	0.084	0.130	0.11
siphonuridae	0.010	0.181	0.029	0.673	0.044	0.050	0.03
leptophlebiidae	0.838	0.119	0.024	0.000	0.008	0.000	0.03
heptageniidae	0.625	0.000	0.038	0.078	0.026	0.232	0.00
goeridae	0.042	0.496	0.247	0.103	0.102	0.002	0.00
leptoceridae	0.052	0.564	0.033	0.067	0.222	0.001	0.00
polycentropodidae	0.102	0.439	0.155	0.015	0.035	0.157	0.09
sericostomatidae	0.082	0.036	0.525	0.199	0.111	0.048	0.00
philopotamidae	0.040	0.008	0.115	0.239	0.196	0.288	0.11
psychomyiidae	0.221	0.116	0.215	0.209	0.020	0.218	0.00
limnephilidae	0.020	0.238	0.122	0.163	0.355	0.096	0.00
hydropsychidae	0.327	0.163	0.059	0.373	0.004	0.071	0.00
simuliidae	0.438	0.071	0.440	0.034	0.006	0.008	0.00
chrironomidae	0.579	0.057	0.015	0.072	0.003	0.042	0.23
coenagrionidae	0.538	0.125	0.162	0.112	0.053	0.008	0.00
calopterygidae	0.024	0.160	0.629	0.011	0.088	0.088	0.00
lestidae	0.433	0.475	0.000	0.029	0.023	0.001	0.03
dytiscidae	0.630	0.022	0.001	0.344	0.000	0.002	0.00
gyrinidae	0.006	0.365	0.022	0.312	0.245	0.042	0.00
hydrophilidae	0.182	0.589	0.091	0.001	0.093	0.034	0.03
pleidae	0.328	0.108	0.002	0.534	0.022	0.002	0.00
notonectidae	0.107	0.358	0.000	0.284	0.001	0.170	0.00
corixidae	0.118	0.481	0.122	0.145	0.028	0.101	0.00
mesoveliidae	0.007	0.148	0.645	0.059	0.052	0.038	0.09
oligochaeta	0.002	0.013	0.552	0.013	0.011	0.348	0.00

APPENDIX VII cont'd

Contributions (Objects):									
	F1	F2	F3	F4	F5	F6	F7		
sphaeriidae	0.010	0.006	0.040	0.030	0.013	0.019	0.00		
unionidae	0.007	0.002	0.001	0.002	0.007	0.004	0.00		
asellidae	0.001	0.058	0.013	0.019	0.001	0.002	0.11		
gammaridae	0.002	0.066	0.005	0.000	0.001	0.015	0.00		
lymnaeidae	0.034	0.040	0.036	0.002	0.007	0.004	0.00		
viviparidae	0.003	0.020	0.059	0.003	0.001	0.041	0.00		
hydrobiidae	0.069	0.107	0.001	0.000	0.034	0.096	0.00		
piscicolidae	0.005	0.124	0.001	0.002	0.052	0.017	0.00		
glossiphoniidae	0.014	0.000	0.091	0.001	0.002	0.086	0.00		
erpobdellidae	0.002	0.014	0.025	0.000	0.022	0.003	0.00		
sialidae	0.037	0.029	0.000	0.010	0.002	0.013	0.00		
baetidae	0.021	0.013	0.002	0.000	0.002	0.012	0.00		
caenidae	0.001	0.033	0.003	0.043	0.350	0.008	0.00		
ephemeridae	0.002	0.028	0.000	0.054	0.021	0.041	0.00		
siphonuridae	0.000	0.001	0.000	0.017	0.001	0.002	0.00		
leptophlebiidae	0.332	0.058	0.016	0.000	0.013	0.000	0.00		
heptageniidae	0.041	0.000	0.004	0.019	0.007	0.081	0.00		
goeridae	0.002	0.034	0.023	0.022	0.025	0.001	0.00		
leptoceridae	0.001	0.018	0.001	0.006	0.024	0.000	0.00		
polycentropodidae	0.007	0.038	0.018	0.004	0.010	0.059	0.00		
sericostomatidae	0.012	0.006	0.128	0.109	0.068	0.037	0.00		
philopotamidae	0.001	0.000	0.006	0.026	0.024	0.044	0.00		
psychomyiidae	0.009	0.006	0.015	0.032	0.004	0.047	0.00		
limnephilidae	0.001	0.016	0.012	0.035	0.085	0.029	0.00		
hydropsychidae	0.025	0.015	0.008	0.107	0.001	0.029	0.00		
simuliidae	0.092	0.018	0.155	0.027	0.006	0.008	0.00		
chironomidae	0.062	0.008	0.003	0.029	0.002	0.024	0.24		
coenagrionidae	0.099	0.028	0.050	0.078	0.042	0.008	0.00		
calopterygidae	0.004	0.031	0.169	0.007	0.060	0.075	0.00		
lestidae	0.008	0.011	0.000	0.002	0.002	0.000	0.00		
dytiscidae	0.050	0.002	0.000	0.103	0.000	0.001	0.00		
gyrinidae	0.000	0.017	0.001	0.044	0.039	0.008	0.00		
hydrophilidae	0.024	0.094	0.020	0.001	0.052	0.023	0.00		
pleidae	0.010	0.004	0.000	0.062	0.003	0.000	0.00		
notonectidae	0.008	0.031	0.000	0.076	0.000	0.064	0.00		
corixidae	0.003	0.013	0.005	0.012	0.003	0.012	0.00		
mesoveliidae	0.000	0.009	0.053	0.011	0.011	0.010	0.00		
oligochaeta	0.000	0.001	0.037	0.002	0.002	0.074	0.00		

APPENDIX VII cont'd

asellidae	-0.030	-0.238	-0.098	-0.078	0.012	-0.021	0.1
gammaridae	0.026	0.132	0.030	0.000	0.010	0.030	0.0
lymnaeidae	-1.673	1.638	1.335	0.216	0.376	0.241	-0.5
viviparidae	-0.149	-0.327	-0.478	-0.058	-0.047	0.224	-0.0
hydrobiidae	-0.474	0.535	0.040	0.000	0.161	-0.242	-0.0
piscicolidae	0.167	-0.763	-0.053	0.054	-0.255	-0.136	-0.0
glossiphoniidae	0.352	-0.036	0.707	-0.050	0.060	0.386	0.2
erpobdellidae	-0.171	0.366	-0.415	-0.024	-0.248	-0.076	-0.2
sialidae	-0.320	-0.256	-0.025	-0.033	0.038	-0.081	-0.0
baetidae	-0.205	-0.147	0.049	-0.010	0.028	0.068	0.0
caenidae	0.120	0.581	-0.139	-0.377	-1.010	0.137	-0.0
ephemeridae	0.114	0.429	0.032	0.338	0.198	0.247	-0.2
siphonuridae	0.053	0.222	-0.089	0.428	-0.109	-0.117	0.0
leptophlebiidae	-1.124	-0.424	0.191	-0.022	0.110	-0.014	-0.1
heptageniidae	0.638	-0.015	0.157	-0.226	0.130	-0.389	-0.0
goeridae	0.111	-0.381	0.268	-0.174	-0.173	-0.026	-0.0
leptoceridae	-0.181	0.597	0.145	0.206	-0.375	-0.020	-0.1
polycentropodidae	0.160	-0.331	-0.197	-0.051	0.093	0.198	-0.1
sericostomatidae	0.358	0.238	0.906	-0.558	0.416	-0.273	0.0
philopotamidae	0.079	-0.035	-0.134	0.192	-0.174	-0.211	-0.1
psychomyiidae	-0.249	-0.180	0.246	0.242	-0.075	0.247	0.0
limnephilidae	-0.058	0.236	0.169	0.195	-0.238	-0.150	-0.0
hydropsychidae	0.639	-0.452	-0.272	0.682	0.074	-0.298	-0.0
simuliidae	0.295	-0.119	0.295	0.082	0.036	0.039	-0.0
chironomidae	-0.491	-0.155	-0.079	0.174	0.038	-0.132	0.3
coenagrionidae	0.595	0.287	-0.327	-0.271	0.187	-0.074	-0.0
calopterygidae	-0.121	0.313	-0.620	0.083	0.232	0.231	0.0
lestidae	-0.403	0.422	-0.011	0.104	0.092	-0.014	-0.1
dytiscidae	0.853	-0.159	-0.030	0.630	0.017	-0.052	0.0
gyrinidae	-0.041	0.320	-0.079	-0.236	-0.253	0.109	0.0
hydrophilidae	-0.404	0.727	0.285	0.032	-0.290	0.174	-0.0
pleidae	1.578	-0.905	-0.137	2.013	-0.407	0.130	-0.1
notonectidae	-1.357	-2.506	0.039	-2.234	0.095	1.726	-1.1
corixidae	-0.091	-0.185	-0.093	0.101	-0.045	-0.085	-0.0
mesoveliidae	0.085	0.389	-0.811	0.245	0.230	0.196	0.2
oligochaeta	0.025	0.070	-0.451	-0.059	0.064	-0.358	-0.1
Standard coordinates (Objects):							
	F1	F2	F3	F4	F5	F6	F7
sphaeriidae	0.345	-0.265	-0.677	-0.592	-0.394	-0.468	0.1
unionidae	0.648	0.329	0.205	0.321	0.647	0.461	-1.5
asellidae	-0.101	-0.890	-0.429	-0.514	0.085	-0.152	1.4
gammaridae	0.086	0.492	0.132	0.001	0.068	0.236	0.2
lymnaeidae	-5.647	6.115	5.832	1.419	2.616	-1.881	-5.7
viviparidae	-0.504	-1.222	-2.088	-0.445	-0.330	1.746	-0.3
hydrobiidae	-1.600	1.996	0.173	0.001	1.123	-1.887	-0.2
piscicolidae	0.565	-2.847	-0.233	0.358	-1.846	-1.050	-0.7
glossiphoniidae	1.189	-0.135	3.088	-0.392	0.421	3.006	2.2
erpobdellidae	-0.578	1.368	-1.815	-0.158	-1.729	-0.596	-2.7
sialidae	-1.081	-0.955	-0.111	-0.547	0.267	-0.634	-0.8
baetidae	-0.692	-0.549	0.215	-0.057	0.193	0.532	0.1
caenidae	0.406	2.170	-0.607	-2.475	-7.030	1.064	-0.3
ephemeridae	0.386	1.603	0.141	2.220	1.375	1.922	-2.4
siphonuridae	0.179	0.830	-0.390	2.813	-0.752	-0.915	0.6
leptophlebiidae	-3.794	-1.583	0.834	-0.142	0.765	-0.113	-1.3
heptageniidae	2.155	-0.056	0.687	-1.433	0.903	-3.031	-0.2
goeridae	0.376	-1.421	1.173	-1.141	-1.203	-0.202	-0.4
leptoceridae	-0.611	2.228	0.635	1.350	-2.606	-0.155	-2.0
polycentropodidae	0.539	-1.235	-0.859	-0.399	0.646	1.540	-1.6
sericostomatidae	1.210	0.887	3.961	-3.654	2.896	-2.128	0.1
philopotamidae	0.267	-0.132	-0.584	1.264	-1.213	-1.647	-1.3
psychomyiidae	-0.840	-0.672	1.073	1.590	-0.525	1.929	0.1
limnephilidae	-0.230	0.831	0.739	1.281	-2.004	-1.171	-0.4
hydropsychidae	2.158	-1.686	-1.189	4.478	0.514	-2.326	-0.4
simuliidae	0.994	-0.443	1.291	0.536	0.249	0.302	-0.2
chironomidae	-1.659	-0.578	-0.345	1.140	0.263	-1.031	3.2
coenagrionidae	2.010	1.072	-1.429	-1.783	1.304	-0.578	-0.3
calopterygidae	-0.408	1.168	-2.709	0.547	1.615	1.803	0.0
lestidae	-1.360	1.575	-0.049	0.681	0.642	-0.112	-1.2
dytiscidae	2.878	-0.594	-0.132	4.139	0.121	-0.409	0.1
gyrinidae	-0.138	1.196	-0.344	-1.945	-1.827	0.848	0.5
hydrophilidae	-1.355	2.714	1.247	0.208	-2.014	1.354	1.0
pleidae	5.326	-3.377	-0.599	13.221	-2.829	1.014	-1.8
notonectidae	-4.616	-9.353	0.169	-14.674	0.660	13.459	-12.5
corixidae	-0.309	-0.690	-0.407	0.665	-0.312	-0.651	-0.1
mesoveliidae	0.285	1.452	-3.544	1.608	1.601	1.527	2.4
oligochaeta	0.085	0.260	-1.970	-0.454	0.443	-2.790	-1.5

APPENDIX VII cont'd

Squared cosines (Sites):								
	F1	F2	F3	F4	F5	F6	F7	
1	0.028	0.395	0.047	0.474	0.028	0.025	0.00	
2	0.215	0.692	0.072	0.002	0.002	0.003	0.00	
3	0.757	0.096	0.013	0.069	0.028	0.037	0.00	
4	0.225	0.003	0.001	0.337	0.062	0.360	0.00	
5	0.111	0.848	0.005	0.024	0.006	0.000	0.00	
6	0.788	0.081	0.046	0.073	0.003	0.001	0.00	
7	0.013	0.212	0.105	0.228	0.399	0.003	0.00	
8	0.052	0.000	0.004	0.465	0.168	0.308	0.00	
9	0.029	0.336	0.050	0.052	0.018	0.502	0.00	
10	0.581	0.156	0.003	0.250	0.009	0.001	0.00	
1	0.001	0.500	0.205	0.054	0.171	0.064	0.00	
2	0.182	0.242	0.533	0.003	0.014	0.003	0.00	
3	0.004	0.703	0.134	0.017	0.129	0.001	0.00	
4	0.134	0.256	0.435	0.129	0.000	0.045	0.00	
5	0.030	0.537	0.372	0.001	0.003	0.045	0.00	
6	0.110	0.064	0.417	0.126	0.098	0.178	0.00	
7	0.279	0.021	0.103	0.005	0.143	0.004	0.44	
8	0.181	0.187	0.266	0.196	0.009	0.162	0.00	
9	0.070	0.194	0.330	0.116	0.024	0.257	0.00	
10	0.391	0.125	0.350	0.126	0.008	0.000	0.00	
1	0.082	0.066	0.712	0.128	0.000	0.005	0.00	
2	0.207	0.569	0.000	0.146	0.000	0.062	0.00	
3	0.524	0.002	0.304	0.001	0.123	0.046	0.00	
4	0.001	0.083	0.751	0.126	0.034	0.001	0.00	
5	0.455	0.357	0.173	0.002	0.005	0.002	0.00	
6	0.757	0.001	0.152	0.063	0.015	0.008	0.00	
7	0.003	0.000	0.135	0.011	0.360	0.376	0.11	
8	0.431	0.233	0.275	0.040	0.018	0.004	0.00	
9	0.570	0.256	0.092	0.004	0.041	0.021	0.00	
10	0.293	0.046	0.098	0.026	0.521	0.013	0.00	
Regression coefficients:								
	F1	F2	F3	F4	F5	F6	F7	
month	0.388	-0.200	-1.023	0.072	-0.142	-0.287	0.00	
ph	0.239	0.101	-0.059	0.145	-0.435	-0.065	-0.8	
N	0.028	0.081	0.226	0.959	0.288	-0.973	0.00	
P	0.585	0.584	0.036	-0.340	-0.441	0.972	0.20	
DO	-0.041	0.894	0.264	-0.365	0.718	0.115	-0.2	
cond	-0.628	0.561	-0.024	-0.442	-0.429	-0.255	0.00	
TDS	-0.497	0.519	-0.044	0.222	0.307	0.950	-0.2	

APPENDIX VII cont'd

Standard coordinates (Sites):								
	F1	F2	F3	F4	F5	F6	F7	
1	0.053	0.222	-0.089	0.428	-0.109	-0.117	0.00	
2	-1.105	-2.194	-0.826	-0.203	0.233	-0.323	-0.8	
3	0.877	0.345	0.151	-0.517	0.345	-0.449	0.0	
4	0.547	0.072	0.033	-1.302	-0.592	-1.597	0.4	
5	-0.546	1.665	0.148	0.489	0.254	-0.013	-0.4	
6	-1.620	-0.573	-0.507	0.962	0.213	-0.124	-0.5	
7	-0.109	-0.480	-0.396	0.875	-1.227	-0.109	0.5	
8	0.350	-0.017	-0.119	-2.040	-1.301	-1.971	0.2	
9	0.166	0.621	-0.279	-0.431	-0.265	1.584	-0.3	
10	1.578	-0.905	-0.137	2.013	-0.407	0.130	-0.1	
1	-0.056	1.224	-0.918	0.708	1.333	-0.917	-0.3	
2	-0.869	-1.109	-1.926	-0.213	0.505	0.278	-0.9	
3	0.121	1.786	-0.913	0.487	1.424	0.109	-0.6	
4	0.511	0.782	-1.193	-0.976	0.054	-0.688	-0.0	
5	-0.298	1.402	-1.357	-0.117	0.195	0.850	-0.5	
6	-0.528	-0.444	-1.331	1.099	1.029	-1.550	-0.4	
7	-1.454	-0.441	-1.147	-0.377	2.145	0.406	5.7	
8	0.712	0.800	-1.117	-1.441	-0.326	-1.553	0.0	
9	0.612	1.123	-1.715	-1.528	-0.732	2.702	-0.6	
10	1.205	-0.752	-1.475	1.331	-0.354	-0.086	0.1	
1	0.324	-0.322	1.236	0.787	0.024	-0.176	0.2	
2	-1.357	-2.506	0.039	-2.234	0.095	1.726	-1.1	
3	1.592	0.110	1.569	0.154	1.589	1.090	-0.1	
4	0.032	0.383	1.346	-0.828	0.454	-0.092	0.2	
5	-1.673	1.638	1.335	0.216	0.376	-0.241	-0.5	
6	-1.612	-0.056	0.936	0.903	0.465	0.389	-0.3	
7	-0.084	-0.033	0.722	0.309	-1.878	2.150	1.6	
8	1.203	-0.977	1.243	-0.710	0.501	-0.254	-0.0	
9	-1.608	-1.191	0.834	0.263	0.891	-0.715	-0.8	
10	-1.030	0.451	0.773	0.593	-2.829	-0.509	0.2	
Contributions (Sites):								
	F1	F2	F3	F4	F5	F6	F7	
1	0.000	0.002	0.000	0.008	0.001	0.001	0.00	
2	0.036	0.143	0.020	0.001	0.002	0.003	0.00	
3	0.033	0.005	0.001	0.011	0.005	0.009	0.00	
4	0.011	0.000	0.000	0.064	0.013	0.096	0.00	
5	0.011	0.103	0.001	0.009	0.002	0.000	0.00	
6	0.067	0.008	0.007	0.024	0.001	0.000	0.00	
7	0.000	0.008	0.005	0.025	0.049	0.000	0.00	
8	0.004	0.000	0.000	0.127	0.052	0.118	0.00	
9	0.001	0.014	0.003	0.007	0.002	0.088	0.00	
10	0.119	0.039	0.001	0.194	0.008	0.001	0.00	
1	0.000	0.032	0.018	0.011	0.038	0.018	0.00	
2	0.016	0.027	0.080	0.001	0.006	0.002	0.00	
3	0.000	0.083	0.022	0.006	0.052	0.000	0.00	
4	0.007	0.016	0.037	0.025	0.000	0.012	0.00	
5	0.002	0.044	0.042	0.000	0.001	0.016	0.00	
6	0.006	0.005	0.041	0.028	0.024	0.055	0.00	
7	0.046	0.004	0.028	0.003	0.099	0.004	0.7	
8	0.011	0.014	0.027	0.045	0.002	0.052	0.00	
9	0.011	0.036	0.084	0.067	0.015	0.210	0.00	
10	0.064	0.025	0.096	0.079	0.006	0.000	0.00	
1	0.004	0.004	0.052	0.021	0.000	0.001	0.00	
2	0.051	0.171	0.000	0.136	0.000	0.081	0.00	
3	0.106	0.001	0.103	0.001	0.106	0.050	0.00	
4	0.000	0.006	0.077	0.029	0.009	0.000	0.00	
5	0.110	0.106	0.070	0.002	0.006	0.002	0.00	
6	0.080	0.000	0.027	0.025	0.007	0.005	0.00	
7	0.000	0.000	0.016	0.003	0.111	0.146	0.00	
8	0.090	0.060	0.096	0.031	0.016	0.004	0.00	
9	0.066	0.036	0.018	0.002	0.020	0.013	0.00	
10	0.046	0.009	0.026	0.015	0.346	0.011	0.00	

APPENDIX VII cont'd

CCA results:								
Principal coordinates (Sites):								
	F1	F2	F3	F4	F5	F6	F7	
1	0.016	0.060	-0.020	0.065	-0.016	-0.015	0.00	
2	-0.327	-0.588	-0.189	-0.031	0.033	-0.041	-0.0	
3	0.260	0.092	0.035	-0.079	0.050	-0.058	0.0	
4	0.162	0.019	0.008	-0.198	-0.085	-0.205	0.0	
5	-0.162	0.446	0.034	0.074	0.037	-0.002	-0.0	
6	-0.480	-0.154	-0.116	0.146	0.031	-0.016	-0.0	
7	-0.032	-0.129	-0.091	0.133	-0.176	-0.014	0.0	
8	0.104	-0.005	-0.027	-0.311	-0.187	-0.253	0.0	
9	0.049	0.166	-0.064	-0.066	-0.038	0.203	-0.0	
10	0.467	-0.242	-0.031	0.307	-0.058	0.017	-0.0	
1	-0.017	0.328	-0.210	0.108	0.192	-0.118	-0.0	
2	-0.257	-0.297	-0.441	-0.032	0.073	0.036	-0.0	
3	0.036	0.479	-0.209	0.074	0.205	0.014	-0.0	
4	0.152	0.209	-0.273	-0.149	0.008	-0.088	-0.0	
5	-0.088	0.375	-0.313	-0.018	0.028	0.109	-0.0	
6	-0.156	-0.119	-0.305	0.167	0.148	-0.199	-0.0	
7	-0.431	-0.118	-0.262	-0.057	0.308	0.052	0.5	
8	0.211	0.214	-0.256	-0.219	-0.047	-0.199	0.0	
9	0.181	0.301	-0.392	-0.233	-0.105	0.347	-0.0	
10	0.357	-0.202	-0.338	0.203	-0.052	-0.011	0.0	
1	0.096	-0.086	0.283	0.120	0.003	-0.023	0.0	
2	-0.405	-0.671	0.009	-0.340	0.014	0.221	-0.1	
3	0.471	0.030	0.359	0.023	0.228	0.140	-0.0	
4	0.010	0.103	0.308	-0.126	0.065	-0.012	0.0	
5	-0.496	0.439	0.305	0.033	0.054	-0.031	-0.0	
6	-0.478	-0.018	0.214	0.137	0.067	0.050	-0.0	
7	-0.025	-0.009	0.165	0.047	-0.270	0.276	0.1	
8	0.356	-0.262	0.284	-0.108	0.072	-0.034	-0.0	
9	-0.476	-0.319	0.191	0.040	0.128	-0.092	-0.0	
10	-0.305	0.121	0.177	0.090	-0.407	-0.065	0.0	

APPENDIX VII cont'd

	Value	%
Total	1.104	100.000
Constrained	0.281	25.473
Unconstrained	0.823	74.527

Results of the permutation test:

Permutation	1000
Pseudo F	1.074
p-value	0.437
alpha	0.050

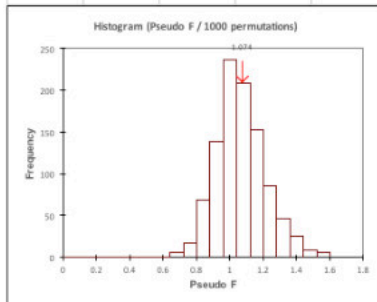
Test interpretation:

H0: The sites/objects data are not linearly related to the sites/variables data.

Ha: The sites/objects data are linearly related to the sites/variables data.

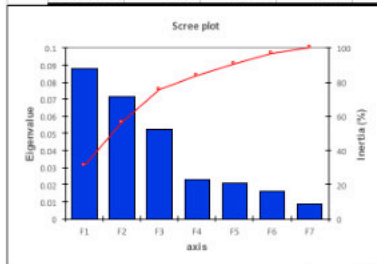
As the computed p-value is greater than the significance level $\alpha=0.05$, one cannot reject the null hypothesis H0.

The risk to reject the null hypothesis H0 while it is true is 43.70%.



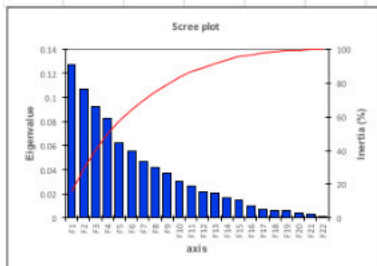
Eigenvalues and percentages of inertia (CCA):

	F1	F2	F3	F4	F5	F6	F7
Eigenvalue	0.088	0.072	0.052	0.023	0.021	0.016	0.009
Constrained	31.208	25.529	18.623	8.245	7.348	5.852	3.196
Cumulative %	31.208	56.736	75.359	83.604	90.952	96.804	100.000
Total inertia	7.950	6.503	4.744	2.100	1.872	1.491	0.814
Cumulative %	7.950	14.452	19.196	21.297	23.168	24.659	25.473



Eigenvalues and percentages of inertia (Unconstrained CCA):

	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14	F15	F16	F17	F18	F19	F20	F21	F22
Eigenvalue	0.127	0.107	0.092	0.083	0.062	0.055	0.047	0.042	0.037	0.031	0.027	0.022	0.021	0.017	0.015	0.010	0.007	0.006	0.006	0.004	0.003	0.001
Unconstrained	15.483	13.013	11.207	10.069	7.576	6.721	5.705	5.105	4.527	3.736	3.267	2.676	2.556	2.056	1.800	1.169	0.896	0.739	0.719	0.487	0.406	0.087
Cumulative %	15.483	28.496	39.703	49.772	57.348	64.069	69.774	74.879	79.406	83.142	86.409	89.085	91.641	93.697	95.497	96.666	97.562	98.301	99.020	99.507	99.913	100.000
Total inertia	11.539	9.698	8.352	7.504	5.646	5.009	4.252	3.805	3.374	2.785	2.435	1.994	1.905	1.532	1.341	0.872	0.668	0.551	0.536	0.363	0.303	0.065
Cumulative %	11.539	21.237	29.589	37.094	42.740	47.749	52.001	55.805	59.179	61.963	64.398	66.393	68.297	69.830	71.171	72.042	72.710	73.261	73.796	74.160	74.462	74.527



APPENDIX VIII

Case Processing Summary^b

Cases							
Valid		Rejected				Total	
		Missing Value		Out of Range Binary Value ^a			
N	Percent	N	Percent	N	Percent	N	Percent
30	100,0	0	,0	0	,0	30	100,0

a. Value different from both 1 and 0.

b. Average Linkage (Between Groups)

Proximity Matrix

Case	Jaccard Measure							
	1	2	3	4	5	6	7	8
1	1,000	,500	,400	,400	,346	,375	,231	,500
2	,500	1,000	,458	,667	,458	,500	,391	,500
3	,400	,458	1,000	,360	,360	,280	,292	,346
4	,400	,667	,360	1,000	,478	,524	,476	,400
5	,346	,458	,360	,478	1,000	,455	,348	,296
6	,375	,500	,280	,524	,455	1,000	,318	,435
7	,231	,391	,292	,476	,348	,318	1,000	,391
8	,500	,500	,346	,400	,296	,435	,391	1,000
9	,385	,500	,400	,400	,346	,435	,455	,500
10	,423	,480	,440	,440	,286	,417	,500	,480
11	,435	,500	,455	,391	,280	,500	,318	,435
12	,375	,650	,600	,600	,391	,429	,381	,435
13	,370	,370	,333	,286	,241	,417	,269	,682
14	,346	,591	,360	,417	,360	,333	,476	,458
15	,250	,304	,381	,381	,381	,350	,238	,304
16	,435	,571	,455	,391	,455	,429	,318	,435
17	,375	,650	,600	,600	,391	,429	,381	,435
18	,435	,500	,455	,391	,280	,500	,318	,435
19	,417	,417	,650	,435	,320	,292	,200	,478
20	,448	,500	,519	,414	,414	,393	,407	,500
21	,320	,571	,333	,455	,455	,364	,706	,375
22	,423	,762	,440	,636	,500	,417	,500	,370
23	,458	,458	,417	,417	,360	,455	,348	,750
24	,423	,423	,440	,385	,385	,308	,435	,423
25	,346	,400	,417	,360	,619	,333	,240	,346
26	,565	,636	,522	,522	,458	,320	,280	,500
27	,385	,500	,346	,522	,522	,500	,280	,385
28	,423	,423	,440	,440	,286	,360	,435	,480
29	,292	,550	,364	,500	,364	,647	,227	,409
30	,440	,440	,667	,346	,400	,375	,231	,333

This is a similarity matrix

APPENDIX VIII cont'd

Proximity Matrix								
Case	Jaccard Measure							
	9	10	11	12	13	14	15	16
1	,385	,423	,435	,375	,370	,346	,250	,435
2	,500	,480	,500	,650	,370	,591	,304	,571
3	,400	,440	,455	,600	,333	,360	,381	,455
4	,400	,440	,391	,600	,286	,417	,381	,391
5	,346	,286	,280	,391	,241	,360	,381	,455
6	,435	,417	,500	,429	,417	,333	,350	,429
7	,455	,500	,318	,381	,269	,476	,238	,318
8	,500	,480	,435	,435	,682	,458	,304	,435
9	1,000	,609	,500	,375	,480	,400	,304	,375
10	,609	1,000	,417	,417	,407	,333	,348	,417
11	,500	,417	1,000	,429	,478	,455	,227	,304
12	,375	,417	,429	1,000	,417	,391	,421	,579
13	,480	,407	,478	,417	1,000	,333	,292	,308
14	,400	,333	,455	,391	,333	1,000	,208	,455
15	,304	,348	,227	,421	,292	,208	1,000	,421
16	,375	,417	,304	,579	,308	,455	,421	1,000
17	,375	,417	,429	1,000	,417	,391	,421	,579
18	,500	,417	1,000	,429	,478	,455	,227	,304
19	,360	,346	,348	,550	,458	,320	,400	,409
20	,556	,593	,444	,444	,536	,464	,385	,444
21	,375	,417	,304	,364	,259	,600	,227	,429
22	,542	,462	,478	,545	,267	,565	,240	,478
23	,346	,440	,391	,455	,565	,478	,318	,524
24	,682	,462	,417	,308	,357	,385	,292	,308
25	,346	,241	,333	,391	,333	,308	,381	,391
26	,440	,423	,435	,571	,423	,458	,429	,571
27	,385	,321	,435	,435	,423	,522	,304	,375
28	,609	,727	,360	,417	,407	,286	,409	,360
29	,240	,280	,474	,556	,391	,429	,389	,474
30	,500	,370	,500	,435	,370	,346	,304	,435

This is a similarity matrix

APPENDIX VIII cont'd

Proximity Matrix								
Case	Jaccard Measure							
	17	18	19	20	21	22	23	24
1	,375	,435	,417	,448	,320	,423	,458	,423
2	,650	,500	,417	,500	,571	,762	,458	,423
3	,600	,455	,650	,519	,333	,440	,417	,440
4	,600	,391	,435	,414	,455	,636	,417	,385
5	,391	,280	,320	,414	,455	,500	,360	,385
6	,429	,500	,292	,393	,364	,417	,455	,308
7	,381	,318	,200	,407	,706	,500	,348	,435
8	,435	,435	,478	,500	,375	,370	,750	,423
9	,375	,500	,360	,556	,375	,542	,346	,682
10	,417	,417	,346	,593	,417	,462	,440	,462
11	,429	1,000	,348	,444	,304	,478	,391	,417
12	1,000	,429	,550	,444	,364	,545	,455	,308
13	,417	,478	,458	,536	,259	,267	,565	,357
14	,391	,455	,320	,464	,600	,565	,478	,385
15	,421	,227	,400	,385	,227	,240	,318	,292
16	,579	,304	,409	,444	,429	,478	,524	,308
17	1,000	,429	,550	,444	,364	,545	,455	,308
18	,429	1,000	,348	,444	,304	,478	,391	,417
19	,550	,348	1,000	,481	,240	,296	,500	,296
20	,444	,444	,481	1,000	,500	,483	,577	,433
21	,364	,304	,240	,500	1,000	,545	,455	,360
22	,545	,478	,296	,483	,545	1,000	,385	,520
23	,455	,391	,500	,577	,455	,385	1,000	,333
24	,308	,417	,296	,433	,360	,520	,333	1,000
25	,391	,333	,435	,414	,333	,333	,360	,385
26	,571	,435	,478	,500	,375	,609	,591	,480
27	,435	,435	,360	,448	,435	,480	,522	,370
28	,417	,360	,346	,536	,308	,407	,385	,583
29	,556	,474	,381	,321	,333	,391	,500	,231
30	,435	,500	,619	,556	,320	,423	,400	,423

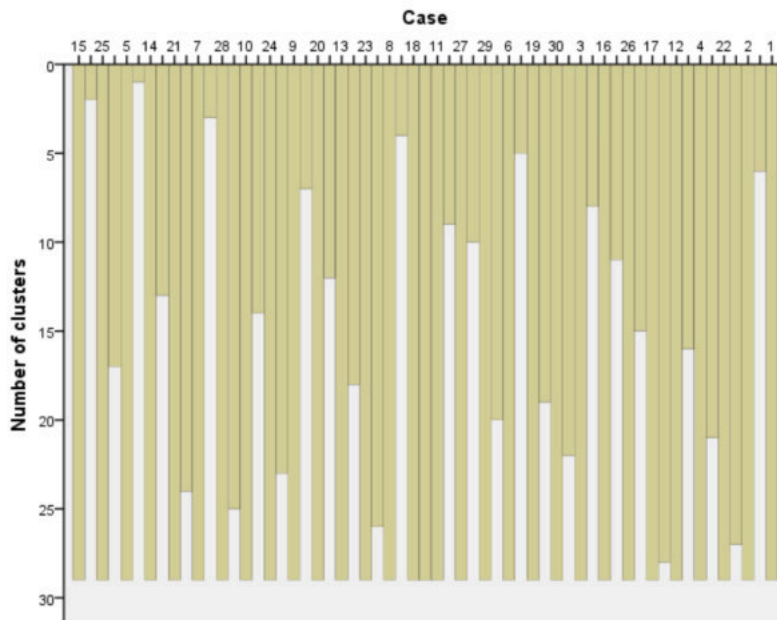
This is a similarity matrix

APPENDIX VIII cont'd

Proximity Matrix

Case	Jaccard Measure					
	25	26	27	28	29	30
1	,346	,565	,385	,423	,292	,440
2	,400	,636	,500	,423	,550	,440
3	,417	,522	,346	,440	,364	,667
4	,360	,522	,522	,440	,500	,346
5	,619	,458	,522	,286	,364	,400
6	,333	,320	,500	,360	,647	,375
7	,240	,280	,280	,435	,227	,231
8	,346	,500	,385	,480	,409	,333
9	,346	,440	,385	,609	,240	,500
10	,241	,423	,321	,727	,280	,370
11	,333	,435	,435	,360	,474	,500
12	,391	,571	,435	,417	,556	,435
13	,333	,423	,423	,407	,391	,370
14	,308	,458	,522	,286	,429	,346
15	,381	,429	,304	,409	,389	,304
16	,391	,571	,375	,360	,474	,435
17	,391	,571	,435	,417	,556	,435
18	,333	,435	,435	,360	,474	,500
19	,435	,478	,360	,346	,381	,619
20	,414	,500	,448	,536	,321	,556
21	,333	,375	,435	,308	,333	,320
22	,333	,609	,480	,407	,391	,423
23	,360	,591	,522	,385	,500	,400
24	,385	,480	,370	,583	,231	,423
25	1,000	,400	,400	,333	,304	,458
26	,400	1,000	,565	,423	,409	,440
27	,400	,565	1,000	,321	,550	,385
28	,333	,423	,321	1,000	,231	,370
29	,304	,409	,550	,231	1,000	,348
30	,458	,440	,385	,370	,348	1,000

This is a similarity matrix



APPENDIX IX

Coding for cluster analysis in R

```
> data4=read.table("data.txt", header=TRUE)

> str(data4)

'data.frame': 39 obs. of 10 variables:
 $ A: int 10 0 0 29 0 0 0 0 4 0 ...
 $ B: int 5 0 4 14 0 0 2 1 3 0 ...
 $ C: int 11 2 9 36 0 0 0 2 0 4 ...
 $ D: int 7 4 12 42 0 0 0 0 3 1 ...
 $ E: int 2 0 0 36 0 3 0 13 0 0 ...
 $ F: int 9 6 3 11 0 0 2 2 0 3 ...
 $ G: int 10 0 17 20 0 0 1 4 0 5 ...
 $ H: int 12 6 14 49 0 0 1 0 2 6 ...
 $ I: int 8 0 11 24 0 0 0 1 0 0 ...
 $ J: int 6 1 6 38 0 0 3 0 0 0 ...

> cluster_similarity(data4$A,data4$B,similarity="jaccard", method="independence")
[1] 0.3926554

> cluster_similarity(data4$A,data4$C,similarity="jaccard", method="independence")
[1] 0.3589744

> cluster_similarity(data4$A,data4$D,similarity="jaccard", method="independence")
[1] 0.2743142

> cluster_similarity(data4$A,data4$E,similarity="jaccard", method="independence")
[1] 0.2644231

> cluster_similarity(data4$A,data4$F,similarity="jaccard", method="independence")
[1] 0.275

> cluster_similarity(data4$A,data4$G,similarity="jaccard", method="independence")
[1] 0.3246753

> cluster_similarity(data4$A,data4$H,similarity="jaccard", method="independence")
[1] 0.2034739

> cluster_similarity(data4$A,data4$I,similarity="jaccard", method="independence")
[1] 0.3432836

> cluster_similarity(data4$A,data4$J,similarity="jaccard", method="independence")
```

[1] 0.2371638
> cluster_similarity(data4\$B,data4\$C,similarity="jaccard", method="independence")

[1] 0.2297297
> cluster_similarity(data4\$B,data4\$D,similarity="jaccard", method="independence")

[1] 0.2823529
> cluster_similarity(data4\$B,data4\$E,similarity="jaccard", method="independence")

[1] 0.2058824
> cluster_similarity(data4\$B,data4\$F,similarity="jaccard", method="independence")

[1] 0.3987138
> cluster_similarity(data4\$B,data4\$G,similarity="jaccard", method="independence")

[1] 0.2831858
> cluster_similarity(data4\$B,data4\$H,similarity="jaccard", method="independence")

[1] 0.2275449
> cluster_similarity(data4\$B,data4\$I,similarity="jaccard", method="independence")

[1] 0.3058824
> cluster_similarity(data4\$B,data4\$J,similarity="jaccard", method="independence")

[1] 0.2602339
> cluster_similarity(data4\$C,data4\$D,similarity="jaccard", method="independence")

[1] 0.2066327
> cluster_similarity(data4\$C,data4\$E,similarity="jaccard", method="independence")

[1] 0.257732
> cluster_similarity(data4\$C,data4\$F,similarity="jaccard", method="independence")

[1] 0.4131737
> cluster_similarity(data4\$C,data4\$G,similarity="jaccard", method="independence")

[1] 0.3563218
> cluster_similarity(data4\$C,data4\$H,similarity="jaccard", method="independence")

[1] 0.228022
> cluster_similarity(data4\$C,data4\$I,similarity="jaccard", method="independence")

[1] 0.426506
> cluster_similarity(data4\$C,data4\$J,similarity="jaccard", method="independence")

[1] 0.2614555
> cluster_similarity(data4\$D,data4\$E,similarity="jaccard", method="independence")

[1] 0.2213542
> cluster_similarity(data4\$D,data4\$F,similarity="jaccard", method="independence")

[1] 0.2942857
> cluster_similarity(data4\$D,data4\$G,similarity="jaccard", method="independence")

[1] 0.1984127
> cluster_similarity(data4\$D,data4\$H,similarity="jaccard", method="independence")

[1] 0.3416928
> cluster_similarity(data4\$D,data4\$I,similarity="jaccard", method="independence")

[1] 0.2217484
> cluster_similarity(data4\$D,data4\$J,similarity="jaccard", method="independence")

[1] 0.226776
> cluster_similarity(data4\$E,data4\$F,similarity="jaccard", method="independence")

[1] 0.2717391
> cluster_similarity(data4\$E,data4\$G,similarity="jaccard", method="independence")

[1] 0.2614555
> cluster_similarity(data4\$E,data4\$H,similarity="jaccard", method="independence")

[1] 0.200542
> cluster_similarity(data4\$E,data4\$I,similarity="jaccard", method="independence")

[1] 0.2866521
> cluster_similarity(data4\$E,data4\$J,similarity="jaccard", method="independence")

[1] 0.3257143
> cluster_similarity(data4\$F,data4\$G,similarity="jaccard", method="independence")

[1] 0.369697
> cluster_similarity(data4\$F,data4\$H,similarity="jaccard", method="independence")

[1] 0.2521994
> cluster_similarity(data4\$F,data4\$I,similarity="jaccard", method="independence")

[1] 0.3333333
> cluster_similarity(data4\$F,data4\$J,similarity="jaccard", method="independence")

[1] 0.28
> cluster_similarity(data4\$G,data4\$H,similarity="jaccard", method="independence")

[1] 0.1666667
> cluster_similarity(data4\$G,data4\$I,similarity="jaccard", method="independence")

[1] 0.4444444
> cluster_similarity(data4\$G,data4\$J,similarity="jaccard", method="independence")

[1] 0.2240437
> cluster_similarity(data4\$H,data4\$I,similarity="jaccard", method="independence")

[1] 0.1969365
> cluster_similarity(data4\$H,data4\$J,similarity="jaccard", method="independence")

[1] 0.2051282
> cluster_similarity(data4\$I,data4\$J,similarity="jaccard", method="independence")

[1] 0.2968037
> cluster_similarity(data4\$A,data4\$B,similarity="jaccard", method="independence")

[1] 0.3222892
> cluster_similarity(data4\$A,data4\$C,similarity="jaccard", method="independence")

[1] 0.2686981
> cluster_similarity(data4\$A,data4\$D,similarity="jaccard", method="independence")

[1] 0.2596685
> cluster_similarity(data4\$A,data4\$E,similarity="jaccard", method="independence")

[1] 0.2180851
> cluster_similarity(data4\$A,data4\$F,similarity="jaccard", method="independence")

[1] 0.2825
> cluster_similarity(data4\$A,data4\$G,similarity="jaccard", method="independence")

[1] 0.1890661
> cluster_similarity(data4\$A,data4\$H,similarity="jaccard", method="independence")

[1] 0.316092
> cluster_similarity(data4\$A,data4\$I,similarity="jaccard", method="independence")

[1] 0.2354571
> cluster_similarity(data4\$A,data4\$J,similarity="jaccard", method="independence")

[1] 0.2463768
> cluster_similarity(data4\$B,data4\$C,similarity="jaccard", method="independence")

[1] 0.3061798

> cluster_similarity(data4\$B,data4\$D,similarity="jaccard", method="independence")

[1] 0.4983819

> cluster_similarity(data4\$B,data4\$E,similarity="jaccard", method="independence")

[1] 0.2988827

> cluster_similarity(data4\$B,data4\$F,similarity="jaccard", method="independence")

[1] 0.3720317

> cluster_similarity(data4\$B,data4\$G,similarity="jaccard", method="independence")

[1] 0.3094059

> cluster_similarity(data4\$B,data4\$H,similarity="jaccard", method="independence")

[1] 0.3210227

> cluster_similarity(data4\$B,data4\$I,similarity="jaccard", method="independence")

[1] 0.3323529

> cluster_similarity(data4\$B,data4\$J,similarity="jaccard", method="independence")

[1] 0.3083832

> cluster_similarity(data4\$C,data4\$D,similarity="jaccard", method="independence")

[1] 0.2519481

> cluster_similarity(data4\$C,data4\$E,similarity="jaccard", method="independence")

[1] 0.2410256

> cluster_similarity(data4\$C,data4\$F,similarity="jaccard", method="independence")

[1] 0.2476852

> cluster_similarity(data4\$C,data4\$G,similarity="jaccard", method="independence")

[1] 0.2714617

> cluster_similarity(data4\$C,data4\$H,similarity="jaccard", method="independence")

[1] 0.2378517

> cluster_similarity(data4\$C,data4\$I,similarity="jaccard", method="independence")

[1] 0.2620321

> cluster_similarity(data4\$C,data4\$J,similarity="jaccard", method="independence")
[1] 0.273743

> cluster_similarity(data4\$D,data4\$E,similarity="jaccard", method="independence")
[1] 0.3426184

> cluster_similarity(data4\$D,data4\$F,similarity="jaccard", method="independence")
[1] 0.432

> cluster_similarity(data4\$D,data4\$G,similarity="jaccard", method="independence")
[1] 0.4

> cluster_similarity(data4\$D,data4\$H,similarity="jaccard", method="independence")
[1] 0.2717678

> cluster_similarity(data4\$D,data4\$I,similarity="jaccard", method="independence")
[1] 0.2912088

> cluster_similarity(data4\$D,data4\$J,similarity="jaccard", method="independence")
[1] 0.2897727

> cluster_similarity(data4\$E,data4\$F,similarity="jaccard", method="independence")
[1] 0.3576826

> cluster_similarity(data4\$E,data4\$G,similarity="jaccard", method="independence")
[1] 0.2955083

> cluster_similarity(data4\$E,data4\$H,similarity="jaccard", method="independence")
[1] 0.2009926

> cluster_similarity(data4\$E,data4\$I,similarity="jaccard", method="independence")
[1] 0.2421053

> cluster_similarity(data4\$E,data4\$J,similarity="jaccard", method="independence")
[1] 0.1692308

> cluster_similarity(data4\$F,data4\$G,similarity="jaccard", method="independence")
[1] 0.3194748

> cluster_similarity(data4\$F,data4\$H,similarity="jaccard", method="independence")

[1] 0.3441397

> cluster_similarity(data4\$F,data4\$I,similarity="jaccard", method="independence")

[1] 0.320802

> cluster_similarity(data4\$F,data4\$J,similarity="jaccard", method="independence")

[1] 0.2839196

> cluster_similarity(data4\$G,data4\$H,similarity="jaccard", method="independence")

[1] 0.3078759

> cluster_similarity(data4\$G,data4\$I,similarity="jaccard", method="independence")

[1] 0.377892

> cluster_similarity(data4\$G,data4\$J,similarity="jaccard", method="independence")

[1] 0.3612565

> cluster_similarity(data4\$H,data4\$I,similarity="jaccard", method="independence")

[1] 0.3184358

> cluster_similarity(data4\$H,data4\$J,similarity="jaccard", method="independence")

[1] 0.3028571

> cluster_similarity(data4\$I,data4\$J,similarity="jaccard", method="independence")

[1] 0.3962264

APPENDIX XI

Coding data for environmental modelling in R

```
> bc1=bioclim(dataclim[,c('ph1','N1','P1','DO1','cond1','TDS1')])
```

```
> bc1
```

```
class   : Bioclim
```

```
variables: ph1 N1 P1 DO1 cond1 TDS1
```

```
presence points: 10
```

```
 _ph1 N1 P1 DO1 cond1 TDS1
```

```
1 6.8 4.2 0.63 11.4 620 430
```

```
2 7.4 2.3 0.07 11.4 600 441
```

```
3 6.9 3.9 0.66 13.1 540 422
```

```
4 6.6 3.8 0.57 12.6 640 411
```

```
5 7.2 4.2 0.50 12.3 700 463
```

```
6 6.9 4.0 0.40 11.2 650 480
```

```
7 5.9 4.1 0.92 9.5 670 462
```

```
8 7.0 3.6 0.53 12.5 580 387
```

```
9 7.2 3.6 0.16 11.7 630 450
```

```
10 7.5 4.4 0.76 9.6 750 444
```

```
_(... ..)
```

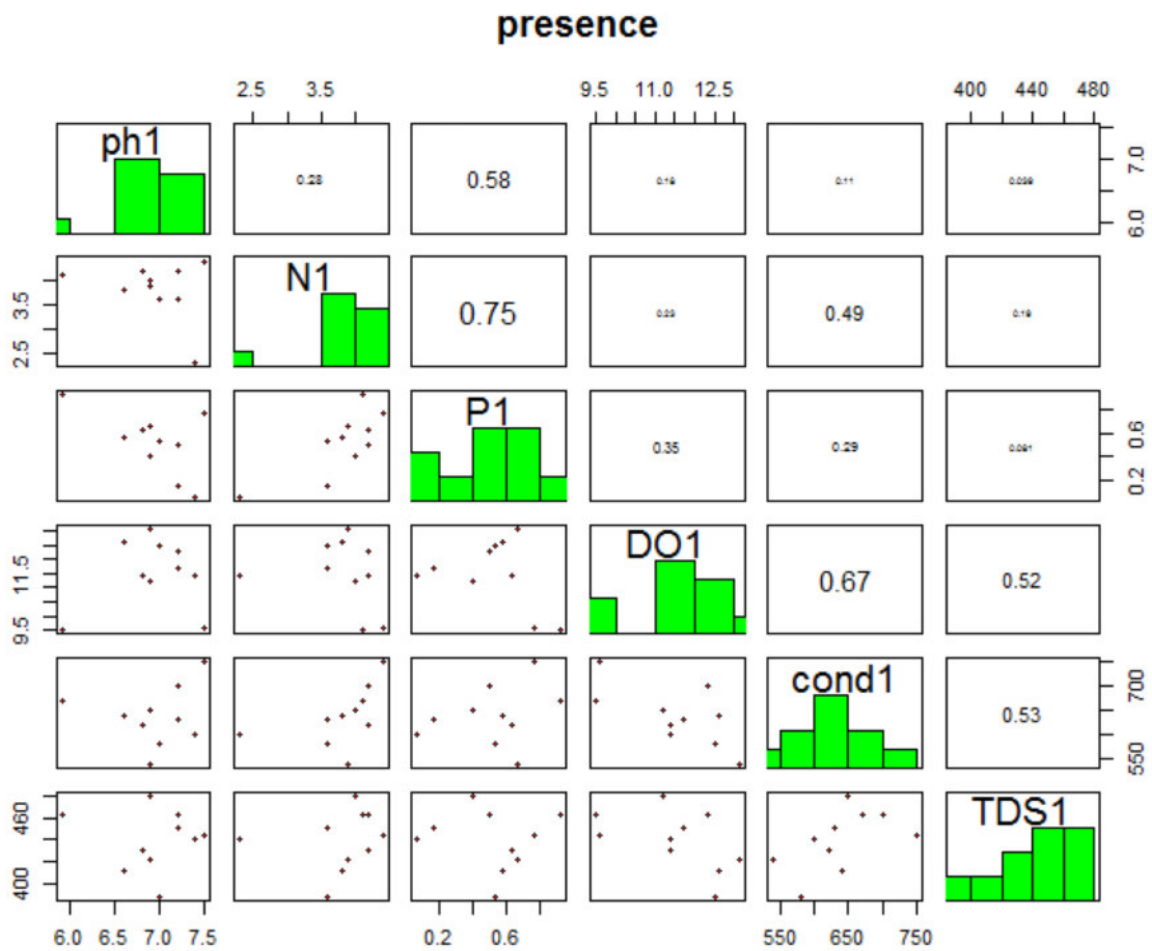
```
> pairs(bc1)
```

The “bioclim” function is preferable here as initial data contains only presence points.

APPENDIX XI

Environmental Modelling

May



The strongest correlations:

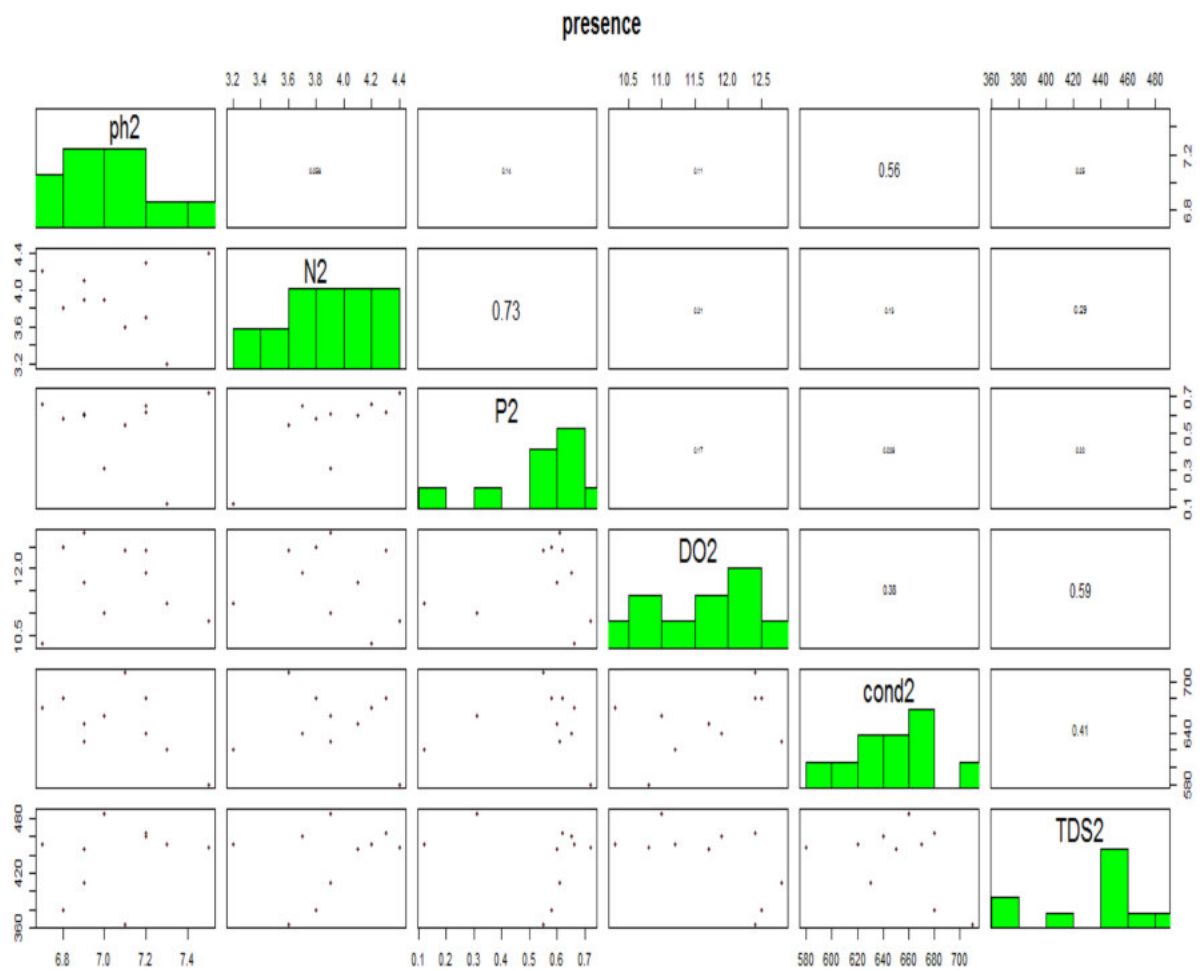
N*P - 0.75

Do*Cond - 0.67

Ph*P - 0.58

APPENDIX XI cont'd

June



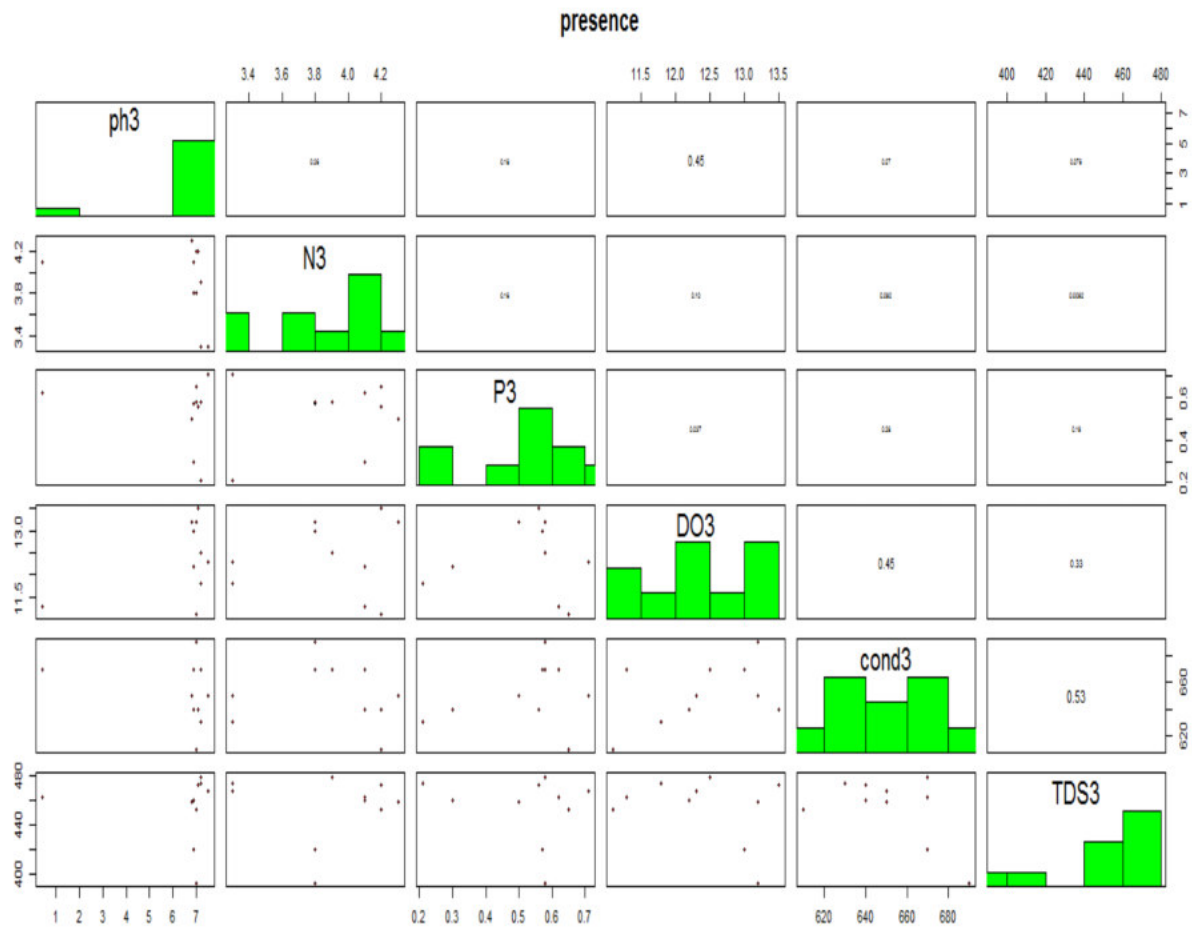
The strongest correlations:

N*P - 0.73

Do*TDS – 0.59

APPENDIX XI cont'd

July



The strongest correlation is Cond*TDS – 0.53, but still it is quite weak .