Investigation on the relationship between organic matter decomposition and macroinvertebrate assemblages using colonisation traps

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Number of words: 9578

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MSc Conservation

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Abstract

1. The important role of organic matter decomposition in reflecting ecosystem functions in the aquatic system has been well recognized. And a new equipment called colonisation trap was developed for measuring the organic matter decomposition rates and associated benthic macroinvertebrate communities.
2. The fundamental aim of this study was to investigate the primary factors regulating the invertebrate decomposition rates. Fieldwork including the deployment of colonisation traps and MoRPH survey was conducted on River Mimram, River Crane and River Ash during May, June and July, respectively. The influence of invertebrate abundance, taxon richness, biotic indicator (ARMI scores) and abundance of different functional feeding groups on the invertebrate decomposition rates was then analysed.
3. Macroinvertebrate assemblages in three rivers were in accordance with water quality and physical habitat conditions. More sensitive taxa such as Ephemerellidae, Trichoptera and Plecoptera were occurred in River Mirmam, indicating relatively better water quality; while pollution–tolerant taxa such as Asellidae and Gastropoda dominated the River Ash.
4. Multivariate analysis revealed that macroinvertebrate assemblages were primarily controlled by the habitat heterogeneity, involving riparian vegetation complexity, riparian physical habitat complexity and channel physical habitat complexity. The negative correlation occurred between invertebrate assemblages and habitat heterogeneity in River Mimram, whereas, a positive correlation was observed in River Crane. Positions of sample sites and subjective MoRPH survey were possible reasons accounting for the different performance of physical habitats.
5. Invertebrate abundance, and the abundance of detritivores such as Gammaridae and Gastropoda in particular, were positively correlated with the invertebrate decomposition rates. The more detritivores in the aquatic system, the faster the organic matter decomposition rates could be. Other factors including taxon richness, ARMI scores were only observed in River Mimram, and no direct factor was found in River Crane.
6. This study suggested the organic matter decomposition rate measured by the colonisation traps could be a useful indicator of ecosystem functions in the aquatic system. And for the next stage, more colonisation traps should be deployed in different rivers to obtain a broad understanding, with the engagement of citizen scientists.

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Chapter 1: Introduction

1.1 Background and context

There has been a dramatic increase in the number of river restoration projects across England, and this trend is more likely to continue, according to England et al (2008). Monitoring is a crucial part of river restoration projects, as it is used to determine whether a project working effectively (Skinner and Bruce-Burgess, 2005). Monitor elements vary from physical parameters to chemical and ecological parameters, and are highly case-specific (England et al, 2008). The weak response of fishes to river rehabilitation (artificial riffles and flow deflectors) was observed and a more directed biological monitoring is required, suggested by Pretty et al (2003). River rehabilitation projects were also found to have no significant impact on taxon richness of the benthos or of the rehabilitated stretch of the rivers as a whole (Harrison et al, 2004), although the invertebrate abundance especially the abundance of rheophilic taxa was higher in the benthos of artificial riffles, compared to the reference benthos. Additionally, invertebrates have also been used together with leaf litter decomposition (Muehlbauer et al, 2009), morphology (Jahnig et al, 2010), and other parameters of ecosystem functions (Young and Collier, 2009).

Formalised national programs such as the Angler's Riverfly Monitoring Initiative in the UK, now at a critical juncture of transferring from detecting pollution to evaluating ecological restoration (Huddart et al, 2016), facing the increased river restoration projects. Monitoring is often constrained by limited resources, while the engagement of citizen scientists is expected to mitigate it (Huddart et al, 2016). ARMI samples monthly on over 900 sites across the UK, which provides a huge database for further analyses on major drivers affecting restoration outcomes. In addition to macroinvertebrates (abundance, taxon richness and diversity), there is a growing interest on process-based indicators of ecosystem functioning, such as leaf litter decomposition and primary production (Young and Collier, 2009; Huddart et al, 2016).

Hence, a new equipment called colonisation trap has been developed to measure the decomposition rates together with the associated invertebrates. The decomposition rate is expected to increase with the increased invertebrate abundance, especially the abundance of detritivores. However, it is still an expectation without evidence support.

Therefore, in prior to the application of colonisation traps in river restoration monitoring by ARMI, research on determining the controlling factors of organic matter decomposition is urgently needed, and it is exactly the fundamental aim of this report. Moreover, there is little research on investigating the relationship between water quality – detritivore density - invertebrate decomposition (Graca, 2001). This research is expected to fill the knowledge gap.

1.2 Literature review

1.2.1 Biotic indices

Benthic macroinvertebrate is of ecologically important within the food chain, by consuming organic matters (wood and leaf debris) and providing food to fishes and other mammals (Herbst, n.d.). It is a commonly used indicator for water quality monitoring due to its ease of sampling as well as varying sensitivities to environmental changes, such as PH, water temperature and pollutants (Huddart et al, 2016). Moreover, benthic macroinvertebrate is not only reflect the effect occur over time in both local and wider scales, may also able to identify the impacts of habitat degradation (Zhang et al, 2014) and flow alteration (Metcalfe-Smith, 1994), compared to chemical methods.

A brief review of different biotic indices was provided by Metcalfe-Smith (1994), which was mainly focused on the indices applied in the Europe. Results (such as scores, index or class) extracted from a list of invertebrate taxa allow non-specialists who need the invertebrate data to make the decisions involving water management (Armitage et al, 1983). Biotic indices including the Trent biotic index, Chandler Biotic Score were frequently used in Great Britain, however, they were not applicable nationwide since they were all designed for a localised area of the country. As a result, Biological Monitoring Working party (BMWP), a standardized score system has been developed and becomes one of the most popular indicators in Great Britain. Invertebrate families will be classified into different groups based on their pollution tolerances. The lower the value, the more tolerant the invertebrate is. BMWP is believed to be less time-consuming by eliminating the invertebrate abundance. However, on the other hand, it magnifies the impact of invertebrate diversity. For example, the sample with a higher number of invertebrates is more likely to achieve a higher final score, even if samples are collected from the same site. BMWP-ASPT (average score per taxon), dividing the BMWP score by the number of scoring taxa, is independent of invertebrate diversity and is always used together with

BMWP (Armitage et al, 1983). The performance of BMWP and ASPT has been tested on 268 sites on 41 rivers across the UK, and it was found that predicted ASPT can explain 65% of the variance, compared to BMWP score (22%, Armitage et al, 1983). And it is suggested that Chironomidae and Oligochaeta should be eliminated from the scoring system, because some of them are tolerant of pollution, while others are sensitive (Pinder and Farr, 1987).

BMWP score system has also been successfully adapted and applied in other countries. In prior to the application outside of the UK, BMWP score system will be modified by deleting the invertebrate taxa that are absent in that country, and adding additional invertebrates that are not in the list within BMWP score system and are potential biotic indicators. A modified BMWP system (BMWP-PL) was applied in Poland, together with chemical data and other biological indicators including saprobic index, diversity index and Belgian biotic index (Cezrniawska-Kusza, 2005). The BMWP-PL score was correlated with other biological indices and chemical parameters, which indicated that BMWP-PL could be an applicable method for water quality monitoring in Poland. Mustow (2002) did the similar research and identified the high potential of applying modified BMWP score system (BMWP-Thailand) in Thailand as well as other subtropical and tropical countries. Furthermore, Zamora-Munoz and Alba-Tercedor (1996) proved that the modified BMWP score system in Spain could be an easy method for water quality assessment, compared with multivariate methods (TWINSPAN and Canonical Correspondence Analysis (CCA)). Successful application of modified BMWP score system was available in Argentina (Capitulo et al, 2001), Chile (Alvial et al, 2012) and Portugal (Blijswijk et al, 2004).

1.2.2 Contribution of citizen science

Citizen science, referring to public participation in scientific research, is now playing an active role in protecting the environment (Sheldon and Ashcroft, 2016). The Angler's Riverfly Monitoring Initiative (ARMI), coordinated by the Riverfly Partnership, is a typical citizen science initiative (The Riverfly Partnership, n.d.). It provides a platform for trained volunteers to assess and monitor their local water quality by recording aquatic invertebrates, which in verse, promote increased awareness of protecting their local freshwater environment. After the training workshop, volunteers conduct a three-minute kick sampling to collect, identify and record the target invertebrates. Eight target invertebrates are selected based on their sensitivity to pollution, distribution and abundance in rivers across the country: Cased caddisfly, Caseless caddisfly, Mayfly, Blue-winged olive, Flat-bodied olive, Olives, Stonefly and Freshwater shrimp. An ARMI score is then produced to compare with designated ‘trigger’ level. Environmental Agency will be informed if the ARMI score drops below the trigger level, and they will respond to ARMI by telling them the results of detailed investigations and subsequent actions being taken. It forms a benign circulation and enables local people to reconnect with local river system.

ARMI with more frequent sampling, complements the routine statutory monitoring of Environment Agency and is expected to detect pollution incidents that might be missed by Environment Agency (Huddart et al, 2016). Enormous data with extended spatial and temporal resolution will be collected at lower cost for further scientific analyses, which is considered as the major benefit of citizen science. And more significantly, new species were reported to be found for the first time in the UK during the regular ARMI sampling (the Riverfly Partnership, n.d.). On 22 December 2010, Caddisfly *Synagapetus dubitans* was found near a small stream near Masham, North Yorkshire. An invasive species of shrimp *Dikerogammarus villosus,* known as killer shrimp, were found for the first time at Grafham Water reservoir in Cambridgeshire in the year 2010. Precautionary biosecurity measures were put in place immediately as this killer shrimp could dominate the habitats it invades and lead to the extinction of a range of native species.

However, in the meantime, the quality of data collected by citizen scientists rather than professionals is always in doubt (Nerbonne and Nelson, 2008). Lower data quality may owe to reasons including limited technical capacity, inappropriate equipment, and inconsistent methodology and purpose (Fiore and Fitch, 2016). But in terms of ARMI score system, it is achieved by simplifying the BMWP methodology, which has proven scientific validity in the UK (Fiore and Fitch, 2016). A smaller number of invertebrate taxa and a coarser taxonomic resolution are included in the monitoring protocol (Huddart et al, 2016), so that users with varying levels of skill (such as citizen scientists) are able to distinguish target invertebrates. Furthermore, trained volunteers carry out kick sampling using the same equipment used by EA ecologists, with the aid of river managers who are professional in riverfly identification. ARMI score system has proven successfully detect the pollution incident in River Rhymney in 2009 (The Riverfly Partnership, 2009), for example. A significant drop in ARMI scores measured in River Rhymney was reported to

Environment Agency, and followed with further investigation and identification of the source of polluters by Environmental Agency. Other documented cases of ARMI success are available in the Riverfly Partnership (n.d.), such as pollution incidents in River Sirhowy, Blackwood in 2007 and in River Kennet in 2013 (Thompson et al, 2013). But at the same time, ARMI score is less likely to detect the restoration success (Huddart et al, 2016), as a higher taxonomic resolution is required. ARMI is now planning to expand their target invertebrates to involve all easily identifiable invertebrate taxa, and as a result to increase the likelihood of detecting restoration outcome (Huddart et al, 2016).

1.2.3 Functional feeding groups

In addition to taxonomic groups, benthic macroinvertebrates could also be classified into different functional feeding groups based on their morpho-behaviour mechanisms of food acquisition, which could reflect the adaptation of species to environmental conditions (Uwadiae, 2010). A total number of five functional feeding groups were identified by Cummins (1974), which are scrapers, shredders, gathering collectors, filtering collectors and predators. One of the benefits is that invertebrate taxa within the same groups could be studied collectively, instead of hundreds of different invertebrate taxa. And many research showed that the distribution pattern of functional feeding groups was correlated to the environmental gradient in the aquatic systems (Uwadiae, 2010).

Different food sources are utilized by benthic macroinvertebrates with the food chain (Cummins, 1974). Detritus is considered as an important energy source for aquatic systems. It consists of two major categories, Coarse Particulate Organic Matter (CPOM, >1mm) and Fine Particulate Organic Matter (FPOM, 0.5μm-1mm), and CPOM could be further divided into the wood and non-woody materials. Aquatic invertebrates feeding on CPOM are shredders, such as Trichoptera and Plecoptera (Graca, 2001), which are responsible for converting COMP into FOMP in aquatic systems. FOMP deposited at stream bottom and suspended in the water column were mainly collected and consumed by gathering collectors and filtering collectors, respectively (Wallace and Webster, 1996). Invertebrates feeding on attached algae and associated detritus are known as scrapers, and invertebrates consuming other animals are called predators. Since the invertebrates are classified according to the way they feed, rather than what they eat, other groups such as scrapers may behave as detritivores in the absence of shredders, to some extent (Graca, 2001). For example, a high shredding effect of the gastropods *Melanopsis praemorsa* and *Physa acuta* on leaves were found in a Morocco stream lacking Trichoptera and Plecoptera (Chergui and Pattee, 1991). In addition to detrital CPOM, shredders may also feed on algal and macrophyte tissues (Friberg and Jacobsen, 1994), and even on other invertebrates (Solem and Johansson, 1991).

1.2.3.1 Functional feeding groups and organic matter decomposition

The important role of shredders in FPOM decomposition, particularly leaf litter decomposition has been well recognized. According to Cummins (1974), physical leaching, microbial degradation and invertebrate decomposition are involved when COMP entering the aquatic system. And as a consequence, more than 75% of CPOM could be converted into FPOM including plant fragments and a significant proportion of faeces, which indicates very low assimilation efficiencies of shredders (Wallace and Webster, 1996; Subekropp and Klug, 1976). Shredders are selective feeders, and Leaf litter decomposition is known as influenced by leaf quality (Graca, 2001; Leroy and Marks, 2006). Richardson et al (2004) implanted leaf litter from two conifer species (western red cedar and western hemlock) and one common deciduous species, in a coastal rainforest stream of British Columbia during summer and autumn. It was found that decomposition rates were positively correlated with the initial nitrogen content of leaf litter, but were negatively correlated with C:N ratios. A similar correlation between nitrogen content and the growth of larvae of *Aedes spp*. was observed by Walker et al (1997). Younger instar of *Anisocentropus kirramus* was found not able to ingest tough leaves (Nolen and Pearson, 1993), indicating the important role of leaf toughness in determining the selections among leaves, which agreed with the results found by Pennings et al (1998). But in most cases, the feeding preference may be influenced by combinations of several factors, rather than a single factor.

It has been reported that shredders prefer well-colonized CPOM (Graca, 2001), and a strong correlation between abundance of shredders and fungal biomass were discovered by Robinson et al (1998). One possible explanation is that fungi help with the assimilation process of shredders. Fungi and bacteria dominate the colonisation of CPOM, digest plant cell walls and transfer plant materials into edible compounds which can be assimilated by shredders (Jenkins and Suberkpopp, 1995). Shredders are known as selective feeders (Graca, 2001), and leave toughness was observed as one of the major contributors (Pennings et al, 1998). This preference could be satisfied by fungal colonization as it was found to decrease the leaf toughness (Jenkins and Suberkpopp, 1995). The other reasonable explanation is that fungi may be a better food source than leaves for shredders, in terms of nutrient contents. Based on the experiment conducted by Slansky and Scriber (1985), nitrogen content ranged from 1 to 7% in fungi mycelia, which was significantly higher than that in the senescent leaves (0.5-1.5%). And the preferential feeding of fungi was observed for both salt marsh snail *Littoraria irrorata* (Graca et al, 2001), caddisfly *Psychoglypha sp.* (Arsuffi and Suberkropp, 1989) and the isopod *Asellus aquaticus* (Graca et al, 1993).

FPOM generated by shredders provides major food sources for collectors, however, there is little evidence on the relationship between FPOM generated by shredders and its use by collectors (Graca, 2001). Both gathering and filtering collectors adapted to feed primary on surface-colonized FPOM, and depend on the microbial biomass associated with particle sizes (Vannote et al, 1980; Cummins and Klug, 1979). The partitioning of food sources with regard to the particle size was well documented for gathering and filtering collectors: filtering collectors collect and ingest the entire size range of fine particles from transport; while the size range of deposited fine particles that gathering collectors can ingest was demonstrated to depend on the morphology of mouth parts of gathering collectors (Cummins and Klug, 1979). Feeding activities of macroinvertebrates may alter the particle size in the aquatic system, and therefore have a significant effect on various collectors feeding in a size-dependent fashion (Wallace and Webster, 1996). For example, atyid shrimp in a Puerto Rican stream reduced the depositional particles, and followed with a reduction in the abundance of smaller collectors (Chironomid larvae). *Hexagenia limbata* nymphs may increase the particle size of deposition in some degree, by gathering and aggregating fine particles before ingestion (Zimmerman et al, 1975).

Some filtering collectors such as Simulium were found greatly contributed to the highest rates of seston removal (Morin et al, 1988). In addition to remove the suspended FPOM, those fine-particle feeders including Simulium and Bivalve, could ingest minute particles and provide large particles by egesting compacted fecal particles (Wallace and Webster, 1996). Some large-particle feeders prefer high-quality food items such as diatoms and animal drift, and thus affect the quantity and type of suspended POM (Benke and Wallace, 1980). Filtering collectors usually spend less energy in searching food, which makes it possible to have higher densities of filters than other functional feeding groups.

Scrapers, grazing on the food that adheres to surfaces, have morpho-behaviour adaptions for keeping their positions on exposed surfaces in turbulent water (Cummins and Klug, 1979). Many studies showed a significant correlation between algae and scraper abundance (Gregory, 1983). Light and nutrients will lead to the increase of algae abundance, which followed by an increase in the scraper abundance (Mulholland et al, 1983); while in verse, the removal of scraper will result in the increase of algae abundance. Yet other studies indicated that scrapers had no influence on algae abundance, especially when algae are light limited (Feminella et al, 1989) or when scraper abundance is low (Jacoby, 1987). Scrapers such as snails were found to increase the fine particle loadings exported downstream.

The relationship between the morpho-behavioural adaptations of invertebrate functional feeding groups and stream sizes were demonstrated in Figure 1 (Vannote et al, 1980). Streamside, riparian vegetation trapped in the stream channel is considered as the major energy input for aquatic systems (Cummins et al, 1989). Shredders were found as the most abundant functional feeding groups occurring at headwater stream, which was strongly influenced by the allochthonous organic matter. Along with the increased stream size, the controlling factor in invertebrate assemblages changed from allochthonous inputs and light in headwater streams to algae or rooted vascular plant production in medium-sized rivers. And therefore, the proportion of scrapers were maximized in medium-sized rivers. FPOM generated by shredders were transported downstream, which contributed to the reduced particle sizes as well as significantly dominance of collectors downstream. Gathering collectors usually are the most abundant invertebrates, and many gathering collectors such as Chironomids were frequently reported as the prey (Wallace and Webster, 1996). Predators, feeding on other animals, are greatly dependent on the abundance of prey.

The use of coarse (5mm) and fine meshed (0.5mm) litter bags are considered as a common method to study the role of benthic macroinvertebrates on leaf decomposition (Graca, 2001). And results showed that the decomposition rates in coarse meshed litter bags were approximately 15 times faster than in fine meshed bags when invertebrate abundances are high (Stewart, 1992); whereas, differences between coarse and fine meshed bags were comparatively low, and some studies even showed no difference with low densities of invertebrates (Stockley et al, 1998). Significant higher numbers of detritivores were observed on red alder and western red cedar with relatively higher decomposition rates in autumn, in a small stream of British Columbia (Richardson et al, 2004). Further convincing evidence was provided by Cuffney et al (1990) and Wallace et al (1995). Insecticide was used to reduce the invertebrates in a stream in North Carolina, USA, but not influence the microbial assemblages. The reduction of invertebrates led to a 50-74% reduction of leaf decomposition, and together with a reduction in FPOM export, indicating the important role of detritivores in leaf decomposition. Cotton strip was also used as an alternative method and the loss in tensile strength was used to measure the decomposition (Huddart et al, 2016).

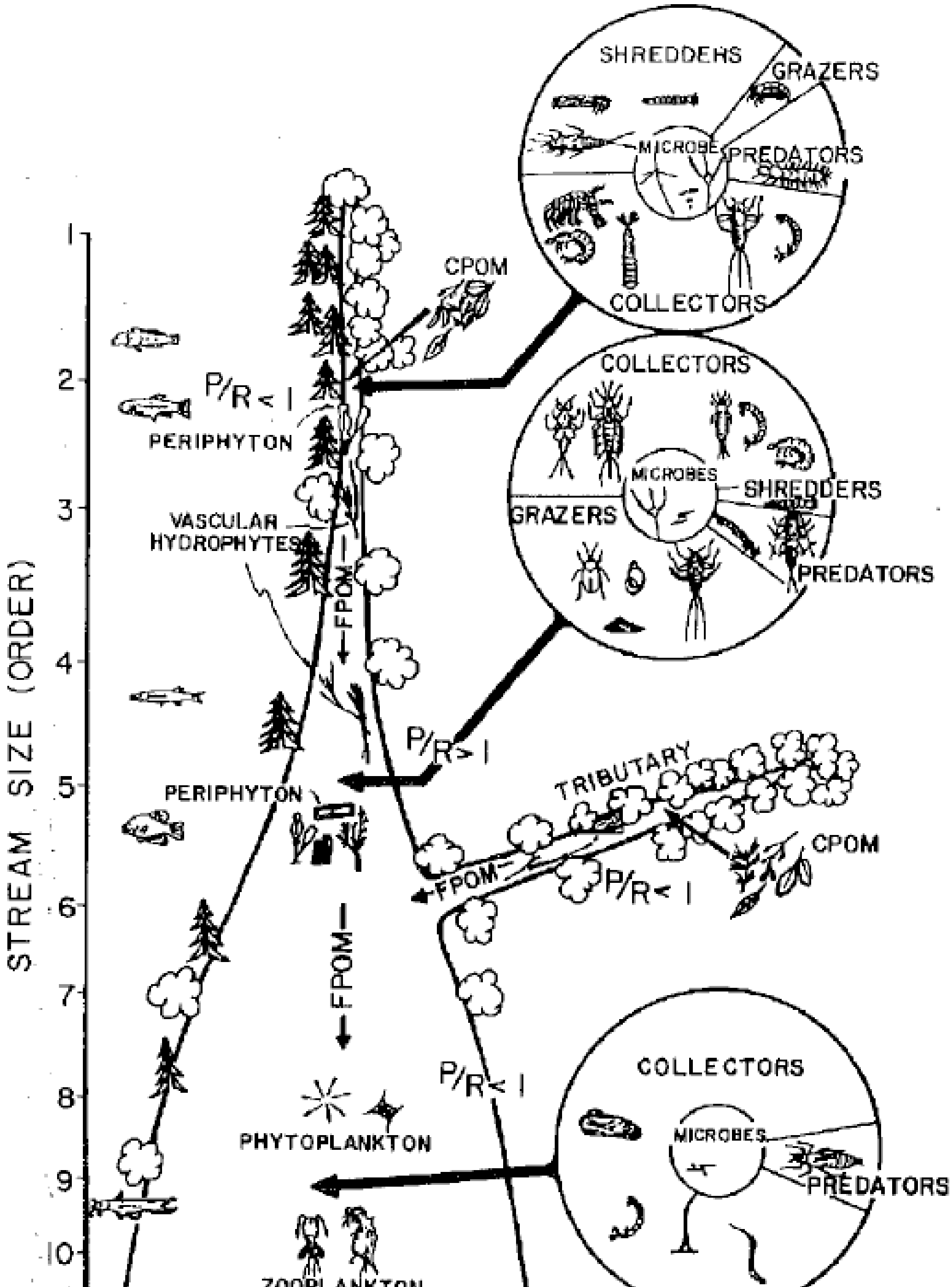


Figure 1: Demonstration of the River Continuum Concept. The change in invertebrate composition correlated with the shift in types and locations of food resources with stream size. Available from Vannote et al (1980).

1.3 Aims and objectives

Since colonisation trap is a brand new method, it is necessary to test whether it is working effectively follow its original design. It is the first time to investigate the interaction among water quality, benthic macroinvertebrates and organic matter decomposition by deploying colonisation traps site by site. And I will provide some personal advice after addressing the following questions: 1) what is the condition of invertebrate assemblages within colonisation traps, with the comparison of three-minute kick sampling? 2) What are the possible variables regulating the invertebrate abundance and diversity? 3) What is the major driver of invertebrate decomposition rates? In order to solve these questions, colonisation traps were deployed in three different rivers, two Countryside River (River Mimram and River Ash) and one urban river (River Crane). Invertebrate communities, biotic parameters and physical habitat conditions were investigated.

Chapter 2: Materials and methods

A total number of 16 sample sites were selected from three rivers: River Mimram (8 sample sites), River Crane (4 sample sites) and River Ash (4 sample sites). Fieldwork including MoRPH survey and deployment and collection of colonisation traps were undertaken on River Mimram, River Crane and River Ash during May, June and July, respectively. Since the MoRPH information of River Mimram was available in Modular River Survey (https://modularriversurvey.org/documents/), the MoRPH survey was only carried on River Crane and River Ash.

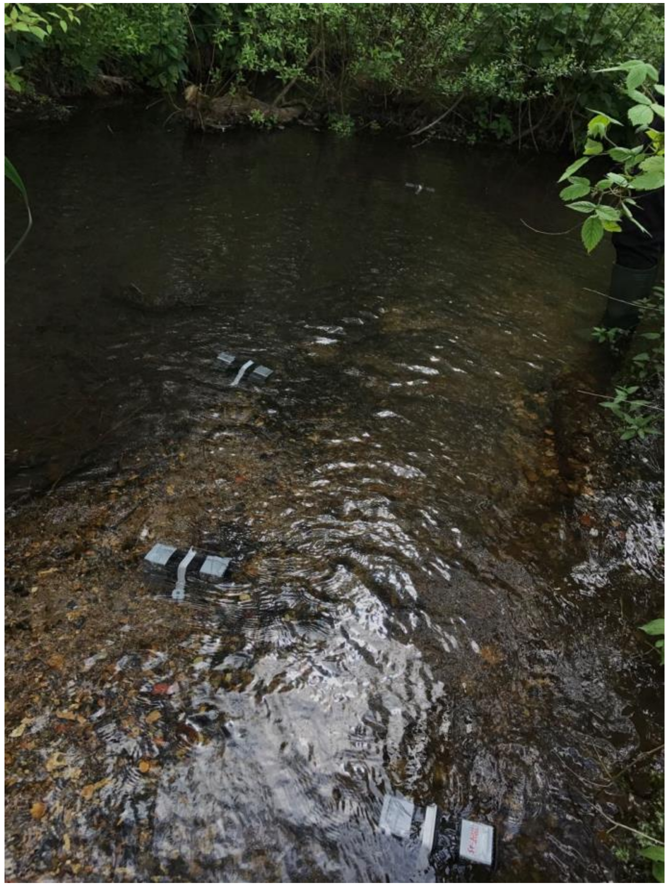
2.1 Study site

River Mimram, a typical chalk stream rises from the Hertfordshire chalk, joins with River Beane to meet the River Lee in Hertford (WWF, n.d.). It holds various habitats such as marsh, fen, meadows, ponds and wet grassland, for wildlife to thrive: River Mimram supports alder-rich woodland at Panshanger Park; one of the selected sample sites, Tewinbury is designated as Site of Specific Scientific Interest (SSSI); diverse species of birds (over 20 species) and fishes could be found in the Mimram (Cole, 2010), which greatly attracting fisherman and bird lovers. As the major sources of water, industry and recreation for people of Hertfordshire, River Mimram has experienced the significant over-abstraction over a long time period. It is estimated that the average daily abstraction amount in River Mimram is up to 14 million litres, and it could reach more than 20 million litres during peak weeks (WWF, n.d.). Even worse, the water demand is likely to increase with the increased households. A dry river through Welwyn garden city was observed in the year 2006. Luckily, people are willing to pay to improve the River Mimram, since the river status is closely linked to their daily life (Jacobs, 2002). ARMI are doing the regular monitoring activities monthly to detect and report any problems in the River Mimram. Eight sample sites were identified for this research, which was Hoo farm, Kimpton mill, Singlers marsh 1, Singlers marsh south end, Digswell meadow, Tewinbury, Panshanger diversion and Panshanger from upstream to downstream of River Mimram (Figure 2).

The second river, River Ash origins from the village of Brent Pelham in Hertfordshire and flows into the River Lea by passing through the Hadhams, Widford and Wareside. Lower Ash with lower scores of fish, macrophytes and river flow is failing to achieve the good ecological status (River Lea Catchment Partnership, n.d.). Hence, there are a great number of ongoing or planned restoration projects along the River Ash aiming to help improving the river, such as the sewage treatments in the upper Ash, installation of a series of deflectors in the river at Waters Place Farm, and installation of Sustainable Drainage Systems (SUDS) for water quality improvement and flood control (River Lea Catchment Partnership, n.d.). Weir removal is an alternative method to ease the fish movement in the lower Ash. Four weirs within a 250m stretch of the river between DS Widford STW and Hoghams are planned to be removed in this September, and therefore the need of pre-project evaluation on river status highlights the important role of River Ash in this research. According to the field investigation, more silty and muddy substrates and higher shading of the river caused by trees were observed in the River Ash (Figure 3), with the comparison of River Mimram. And colonisation trap is more suitable for invertebrate sampling in relatively deep water.

River Crane, the only urban river in this research, is selected mainly for contrast. It is a small tributary of the River Thames with a 35km length of the main channel (Crane Valley Partnership, 2016). A severe pollution incident was recorded on October 2011, when a large volume of sewage poured into the river. Associated sewage treatments followed with monthly monitoring have been conducted to help river returning to the pre-incident level. Four out of eleven monitoring sites were selected due to the limited time, and they are all located in the lower reach of the River Crane: Donkey wood crane, Donkey wood

DNR, Crane park island and Mill road weir (Figure 4). Donkey wood (crane and DNR) are surrounded by forests and the other two sample sites are within the crane park. Camping, swimming, family picnic those recreational activities were observed within the sample sites, together with lots of rubbish.



	Hoo farm	**Kimpton mill**	**Singlers marsh 1**
Left bank (m)	1	0.3	2.5
Right bank (m)	1	0.6	0.8
Bankful width(m)	4.5	3	5
Water width (m)	4.5	3	5
Water depth (m)	0.05	0.02	0.05

	Singlers marsh south end	Digswell meadow
Left bank (m)	1.3	0.6
Right bank (m)	0.4	0.1
Bankful width(m)	12	3.5
Water width (m)	10.75	3
Water depth (m)	0.2	0.05

	Tewinbury	Panshanger	Panshanger diversion
Left bank (m)	0.2	1.5	0.8
Right bank (m)	0.4	2	1
Bankful width (m)	10	4.5	6
Water width (m)	7.75	4	5
Water depth (m)	0.05	0.18	0.2

Figure 2: Photos taken at eight sample sites of River Mimram by author on 4 May 2017, showing different habitat conditions.

	Donkey wood DNR	Donkey wood crane	Crane park island
Left bank (m)	0.8	1.3	0.5
Right bank (m)	0.75	0.5	0.5
Bankful width (m)	7.8	11.2	10
Water width (m)	6	9.6	8.2
Water depth (m)	0.09	0.23	0.25

Figure 3: Photos taken at three sample sites of River Crane by author on 17 June 2017. A weir occurred in Donkey wood DNR, and colonisation traps were deployed at upstream of the weir.

	Weir 1	Weir 2
Left bank (m)	0.9	1.5
Right bank (m)	0.4	1.2
Bankful width (m)	8.6	8.7
Water width (m)	7.6	7.6
Water depth (m)	0.4	0.45

	Weir 3	Weir 4
Left bank (m)	1.3	0.5
Right bank (m)	1.1	0.2
Bankful width (m)	7.6	4.5
Water width (m)	5.5	4
Water depth (m)	0.54	0.34

Figure 4: Photos taken at four sample sites of River Ash by author on 14 July 2017, indicating higher shading level of riparin vegetaiton and deeper water.

2.2 Data collection

2.2.1 MoRPH survey

The Modular River Physical (MoRPH) survey, launched in late spring 2016, was developed by Queen Mary University of London and the Environmental Agency (Gurnell et al, 2016). It is a simple recording method specifically designed for citizen scientists to monitor and assess the quality of local physical habitat of river systems (Shuker et al, 2017). Non-specialists could easily conduct the MoRPH survey with the aid of MoRPH Technical Manual (Gurnell et al, 2016). And by repeating the MoRPH survey, the spatial and temporal changes of physical habitat conditions could be detectable.

A ‘module’ could extend 10m from the bank top edge on both sides of the river and the length of the module (10-40m) could derive from channel width. Such scale is suitable for centring on biological sampling site, and linking physical habitat monitoring to water and biological monitoring. The module is the smallest spatial unit and also is the basic element of the modular river survey. Therefore, it is expected that in combination with invertebrate monitoring, MoRPH survey conducted by citizen scientists could become a means of effectively understanding and monitoring the river restoration activities (Shuker et al, 2017). When it comes to a larger scale, information extracted from at least 10 continuous module surveys could provide a more compressive description of river habitats, across a river sub-reach in a length of 100-400m. Analysis based on aerial images combining with several MoRPH and MultiMoRPH surveys is greatly contributed to the HydroMoRPH survey, which is focused on the reach assessment.

During the fieldwork, basic hydraulic characteristics were measured first: bank height of both sides, deepest water depth, water and bankfull width. As a consequence, the module length could be achieved according to the water width. Anything observed in the river bank top, bank face and channel bed were recorded in the modular field survey sheet (ver. 7).

2.2.2 Colonisation traps

In order to capture the invertebrates and measure the invertebrate decomposition rates at the same time, colonisation trap was developed by Murray Thompson. It is made of the plastic drainage tube, and the two major components, coarse and fine mesh compartments are held together by electrical tape (seen in Figure 5a). Since the fine mesh could effectively keep the invertebrates out, invertebrate decomposition rates could be drawn from the difference between two compartments. Cloth paper is used as the food resources within the colonisation trap, which is considered to be more standardized decomposition substrate, compared to leaf litter. Cloth paper was cut into the similar size as the cross-section of a trap (50mm*50mm), weighed, and covered by a mesh bag. The coarse and fine mesh bags, allowing better collection of paper even it being decomposed into tiny pieces, were enclosed by a stapler (shown in Figure 5a). After that, mesh bag was placed into either end of the trap and corresponding mesh lid was fixed by electrical tape. The hole on the fine mesh compartment should be fully covered, to prevent any invertebrate entering the compartment.

Four colonisation traps were placed perpendicular to the flow randomly at each sample site (an example shown in figure 5b). Here are some tips for placing the traps: give priority to those places where covered by macrophyte to some degree or has less public access, so that traps are not too visible to be destroyed by the public. Avoid places where water is too shallow or too deep since traps should be fixed in the river bottom. Last but not least, traps will be easily lost due to strong currents.

Figure 5: Preparation (a) and deployment (b) of colonisation traps. Black arrow indicated the direction of water flow. Photo b was taken by Author at Tewinbury, River Mimram on 4 May.

Traps were collected after two weeks. The coarse mesh end should be lifted out first to prevent any loss of invertebrates, and the coarse mesh compartment was emptied by pouring contents into hand net. After removing any debris, invertebrates were then transferred to the white tray, counted, identified and recorded. Papers within coarse and fine mesh bags were removed from the trap, cleaned and stored in labelled plastic bags, separately. Above procedures were repeated to empty all the traps. Invertebrates were finally put back to the river, while papers were brought to the laboratory. Papers were released from mesh bags and cleaned carefully to wash out any attached material. They were then placed in labelled Petri dish separately and weighed after drying for one week (Figure 6).

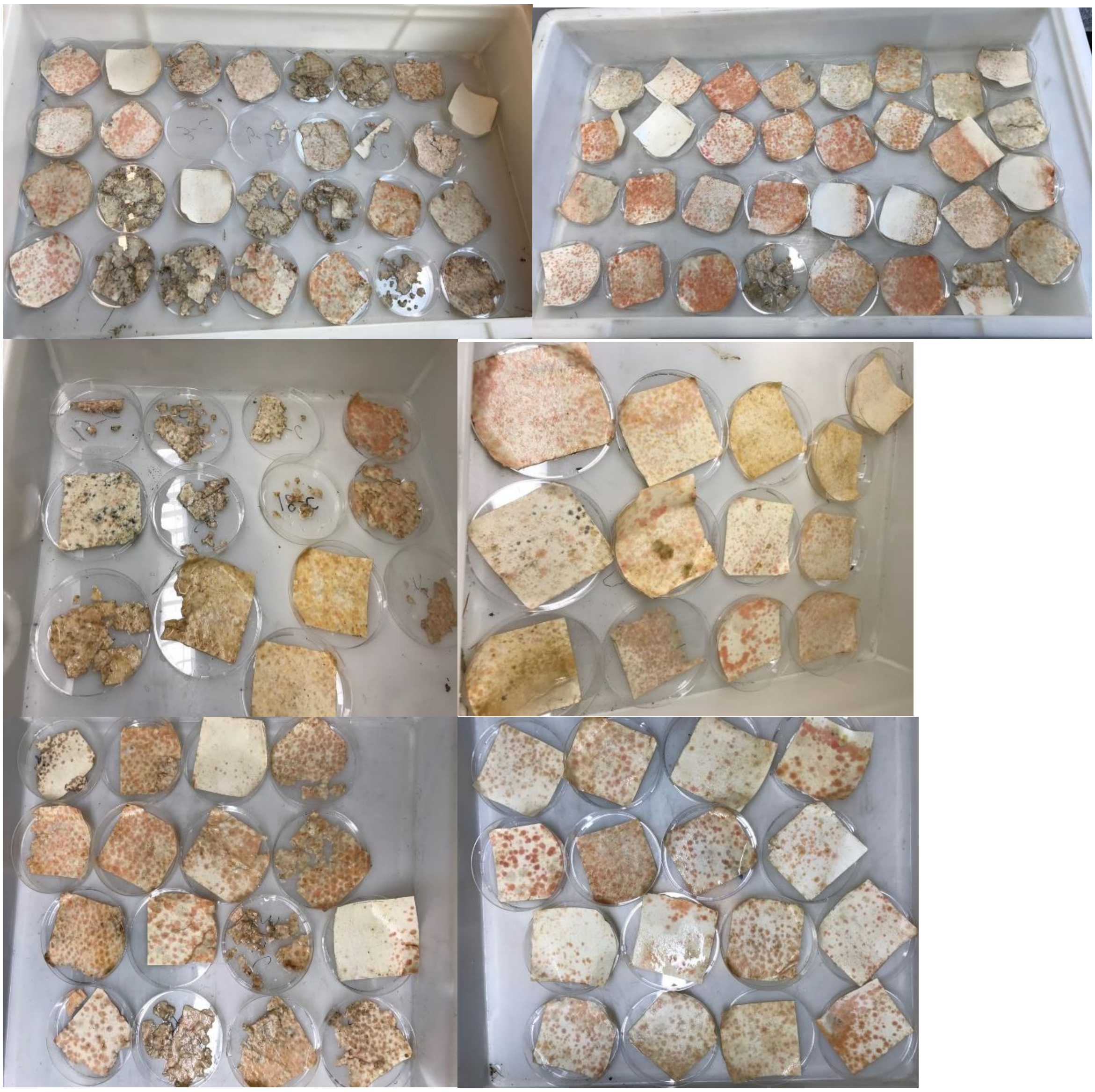


Figure 6: Paper colonised by both invertebrate and micro-organism (left), and paper only colonised by micro-orgnisms (right). Papers were collected from River Mimram, River Crane and River Ash from top to bottom.

2.3 Data analysis

Five variables could be achieved from each trap, invertebrate abundance, taxon richness (number of taxa), ARMI scores, invertebrate decomposition rates and microbial decomposition rates. Designated eight invertebrates with their abundance of a) 1-9; b) 10-99; c) 100-999; d) over 1000 were given scores of a) 1; b) 2; c) 3; d) 4, and thus ARMI score could be achieved by adding all eight scores. The loss of weight per day of papers within fine (Rf) and coarse (Rc) mesh compartments were calculated. As a result, the microbial decomposition rate equals to Rf, while the invertebrate decomposition rate was the difference between two rates (Rc-Rf). A total number of 14 indices could be calculated from each MoRPH survey sheet (Shuker et al, 2017), which covers river channel characteristics, riparian character and human pressures and impacts. However, statistical analysis was only based on 11 indices, with the exception of index 2 - highest energy extensive flow type, index 4 - coarsest extensive bed material particle size and index 6 - average bed material particle size class. Value of each index ranged from 0 to 10.

Invertebrates were classified into five functional feeding groups (FFGs), according to the guide developed by West Virginia Department of Environmental Protection (n.d.), Elliott et al (1988) and Kelly et al (2002). Dominant invertebrate taxa within sample sites or within rivers was determined by comparing the percentage distribution of different functional feeding groups. For River Mimram, invertebrate data collected by kick sampling on May was provided by Simon Stebbings, the Riverfly manager of River Mimram. By making the comparison, similarities and differences in invertebrate assemblages measured by kick sampling and colonisation traps could be characterized.

One-way ANOVA and subsequent post hoc Turkey test were used to determine any significant difference ($P<0.05$) in each variable between sample sites, and also between rivers. Linear regression analyses were carried out to test whether environmental variables had significant impacts on biotic parameters including invertebrate abundance, taxon richness, ARMI scores, invertebrate and microbial decomposition rates as well as the abundances of different functional feeding groups. In addition, linear regression was also used to discover the factors regulating the invertebrate decomposition rates. ANOVA and linear regression analyses were performed in SPSS Statistics 24 (IBM, 2016).

Multivariate analysis was used to investigate how the invertebrate distribution patterns related to environmental variables. Unimodal methods would be appropriate for this dataset, suggested by Detrended Correspondence Analysis (DCA axis 1 length =4.007 >4). Therefore, both Canonical Correspondence Analysis (CCA) and Correspondence Analysis were performed using CANOCO for Windows 4.5 (Leps and Smilauer, 2003). Data were logarithmically transformed to approximate normality and resulting values were Z-transformed for further analysis. As a consequence, three independent variables were identified: riparian physical habitat complexity, channel physical habitat complexity and riparian vegetation complexity.

Chapter 3: Results

Papers of four colonisation traps were found lost in River Mimram when collecting the traps, while even worse, six colonisation traps were removed or destroyed by the public in the River Crane. Therefore, statistical analysis is based on the remaining data.

3.1 Environmental variables

As shown in Table 1, a total number of four MoRPH indices were analysed to be significantly different among three rivers, which were riparian vegetation complexity, extent of non-native invasive plants, average bed material size and extent of bed siltation. River Mimram had a high complexity of vegetation on both river banks, large bed material size and low extent of bed siltation. And River Crane could be characterised as a river with high riparian vegetation complexity, small average bed material size and low extent of bed siltation. Although the average bed material size of River Crane was slightly different from that of River Crane, they all indicated the same bed material class, which was gravel-pebble (Gurnell et al, 2016). In addition, River Ash was low in riparian vegetation complexity, but high in coverage of bed siltation. No invasive plants were found in River Mimram, whereas, River Crane was greatly colonized by non-native invasive species.

Table 1: Comparison of 11 MoRPH indices among River Mimram, River Crane and River Ash. Bold number indicated statistically significant differences at P<0.05 level, and each river differing in the post hoc Turkey tests was given a different letter (a, b). Values are averages ± 1 standard deviation.

MoRPH indices	River Mimram (n=8)	River Crane (n=3)	River Ash (n=4)	F	P
Channel physical habitat complexity	1.538±0.825	3.000±1.179	1.750±0.557	3.378	0.069
Number of aquatic vegetation morphotypes	2.375±0.916	4.000±1.000	2.250±0.957	3.788	0.053
Riparian physical habitat complexity	1.200±0.411	1.400±0.100	1.250±0.311	0.352	0.711
Riparian vegetation complexity	$3.525 \pm 0.875_a$	$3.933 \pm 0.577_a$	$2.050 \pm 0.885_b$	5.551	**0.02**
Degree of human pressure imposed by land cover on the bank top	1.188±1.438	0.333±0.577	0.125±0.250	1.414	0.281
Reinforcement	1.238±1.708	2.667±3.553	0.000	1.606	0.241
Extent of non-native invasive plants	0.000_a	$5.000 \pm 1.803_b$	$0.775 \pm 0.802_a$	39.558	**<0.001**
Number of bed material types	1.750±0.707	2.000±1.000	1.500±0.577	0.4	0.679
Average bed material size (phi)	$0.775 \pm 1.726_a$	$(-2.233) \pm 0.635_b$	$(-1.250) \pm 0.500_{ab}$	6.426	**0.013**
Extent of bed siltation	$0.125 \pm 0.231_a$	0.000_a	$3.125 \pm 1.315_b$	29.164	**<0.001**
Number of flow types	1.000±0	1.333±0.577	1.250±0.5000	1.341	0.298

3.2 Biotic parameters

A number of statistically significant differences at the P< 0.05 level in ARMI scores, invertebrate and microbial decomposition rates were found between rivers (Table 2). Results of Turkey post hoc test indicated that River Ash (1.688±1.138, P= 0.002) had significantly lower ARMI scores than River Mimram (3.464±1.774); invertebrate decomposition rates sampled from River Mimram (0.009±0.014, p= 0.005) were slower compared to that from River Crane (0.026±0.018); and River Mimram (0.007±0.004, P<0.001) and River Ash (0.007±0.005, P<0.001) had significantly slower microbial decomposition rates than River Crane (0.016±0.006).

Table 2**:** Comparison of invertebrate abundance, richness, ARMI scores and decomposition rates among River Mimram, River Crane and River Ash. Bold number indicated statistically significant differences at P<0.05 level, and each river differing in the post hoc Turkey tests was given a different letter (a, b). Values are averages ± 1 standard deviation.

	River Mimram (n=28)	River Crane (n=10)	River Ash (n=16)	F	P
Invertebrate abundance	40.643±39.351	58.800±39.220	38.438±32.688	1.06	0.354
Taxon richness	6.071±2.418	4.400±1.955	5.000±1.713	2.707	0.076
ARMI scores	$3.464 \pm 1.774_a$	$2.300 \pm 1.636_{ab}$	$1.688 \pm 1.138_\mathrm{b}$	6.813	**0.002**
Invertebrate decomposition rates (g/day)	$0.009 \pm 0.014_a$	$0.026 \pm 0.018_b$	$0.013 \pm 0.007_{ab}$	5.786	**0.005**
Microbial decomposition rates (g/day)	$0.007 \pm 0.004_a$	$0.016 \pm 0.006_b$	$0.007 \pm 0.005_a$	0.582	**<0.001**

Variations of invertebrate abundance (Figure 7), ARMI scores (Figure 8) and invertebrate decomposition rates (Figure 9) within rivers, especially within the River Mimram, were greater than between rivers. On the contrary, little variation of taxon richness and microbial decomposition rates were demonstrated in Figure 10 and Figure 11. It is obvious that Kimpton mill had significantly higher invertebrate abundance, ARMI scores and invertebrate decomposition rates compared to other sample sites in the River Mimram. Sample sites within River Crane only significantly differed in invertebrate abundance (Figure 7), which means, Donkey wood crane (105.67±27.54) and Donkey wood DNR (21.5±3.32) had the greatest and smallest invertebrate abundance, respectively. Similarly, no significant difference in biotic parameters was found between sample sites of River Ash, with the exception of invertebrate abundance. Weir 2 had significantly higher invertebrate abundance than other three sites.

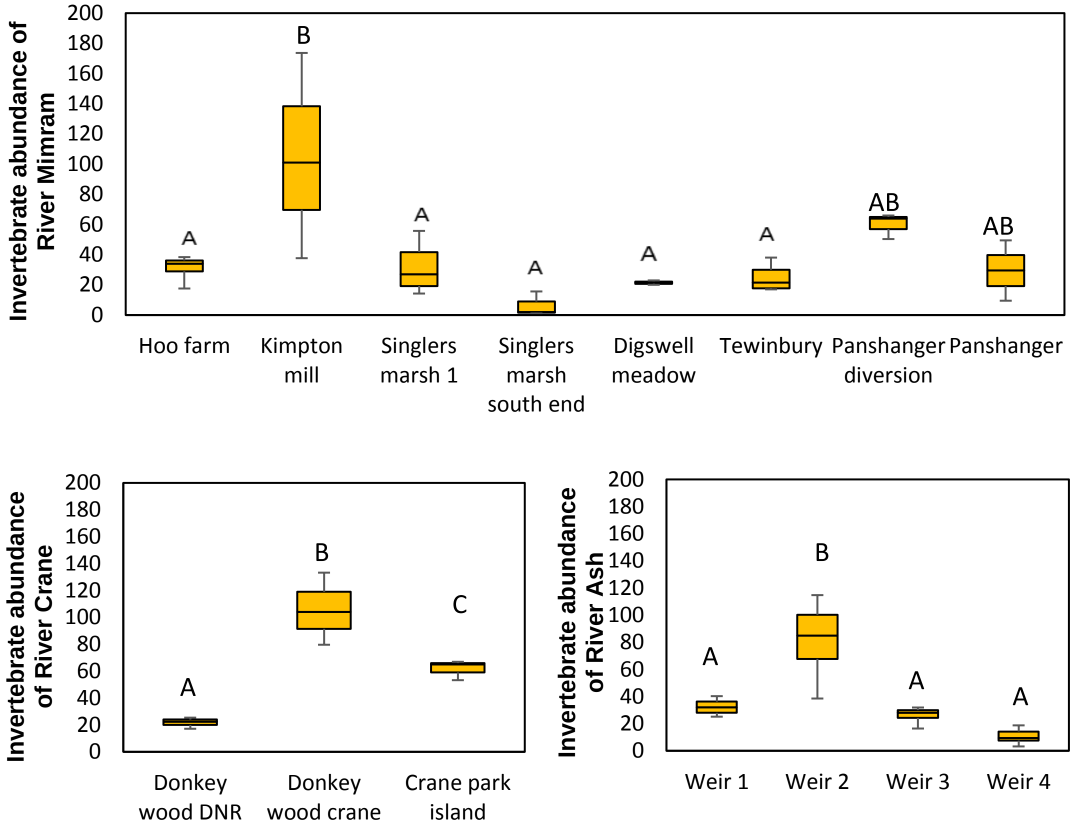


Figure 7: Variations of invertebrate abundance within River Mimram, River Crane and River Ash. The letters (A, B, C) reflect statistically significant differences (P<0.05) based on ANOVA with a subsequent Turkey test. Error bars indicated 95% confidence interval.

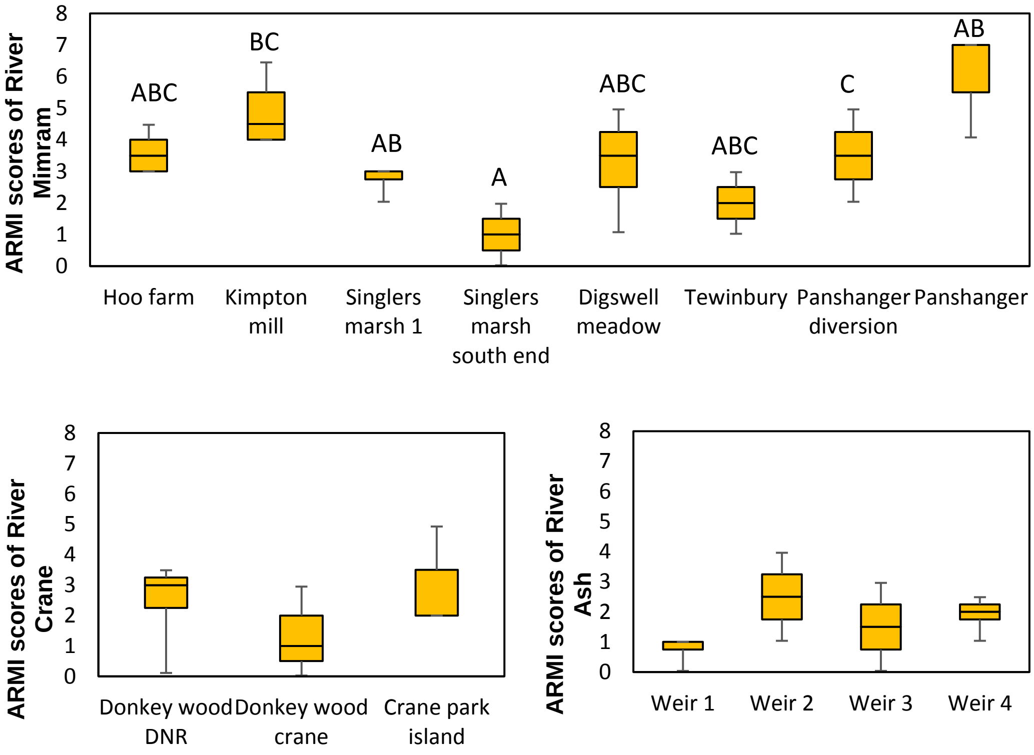


Figure 8: Variations of ARMI scores within River Mimram, River Crane and River Ash. The letters (A, B, C) reflect statistically significant differences (P<0.05) based on ANOVA with a subsequent Turkey test. Error bars indicated 95% confidence interval.

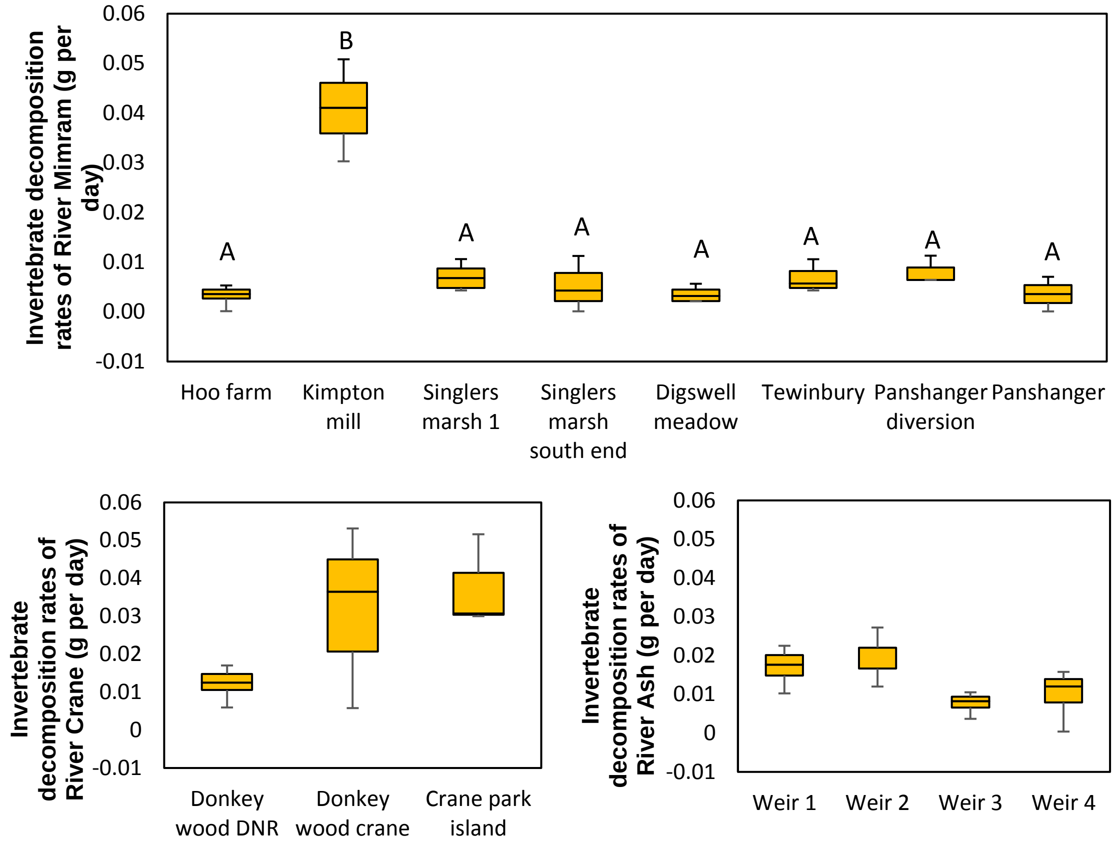


Figure 9: Variations of invertebrate decomposition rates within River Mimram, River Crane and River Ash. The letters (A, B) reflect statistically significant differences (P<0.05) based on ANOVA with a subsequent Turkey test. Error bars indicated 95% confidence interval.

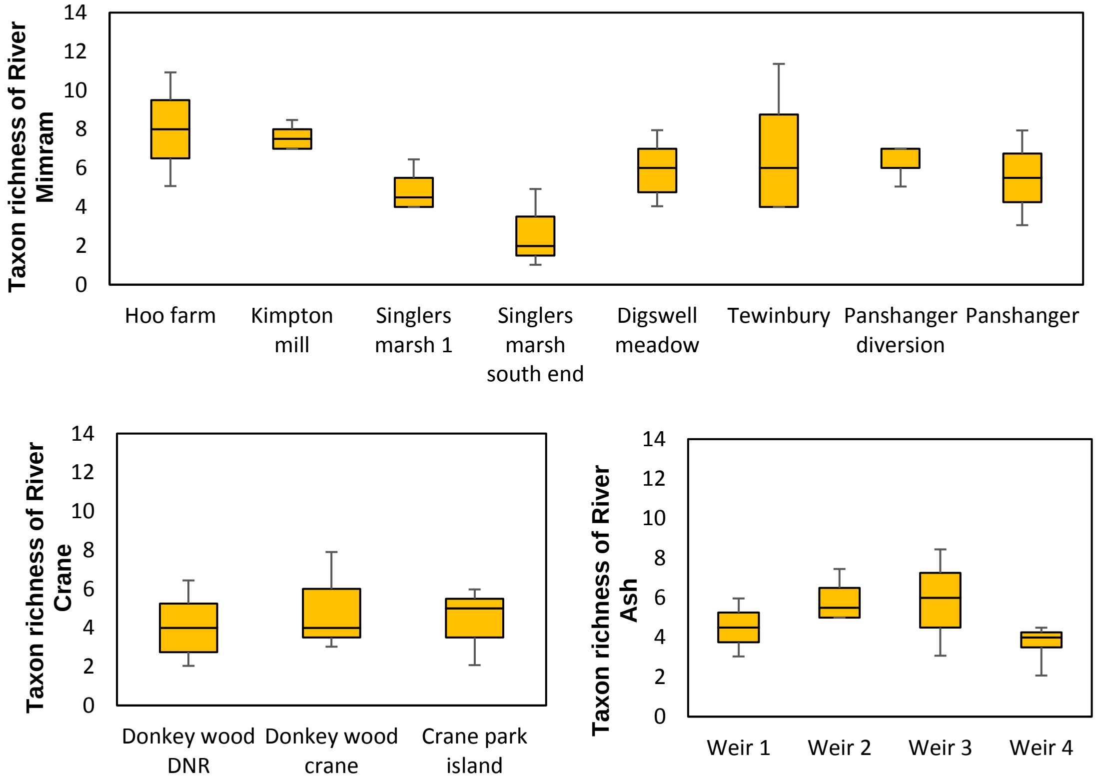


Figure 10: Variations of taxon richness within River Mimram, River Crane and River Ash. Error bars indicated 95% confidence interval.

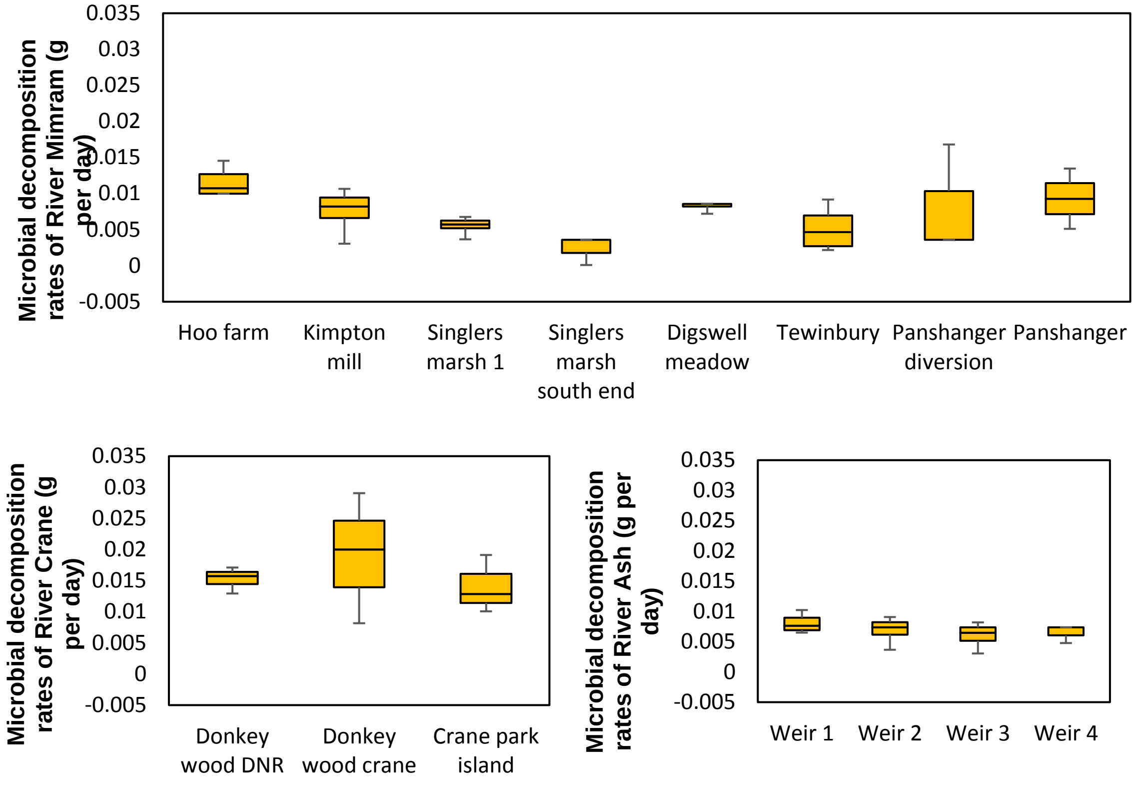


Figure 11: Variations of microbial decomposition rates within River Mimram, River Crane and River Ash. Error bars indicated 95% confidence interval.

3.3 Community composition and diversity

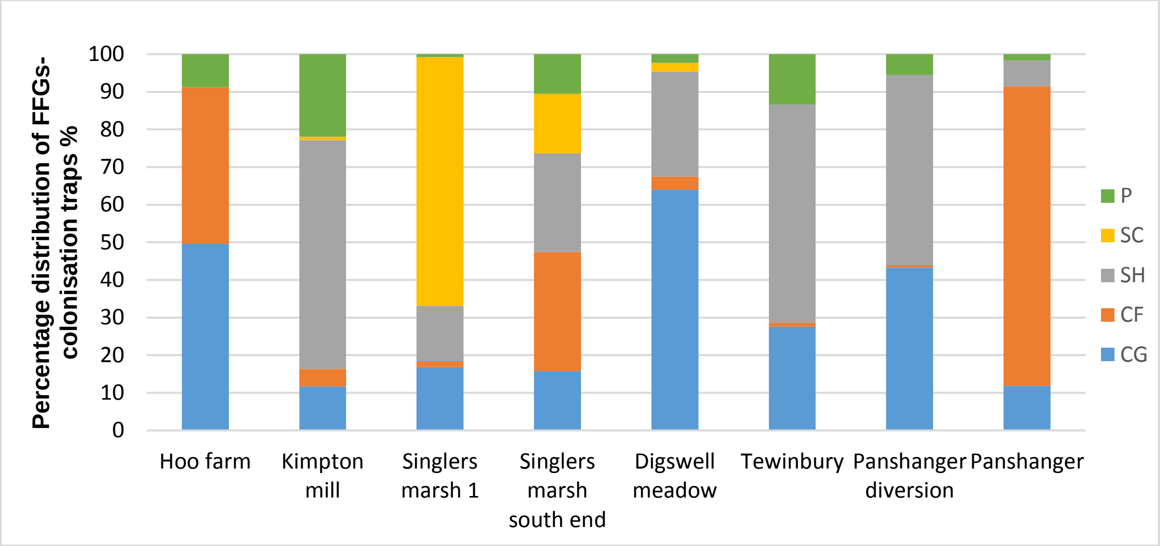
3.3.1 River Mimram

A total number of 21 invertebrate taxa were collected from River Mimram (n=29), including nine gathering collectors, five predators, four filtering collectors, three shredders, and one scraper (Table 3). The frequency of occurrence varied greatly between 1 and 20 out of 28 traps. Ephemerellidae had the highest frequency of occurrence (20 out of 28 traps), followed by Gammaridae (19), Baetidae (16), Cased caddisfly (16) and Turbellaria (15). On the contrary, Ephermeridae (1 out of 28 traps), Plecoptera (1), Corixidae (1) and Beetle larvae (1) were considered as rare taxa in River Mimram. The total abundance of invertebrates was 1138, with an average of 40 per trap. An extremely high abundance of shredders was recorded, which contributed to 40.86 % of the total abundance and together with gathering collectors (26.89%), predators (11.86%) and filtering collectors (11.60%). Gammaridae, accounting for 98.71% of the total abundance of shredders, was the most abundant invertebrate taxa in the River Mimram (40.33%). Turbellaria, Simulium, Ephemerellidae and Gastropoda accounted for most of the abundance of predators, filtering collectors, gathering collectors and scrapers, respectively.

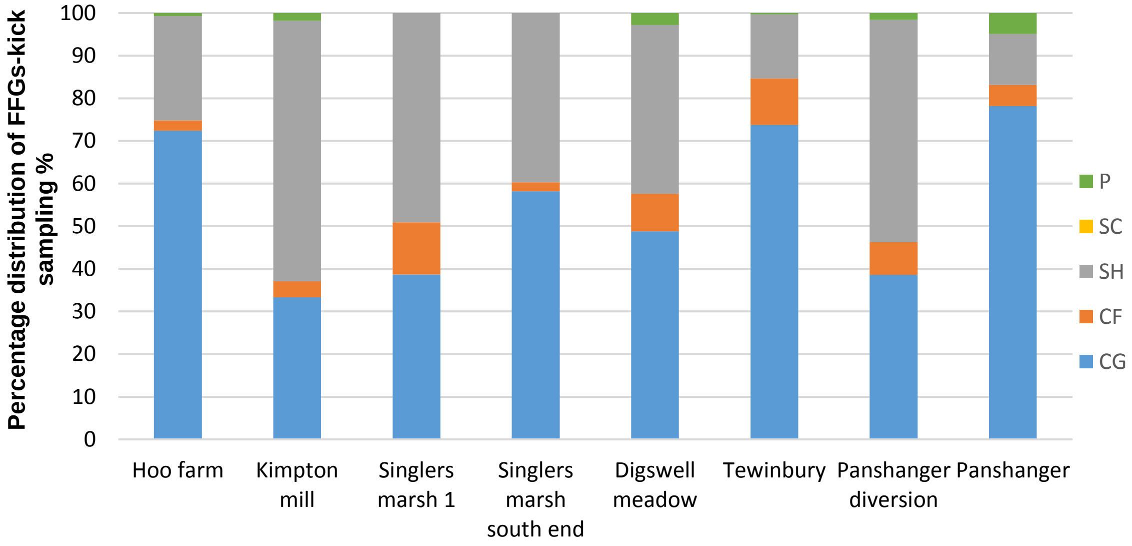
Table 3: Invertebrate abundance and associated frequency of occurrence of each invertebrate taxa collected from three rivers. Each taxa was classified into different functional feeding groups (FFGs). CG= gathering collectors; CF= filtering collectors; SH= shredders; SC= scrapers; P= predators.

FFGs	**Invertebrate taxa**	**River Mimram (n=28)**		**River Crane (n=10)**		**River Ash (n= 16)**	
		Invertebrate abundance	Frequency of occurrence	Invertebrate abundance	Frequency of occurrence	Invertebrate abundance	Frequency of occurrence
CG	Baetidae	32	16	2	2	14	6
CG	Ephemerellidae	111	20	-	-	-	-
CG	Asellidae	17	7	231	6	181	13
CG	Annelids	16	5	49	7	-	-
CG	Chironomidae	47	11	13	2	2	2
CG	Leptophlebiidae	8	6	-	-	-	-
CG	Caenidae	2	2	-	-	-	-
CG	Cased caddisfly	69	16	3	2	2	2
CG	Fly larvae	5	3	1	1	-	-
CF	Ephermeridae	1	1	-	-	-	-
CF	Simulium	111	9	-	-	-	-
CF	Caseless caddisfly	20	13	4	4	6	4
CF	Bivalve	-	-	9	1	37	7
SH	Gammaridae	459	19	225	8	55	12
SH	Plecoptera	4	1	-	-	-	-
SH	Elmidae (adult)	2	2	-	-	6	4
SC	Gastropoda	99	9	41	7	289	14
P	Corixidae	1	1	-	-	-	-
P	Hydrachnidae	4	4	-	-	1	1
P	Hirudinea	13	**9**	10	4	14	9
P	Beetle larvae	1	1	-	-	-	-
P	Turbellaria	116	15	-	-	-	-
P	Sialidae	-	-	-	-	7	5
Sum		**1138**		**588**		**615**	

Further details were presented in the percentage distribution of functional feeding groups of each sample sites (Figure 12), and average values were used due to the uneven number of traps survived in different sample Sites. Invertebrate functional feeding group assemblages varied from upstream to downstream of the River Mimram. Hoo farm was significantly dominated by collectors (91.2%), including gathering collectors (49.6%) and filtering collectors (41.6%). Of all three invertebrate taxa within the group of filtering collectors, Simulium contributed to 80.77% and 100% of the total abundance of filtering collectors at Hoo farm and Panshanger, respectively. The abundance of predators and shredders were maximized in the Kimpton mill, which contributed to 67.14% and 51.90% of the total abundance of predators and shredders, respectively. As the only taxa within the group of scrapers, Gastropoda was the dominant taxa within Sinlgers marsh 1 and was responsible for 90 % of total abundance of gastropods. A relatively even distribution of functional feeding groups occurred at Singlers marsh south end although it had the lowest invertebrate abundance among eight sample sites.



CG	16	13	6	1	14	7	26	4
CF	13	5	1	2	1	0	0	24
SH	0	65	5	2	6	15	30	2
SC	0	1	23	1	1	0	0	0
P	3	24	0	1	1	4	3	1



CG	302	331	63	220	259	494	166	79
CF	10	37	20	8	47	73	33	5
SH	102	607	80	150	210	101	224	12
SC	0	0	0	0	0	0	0	0
P	3	18	0	0	15	2	7	5

Figure 12: Abundance and associated percentage distribution of functional feeding groups along River Mimram. Invertebrate data measured by colonisation traps (figure above) and kick sampling (figure below). CG=gathering collectors; CF=filtering collectors; SH=shredders; SC=scrapers; P=predators.

Obviously, the invertebrate data collected by means of kick sampling indicated that scraper was absent in the River Mimram. The total invertebrate abundance was significantly higher along the river according to Figure 12, but more diverse invertebrates were sampled by colonisation traps. Gathering collectors were observed as the dominant feeding groups at Hoo farm, Singlers marsh south end, Digswell meadow, Tewinbury and Panshanger owing to the high abundance of Baetidae, cased caddisfly and Ephemerellidae. Whereas, the other sample sites were mainly occupied by shredders. Kimpton mill was the site with the highest invertebrate abundance because of the occurrence of shredders, which was quite consistent with the results sampled by colonisation traps. The most important taxa of Kimpton mill, Gammaridae contributed 60.51% and 61.13% to the total invertebrate abundance sampled by colonisation traps and kick sampling, respectively. However, results of linear regression analysis suggested that kick sampling and colonisation traps were significant different methods in terms of the invertebrate abundance, taxon richness and ARMI scores (Figure 13).

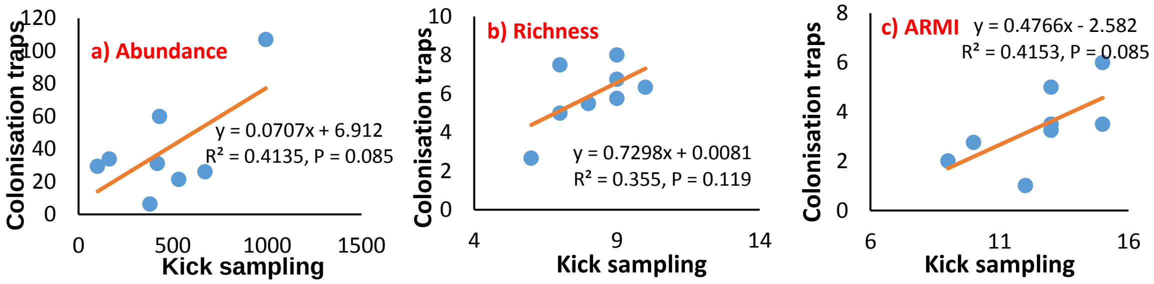
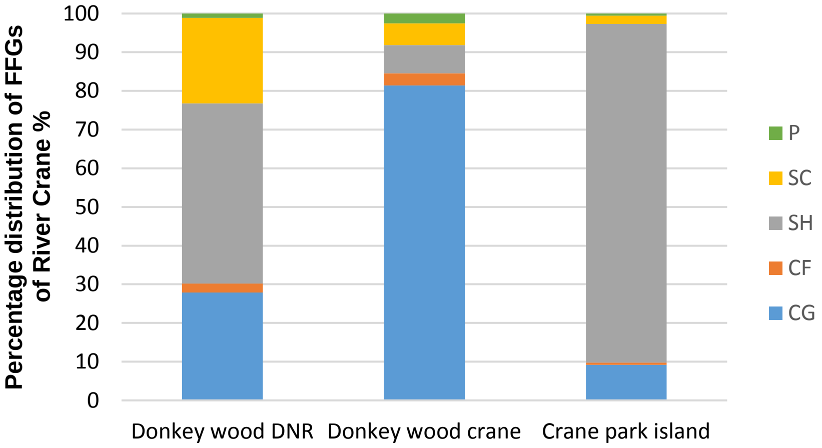


Figure 13: Results of linear regression analysis on invertebrate taxa measured by kick sampling and colonisation traps, in terms of invertebrate abundance (a), taxon richness (b) and ARMI scores (c).

3.3.2 River Crane

Invertebrates sampled from River Crane (n=10) with low diversity had a quite different invertebrate assemblage with that of River Mimram (n=28). 11 invertebrate taxa consisted of six gathering collectors, two filtering collectors, one scraper, one predator and one shredder. Those taxa with high abundance in River Mimram were absent in River Crane: Ephemerellidae, Turbellaria and Simulium. Gammaridae (38.27 %), the secondary abundant taxa, could be easily found in River Crane with the highest frequency of occurrence (8 out of 10 traps). Additionally, Asellidae (39.29%), Annelids (8.33%) and Gastropoda (6.97%) also had relatively high frequencies of occurrence.

Completely different invertebrate assemblages along River Crane were recorded in Figure 14. Compared to other two sample sites, Donkey wood DNR had the lowest invertebrate abundance, which was mainly contributed by gathering collectors (27.27%) and shredders (45.45%). Asellidae was the most dominant taxa within Donkey wood crane and accounted for 87.21% of total abundance of gathering collectors, and 70.15% of total invertebrate abundance. An extremely high abundance of shredders (88.52%), represented by Gammaridae, was observed at Crane park island. Neither filtering collectors nor predators play an important role in invertebrate assemblages of River Crane.



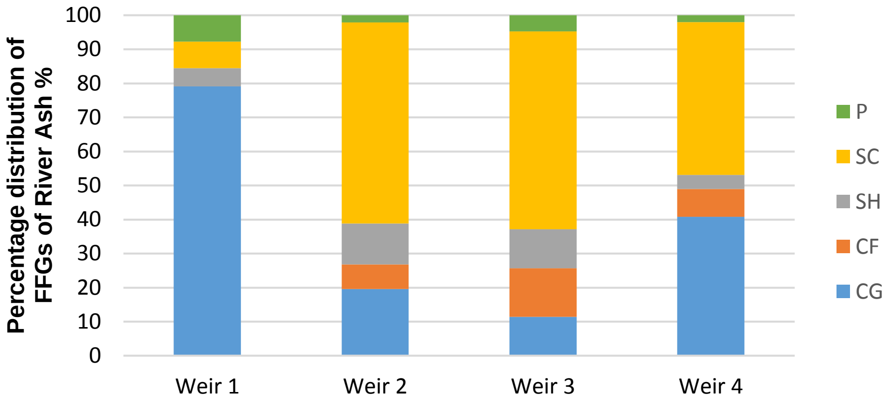
CG	6	86	6
CF	1	3	0
SH	10	8	54
SC	5	6	1
P	0	3	0

Figure 14: Abundance and associated percentage distribution of different functional feeding groups within River Crane. CG=gathering collectors; CF=filtering collectors; SC=scrapers; SH=shredders; P=predators.

3.3.3 River Ash

Unlike other two rivers, the total invertebrate abundance sampled from River Ash was partially controlled by Gastropoda (46.99%). It was also recorded as the most widely distributed taxa in River Ash, with the frequency of occurrence of 14 out of 16 traps. (Gathering collectors including four invertebrate taxa were ranged as the secondary dominant group (32.36%), and Asellidae best represented gathering collectors with a frequency of occurrence of 13. Gammaridae with a high frequency of occurrence had a total abundance of 55, which was significantly lower than other two rives.

Scrapers and gathering collectors were two major contributors, as reflected in Figure 15. Asellidae in Weir 1 contributed to 76.47% of the total invertebrate abundance of Weir 1, and 52% of the total abundance of collector gathering, respectively. Gastropoda was found mainly occurred in Weir 2, with a total number of 49. The number of Gastropoda gradually decreased downstream, and Weir 4 in the lower stream had the lowest invertebrate abundance as well.



CG	26	16	3	5
CF	0	6	4	1
SH	2	10	3	1
SC	3	49	15	6
P	3	2	1	0

Figure 15: Abundance and associated percentage distribution of functional feeding groups along River Ash. CG=gathering collectors; CF=filtering collectors; SH=shredders; SC=scrapers; P=predators.

3.4 Multivariate analysis

A PCA of the environmental variables only resulted in one principal component (Eigenvalues > 1, Table 4), which explained 54.25% of the total variability in the data. This principle component represented chiefly channel physical habitat complexity (loading: 0.847) and to a lesser degree riparian physical habitat complexity (0.711), and riparian vegetation complexity (0.636). Performance of three environmental variables within PC1 on biotic parameters was demonstrated in Table 5, and it varies between rivers. Negative correlations were observed in River Mimram. The change of invertebrate abundance, ARMI scores and invertebrate decomposition rates were significantly negatively correlated to environmental variables; while the change of taxon richness was only influenced by channel physical habitat complexity. Of all five functional feeding groups, gathering collectors were likely best represented the total invertebrate abundance. The abundance of shredders was strongly linked to the channel physical habitat complexity and riparian vegetation complexity, owing to the contribution of Gammaridae. Riparian vegetation complexity was also found to influence the abundance of predators.

In terms of River Crane, the total invertebrate abundance and abundance of gathering collectors were likely to increase with the increase of riparian physical habitat complexity; whereas, the riparian vegetation complexity was negatively correlated to total invertebrate abundance and invertebrate decomposition rates as well. There was no relationship occurred between riparian physical habitat complexity and biotic parameters from River Ash. The total invertebrate abundance, invertebrate decomposition rates and the abundance of gathering collectors were found to increase with the increased complexities of channel physical habitat and riparian vegetation. Additionally, the riparian vegetation complexity had a positive impact on the abundance of predators.

Table 4: Factor loadings of each environmental parameter in a Principle Components Analysis.

Parameter	Loadings on PC1
Channel physical habitat complexity	0.847
Riparian physical habitat complexity	0.711
Riparian vegetation complexity	0.636
Explained variance	2.194

Table 5: Results of regression analysis between biotic parameters and environmental variables. The abundance of different functional feeding groups together with the abundance of the characteristic taxa, Gammaridae was also included. Bold number indicated statistically significant relationships at P<0.05 level. CG=gathering collectors; CF=filtering collectors; SH=shredders; SC=scrapers; P=predators.

	Channel physical habitat complexity			Riparian physical habitat complexity			Riparian vegetation complexity		
	Mimram	Crane	Ash	Mimram	Crane	Ash	Mimram	Crane	Ash
Invertebrate abundance	**-0.336**	0.248	**0.547**	**-0.402**	**0.938**	-0.08	**-0.534**	**-0.819**	**0.503**
CG abundance	**-0.515**	**0.684**	**0.647**	**-0.431**	**0.851**	0.062	**-0.474**	-0.523	**0.857**
CF abundance	0.302	0.331	0.068	0.213	0.382	0.017	0.116	-0.223	0.001
SH abundance	**-0.253**	-0.908	0.469	-0.246	0.03	-0.083	**-0.461**	-0.466	0.386
SC abundance	-0.04	0.368	0.326	-0.284	0.073	-0.131	0.083	0.104	0.191
P abundance	-0.266	0.408	0.26	-0.269	0.538	0.337	**-0.398**	-0.342	**0.592**
Gammaridae	**-0.264**	-0.908	0.508	-0.256	0.03	-0.166	**-0.461**	-0.466	0.361
Taxon richness	**-0.45**	0.093	-0.023	-0.25	0.221	0.266	-0.168	-0.176	0.128
ARMI scores	**-0.586**	-0.378	0.159	**-0.474**	-0.287	-0.369	**-0.592**	0.105	-0.174
Invertebrate decomposition rates (g/day)	**-0.335**	-0.285	**0.636**	**-0.347**	0.497	-0.069	**-0.526**	**-0.634**	**0.688**

The invertebrate decomposition rates in River Mimram were influenced by total invertebrate abundance, taxon richness, ARMI scores as well as the abundance of shredders and predators (Table 6). The abundance of Gammaridae was also found related to the invertebrate decomposition rates, which further identified the important role of shredders. The total invertebrate abundance together with the abundance of scrapers were two major contributors in River Ash. With regard to data collected from River Crane, no significant relationship was found between invertebrate decomposition rates and other biotic parameters.

Table 6: Results of regression analysis indicated differernt factors regulating the invertebrate decomposition rates (g per day). CG=gathering collectors; CF=filtering collectors; SH=shredders; SC=scrapers; P=predators. Bold number indicated statistically significant relationships at P<0.05 level

	Invertebrate decomposition rates (g/day)	
	Mimram	Ash
Invertebrate abundance	**0.846**	**0.611**
CG abundance	0.127	0.422
CF abundance	-0.1	0.266
SH abundance	**0.796**	0.417
SC abundance	-0.01	**0.474**
P abundance	**0.815**	0.373
Gammaridae	**0.797**	0.404
Taxon richness	**0.273**	0.144
ARMI scores	**0.51**	0.135

A Clear community association of invertebrate taxa across all rivers was revealed in Figure 16. Plots within River Mimram showed high heterogeneity due to those unique invertebrate taxa, such as Simulium, Beetle larvae, Plecoptera and Turbellaria (see Table 3). High frequency of occurrence of Gammaridae led to the cluster of plots within River Mimram and River Crane, similarly, the majority of plots within River Ash were located near Gastropoda and Asellidae. It was obvious that the invertebrate composition was completely different between River Mimram and River Ash, since they were plotted on the positive and negative sides, respectively.

On the CCA plot (Figure 17), sample sites within River Crane and River Ash were mainly plotted on the positive side, whereas, sample sites within River Mimram were mainly plotted on the negative side, which indicated different physical habitat conditions along Axis 1. The relationship between environmental variables and 23 invertebrate taxa were also demonstrated in Figure 18. Hirudine, Annelids, Beetle larvae and Plecoptera were occurred at sample sites with high riparian physical habitat complexity, while Asellidae was mainly found in the sample sites with high channel riparian physical habitat complexity.

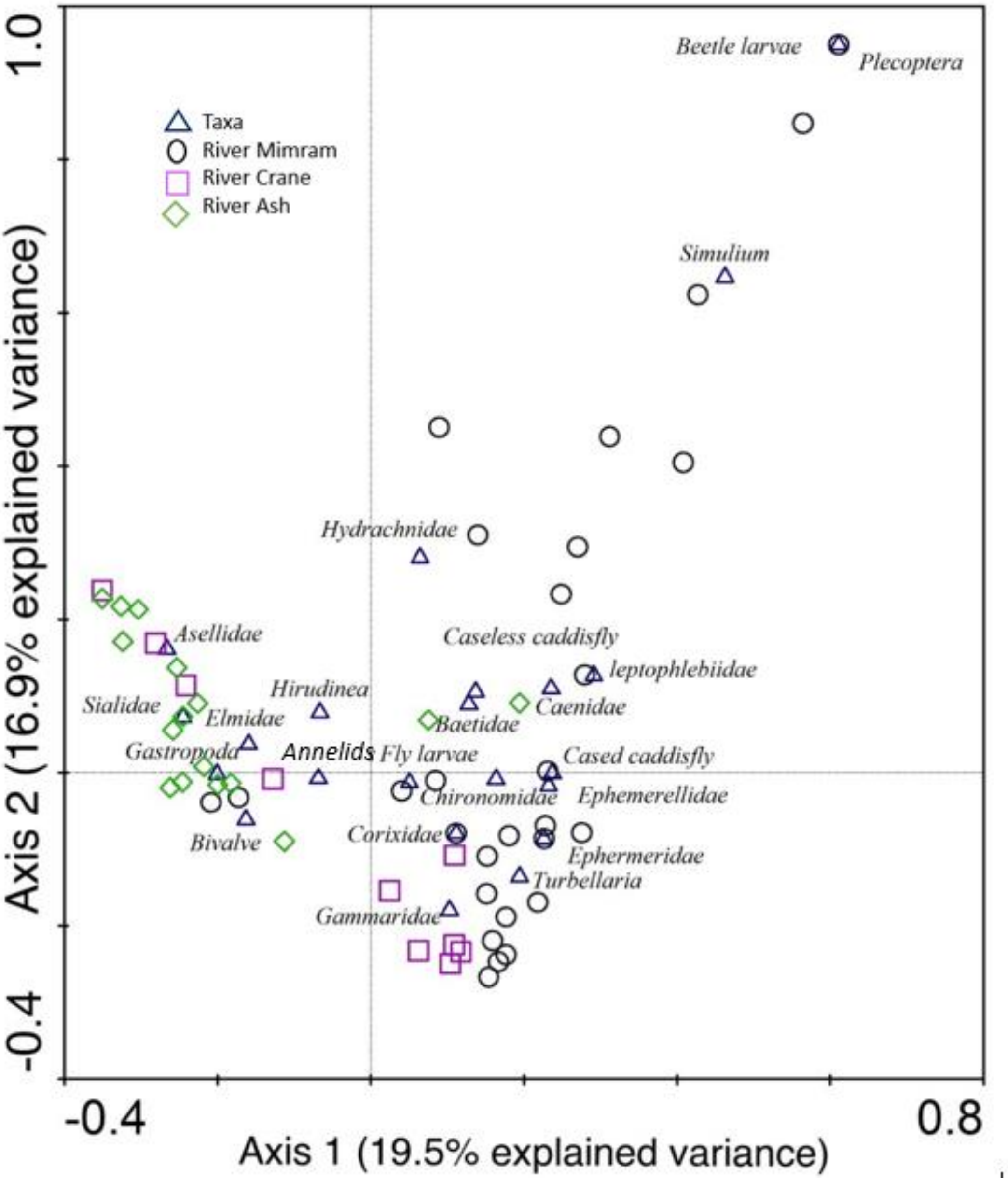


Figure 16: Correspondence Analysis (CA) of invertebrate taxa for three rivers. Taxa was represented by triangles, and plots were coded accroding to different rivers.

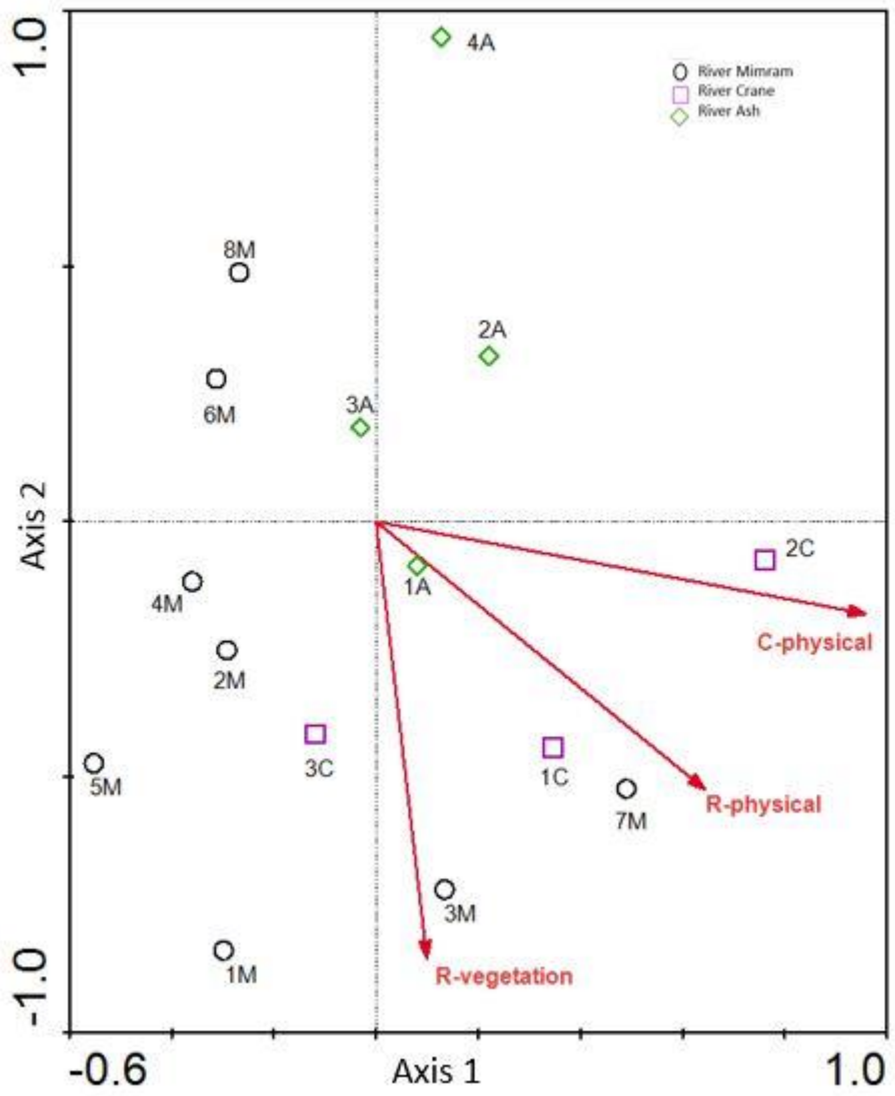


Figure 17: Canonical correspondence analysis of samples and environmental variables. Plots were coded according to different rivers. 1M= Tewinbury, 2M= Digswell meadow, 3M= Singlers marsh south end; 4M= Singlers marsh 1; 5M= Hoo farm, 6M= Kimton mill, 7M= Panshanger, 8M= Panshanger diversion; 1C= Donkey wood DNR, 2C= Donkey wood crane, 3C= Crane park island; 1A= Weir 1, 2A= Weir 2, 3A= Weir 3, 4A= Weir 4.R-physical= Riparian physical habitat complexity; C-physical= Channel physical habitat complexity; R-vegetation= Riparian vegetation complexity.

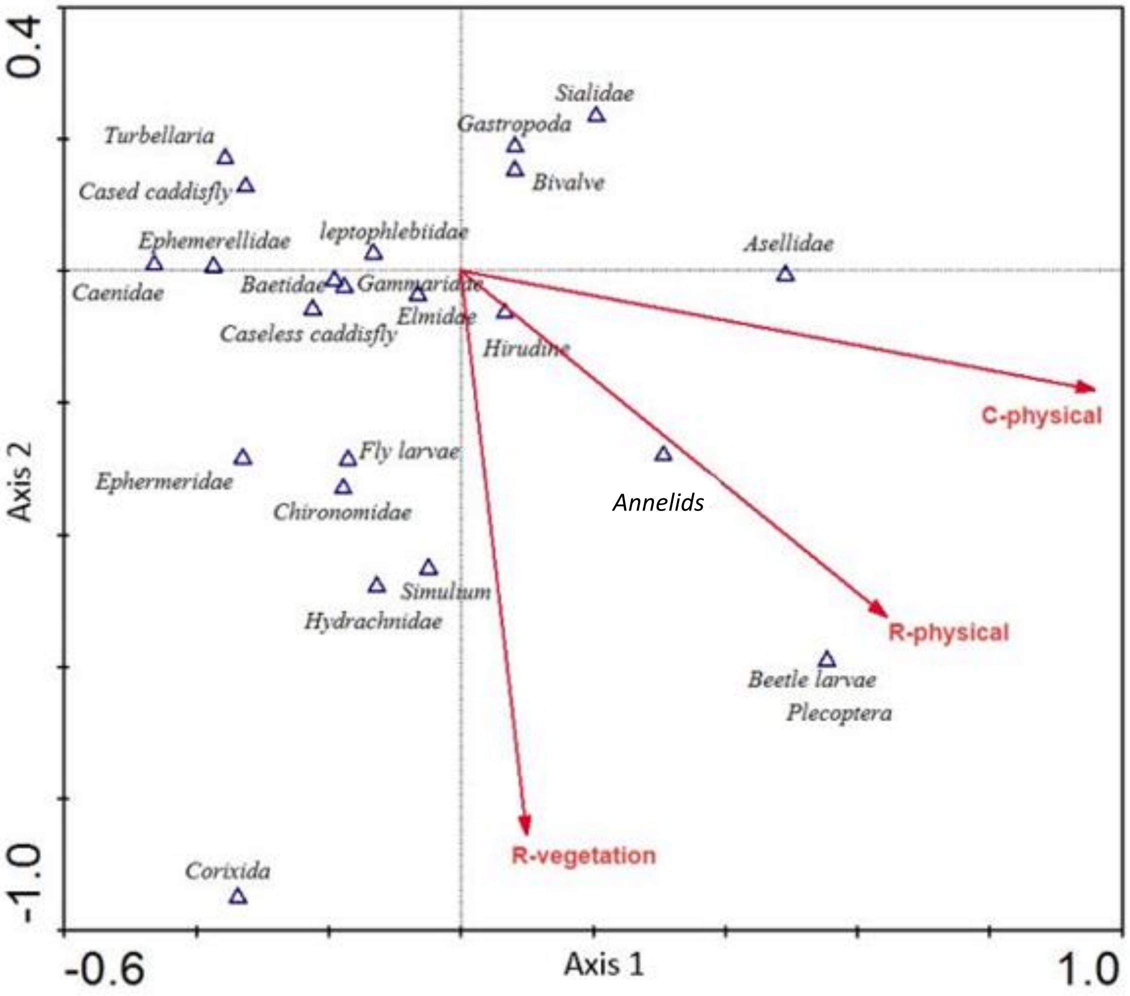


Figure 18: Canonical correspondence analysis of invertebrate taxa and environmental variables. Taxa was represented by triangles. R-physical= Riparian physical habitat complexity; C-physical= Channel physical habitat complexity; R-vegetation= Riparian vegetation complexity.

Chapter 4: Discussion

4.1 Benthic macroinvertebrate characteristics

Multivariate analysis indicated different patterns in benthic macroinvertebrate community structure between two Countryside River (River Mimram and River Ash), associated with different physical habitat conditions. Macroinvertebrate assemblages in River Ash were dominated by Gastropoda and Asellidae. Those taxa are able to live in stressful environments with lower BMWP scores. High densities of Asellidae could be found in the highly degraded system, such as rivers with a high level of organic pollution, or with low oxygen and PH (Maltby, 1995; Moldovan et al, 2001). The relatively low taxon richness and invertebrate abundance and dominance of pollution-tolerant taxa revealed poor water quality of River Ash, which consisted with ARMI scores. Moreover, River Ash had significantly higher extent of bed siltation and a higher level of shading caused by riparian vegetation than other two rivers, which consistent of the field observation (Figure 4). The occurrence of weirs in River Ash is likely to result in the accumulation of sediments and deep water. These factors might able to constrain the macroinvertebrate assemblages in River Ash as well. River Mimram had significantly higher ARMI scores, together with diverse sensitive taxa such as Ephemerellidae, Trichoptera and Plecoptera. Those taxa are known to be sensitive to environmental changes (Zhang et al, 2014), and most of them were found in the upstream such as Hoo farm and Kimpton mill.

Gastropoda was best represented scrapers in this study, and its presence was reported to lead to a large reduction in periphyton biomass, primary productivity and phosphorus uptake (Mulholland et al, 1983). Gammaridae being the dominant part of the benthic macroinvertebrate assemblages in both River Mimram and River Crane (Table 3). The crustacean sub-order Gammaridae includes over 4500 species, and has been found widespread throughout a diverse range of freshwater habitats (Macneil et al, 1997). Gammarus spp. is the amphipod genus with the highest number of freshwater species, and was often found under rocks, among the living and dead vegetation, such places could provide shelter from predators and food resources (Macneil et al, 1997). A negative correlation between the abundance of Gammaridae and Asellidae was observed in this study, that is, higher numbers of Gammaridae were likely to occur at sample site with much fewer Asellidae. Gammaridae, in particular Gammrus spp. were also predators of other invertebrates, such as Isopod (Mninshall, 1967), and therefore have the potential to regulate the density of prey (Macneil et al, 1997). Furthermore, in addition to predator-prey interaction, Williams and Moore (1985) found the competitive advantages could shift from *Gammarus pseudolimnaeus* to *Asellus communis* in response to the increasing cyanide pollution. And as a result, the Gammarus: Asellus ratio could be a possible monitoring indicator of water quality.

According to the proposed longitudinal distribution of invertebrate functional feeding groups (Vannote et al, 1980), the dominant role of shredders is always found in the headwater streams and is strongly linked to the amounts of allochthonous detritus by the riparian vegetation. With the increased export of FPOM generated by shredders, collectors should increase in the importance and dominate the macroinvertebrate assemblages in the lower streams. And the degree of shading becomes the important factor (Minshall, 1978). However, the longitudinal patterns of three rivers did not quite consistent with the proposed distribution pattern. Riparian vegetation is known to strongly link to the invertebrate functional compositions in the aquatic system (Cummins et al, 1989), which is probably the key reason for this inconsistent.

Results suggested that the habitat heterogeneity was the major factors governing the macroinvertebrate assemblages (Table 4), including channel and riparian physical habitat complexity and riparian vegetation complexity. The more complex the habitat, the more living space or surface area, the more diverse the invertebrate communities could be (Shosttell and Williams, 2007; McGoff et al, 2013). The environmental variables used in this paper are only physical habitat conditions, but other factors such as nutrient enrichment, sediment organic matter content were also found significantly affecting the invertebrate assemblages (Zhang et al, 2014).

However, the performance of those environmental factors occurring at three rivers was contradictory. In River MImram, more diverse invertebrate assemblages were tended to occur in less complex habitats, in particular in Kimpton mill. In contrast, positive relationship between invertebrate assemblages and habitat heterogeneity were observed in River Ash. The position of sample sites may matter. Four sample sites of River Ash were located closely and their habitats were similarly affected by weirs, but on the other hand, eight sample sites of River Mimram were located from upstream to downstream of the river, covering a wide range of habitats. The other possibility could be that the MoRPH survey is quiet subjective. The MoRPH survey was only conducted in River Crane and River Ash by myself, and Ellie did the survey in the River Mimram. Furthermore, since colonisation traps were deployed randomly in the sample site, it may not cover all different sub-habitats that recorded in the MoRPH survey. But this reason appears to be less reliable because a negative correlation between ARMI scores and channel physical habitat complexity were also found in the invertebrate data collected by three-minute kick sampling (r= -0.0636, P=0.045).

4.2 Primary factors regulating invertebrate decomposition rates

The processing of organic matter within the aquatic system was concluded as a series of processes of physical leaching, microbial colonisation and invertebrate decomposition. The breakdown of organic matter, in this case, the cloth paper would represent the vegetation fallen from the riparian and could provide a closer description of what actually happened in the river (Menendez et al, 1989). Reddish-brown spots were randomly distributed in the cloth paper as displayed in Figure 3, and it is highly likely due to the action of microorganisms. Micro-organisms such as fungi and bacteria, could soften the paper, increase the nutrient availability and contribute to the digestion of some invertebrates (Cummins and Klug, 1979). In the field experiment, Gastropoda *Physa acuta* was observed dispersed over the whole batch of leaves, while in contrast, Gammaridae only dispersed over some leaves (Chergui and Pattee, 1988). This preference on conditioned leaves might be a possible reason for the uneven distribution of reddish-brown spots in papers, but further analysis on the features of cloth papers are required, such as the strength, thickness and nutrient content of papers, just to name a few. The differences between papers from coarse and fine meshed bags (Figure 3) would suggest that the activity of invertebrate shredders are required for the further breakdown of organic matters.

Overall, factors including current velocity, nutrient availability (nitrogen in particular), microbial species and invertebrate abundance were found directly influence the breakdown of leaves (Chergui and Pattee, 1991). Invertebrates being consumers at intermediate trophic levels, have bottom-up and up-down effects within the aquatic system (Wallace et al, 1999). The fundamental issue that was addressed in this study was the primary factors controlling invertebrate decomposition rates. Results revealed that total invertebrate abundance was directly linked to invertebrate decomposition rates within two countryside rivers. It could be further broken down to the role of specific functional feeding groups, as the functional composition of invertebrate communities has important implications in the trophic system (Uwadiae, 2010).

Shredders, mainly feeding on coarse particles, played a crucial role in the ecosystem functioning of River Mimram. Gammaridae was the most efficient shredder in the River Mimram (Table 6), which was consistent with the results found in the Rhone system (Chergui and Pattee, 1991). It was quite reasonable. Gammaridae is of vital importance in the detritus processing in running waters, and serve as important constituents of fish food (Hou and Sket, 2016). Studies on gut structure indicated that *Gammarus pulex* is able to ingest the plant material in foregut using its own enzymes called cellulases (Macneil et al, 1997). It could be the possible reason for Gammaridae being the most efficient shredder when competing with other invertebrates without enzymes. A significant positive correlation between *G. pulex* in situ feeding rates and associated total leaf decomposition was also observed by Maltby et al (2002), suggesting the *G. pulex* in situ feeding rate could be a useful indicator of ecosystem functions. Moreover, the *G. pulex* in situ feeding rate was also proven to be a suitable indicator of water quality, as it responds differently to a range of effluents (Maltby et al, 2002). Environmental factors such as water temperature, alkalinity and dissolved oxygen were found directly inhabit the *G. pulex* feeding rates. For example, 76% of the variation in the *G. pulex* situ feeding rate could be accounted for by water temperature, and it was demonstrated that a 90% reduction of feeding rate of 8 to 10 mg animals occurred when water temperature decreased from 15 °c to 2 °c (Maltby et al, 2002).

In addition, the organic matter decomposition rate was also directly influenced by taxon richness, water quality (ARMI scores) and even the abundance of predators, in River Mimram. The important role of predators was mainly due to the functional link in the aquatic system (Vannote et al, 1980). Predators are known to feed on other functional groups and highly dependent on the prey abundance. Therefore, increased abundance of other functional feeding groups had the potential to increase the abundance of predators. Water quality has been already reported to be an important factor affecting the decomposition rates (Graca, 2001; Pascoal et al, 2001), and the better the water quality, the faster the invertebrate decomposition rate could be. But studies showed the effects of water quality are contradictory. Pascoal et al (2003) found that nutrient enrichment could accelerate the leaf breakdown rates, together with an increase in abundance and a decrease in richness of macroinvertebrates. In contrast, leaf decomposition rates were observed to be negatively correlated with the mine effluent discharge (Bermingham et al, 1996) and the concentration of dissolved zinc (Niyogi et al, 2001). Further analysis on the water chemistry is required to investigate the interaction between water quality and invertebrate decomposition rates, even though it is not the major aim of this study.

A special shredding effect of Gastropoda was observed in River Ash, in the case of lacking shredders. It agreed with the findings of Chergui and Pattee (1991), and Brady and Turner (2010). Experiments were conducted in the Moulouya system, and Gastropoda *Melanopsis peraemorsa* were proven to be an efficient taxon in terms of the organic matter decomposition. Brady and Turner (2010) discovered the important role of Gastropoda in detritus processing, in particular in the smaller and forest-enclosed lentic systems, which was quite consistent with the status of River Ash. Furthermore, Gastropoda was also found feeding on unconditioned leaf matrix (Chergui and Pattee, 1991), which might contribute to the high shredding effect.

Although significantly highest invertebrate and microbial decomposition rates were demonstrated in Table 2, the invertebrate decomposition rate in River Crane was only found inversely correlated with the riparian vegetation complexity (Table 5). River Crane was observed to have the highest extent of non-native invasive plants, and I would argue the decomposition rates were primarily influenced by litter quality. Litter quality has been determined to account for approximately 97% of the variation in decomposition rates in the first week (Leroy and Marks, 2006), and faster decomposition rates were observed with exotic species (Ashton et al, 2005).

4.3 Limitations

The designation of functional feeding groups could be a problem, since the benthic macroinvertebrates were roughly identified to any easily identifiable level in the field. And also the feeding actions of some invertebrates are highly age/size dependent (Feminella and Stewart, 1986). For example, the larvae of riffle beetle *Elmidae* is assigned as the scraper, while the adult is grouped as the shredder, according to West Virginia Department of Environmental Protection (n.d.). And processing time is an important factor influencing the organic matter decomposition. Within the first week, the average weight lost per day by *M. peraemorsa* was 48mg, which was approximately equivalent to its own dry biomass (Chergui and Pattee, 1987). But after that, the weight lost by *M. peraemorsa* gradually decreased due to the decreased quality of leaf litter. Similarly, litter quality accounted for 97% of the variation in decomposition rates in the first week, but only accounted for 45% by week 8 (Leroy and Marks, 2006). Cloth paper had been decomposed for two weeks in this study, which successfully detects the factors regulating the invertebrate decomposition rate. But different time periods are worthwhile to try, to find a proper processing time that can better reflect the actual trophic system.

Chapter 5: Conclusion

River Mimram had significantly better water quality than River Ash, which was in accordance with the different invertebrate assemblages and physical habitat conditions. Multivariate analysis indicated that habitat heterogeneity was the primary factors influencing the macroinvertebrate assemblages. The more complex the habitat, the less invertebrate abundance and diversity, and also poor water quality in River Mimram. But habitat heterogeneity was positively correlated with macroinvertebrate assemblages in both River Crane and River Ash, with the exception of riparian vegetation complexity in River Crane. Different performances of physical habitat conditions on macroinvertebrate assemblages may due to the factors including the positions of sample sites and the subjective MoRPH survey.

Overall, the invertebrate decomposition rate is a sensitive indicator of detecting the changes in river ecosystem, and the most important factors regulating the invertebrate decomposition rates are invertebrate abundance and the abundance of detritivores such as Gammaridae and Gastropoda, relatively dominated in River Mimram and River Ash. The more detritivores in the aquatic system, the faster the organic matter decomposition rates could be. Taxon richness and ARMI scores were only found correlated with invertebrate decomposition rates in River Mimram, and no direct factor was found in River Crane.

The colonisation trap seems to be an appropriate method that could be applied by citizen scientists. Therefore, for the next stage, more colonisation traps should be deployed at different rivers to obtain a broad scale of relationship. I would give some suggestions based on my experience: first, at least four traps should be deployed at each sample site, and more traps should be prepared for the public site. Mill road weir in the River Crane is a negative example. It is located within the Crane park, which has been widely used for recreation purpose. Three out of four traps were destroyed by the public, so that the sample site was no longer be useful. Secondly, the partition within some colonisation traps was found broken, and some macroinvertebrates were also found in the fine meshed compartments, which to some degree could result in the error in decomposition rates. So it is necessary to make sure the equipment are well prepared. Last but not least, two weeks is a proper processing time period for invertebrates to consume the cloth paper and to display the variations.

Auto-critique

I originally chose this study due to my interest in aquatic invertebrates, and how it could be engaged with citizen science. I really enjoyed in collecting and identifying the invertebrates when we went to the North Norfolk for MSc field course. And I was very keen to work with relevant wildlife conservation organization, and to understand how to apply the knowledge learnt from classes in reality. Consequently, the opportunity appealed to me and I was very keen to participate in the project.

I think strengths of this project was the application of multivariate indicators, involving MoRPH indices, biotic indicators and ecosystem function indicators. It is the first time to deploy the colonisation traps site by site and to investigate the major drivers of invertebrate decomposition rates, which makes it special and meaningful. However, invertebrates were identified into inconsistent levels in the field, as the idea of analysing invertebrate assemblages based on functional feeding groups was came out after the data collection. I probably misclassified several invertebrates.

In hindsight, I would read more literature in prior to the data collection, and most importantly, I would choose the same sample sizes of three rivers to obtain better comparisons.

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