Investigating ecological impacts of invasive freshwater demon shrimp (*Dikerogammarus haemobaphes*, Eichwald, 1841) in Lea Valley and Colne Catchment, UK, using citizen science tools.

CBVX2

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MSc Conservation

I, LEE Dong Jin

hereby declare:

(a) that this MSc Project is my own original work and that all source material used is acknowledged therein;

(b) that it has been prepared specially for the MSc in Conservation of University College London;

(c) that it does not contain any material previously submitted to the Examiners of this or any other University, or any material previously submitted for any other examination.

Signed : ____LEE DONG JIN____

Date : ______27.08.2019_______

Abstract

Dikerogammarus haemobaphes is a species of freshwater gammarid native to the Ponto-Caspian region. Since the 1950s it has spread across much of eastern and western Europe, using the southern and central corridor to reach new freshwater bodies and establishing large, self-sustaining populations. The *D. haemobaphes* invasion is often accompanied by a relative, *D. villosus*, as is the case in the United Kingdom (UK). In 2012, *D. haemobaphes* was discovered in the River Severn, two years after the discovery of *D. villosus*. While there is a healthy body of *D. villosus* research, a knowledge gap exists in understanding the impact of 'demon shrimp', *D. haemobaphes*, even though they now have a wider distribution than *D. villosus* in Great Britain. This study aims to investigate the impact of invasive *D. haemobaphes* in UK's freshwater ecosystems, using established and developing citizen science tools.

Macroinvertebrate surveys conducted with kick sampling as part of this study show that D. haemobaphes has successfully invaded River Stort, with an established population in Bailey Bridge, and has completely displaced the native *Gammarus pulex*. It is also migrating upstream, evident by its presence in Pishiobury Brook, a backwater channel of River Stort. Ordination and linear regression analyses found D. haemobaphes is negatively impacting five taxa from shredder, collector and scraper functional feeding groups (FFGs), including the keystone shredder species *G. pulex*.

A Modular River Physical (MoRPh) survey was used to determine environmental characteristics using key channel, riparian, and human pressure indices. It finds that while channel vegetation and riparian habitat complexities exert influence on macroinvertebrate assemblages, the sites do not differ significantly in a hydromorphological sense. Stream decomposition rate was calculated by measuring the rate of cloth paper breakdown in colonisation traps. Bailey Bridge site has the highest decomposition rate, followed by Pishiobury Brook, Gade and Colne. Multiple comparison tests show that all sites have decomposition rate significantly different from each other, except between Colne and Gade.

The MoRPh and decomposition rate findings appear to contradict with findings from the macroinvertebrate surveys. Given that the stream habitats are similar, and *D. haemobaphes* exert negative pressure on many key shredder and collector taxa, it is counterintuitive that decomposition rate is highest in the stream where they are the predominant gammarid. It is therefore possible that *D. haemobaphes* has a higher detritus processing efficiency than previously thought. The consequent ecosystem impact and practicalities of citizen science methods are also discussed.

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List of abbreviations

ANOVA	- Analysis of Variance
AONB	- Area of Outstanding Natural Beauty
ARMI	- Angler’s Riverfly Monitoring Initiative survey
BB	- Bailey Bridge
BMWP	- Biological Monitoring Working Party survey
CC	- *Chelicorophium curvispinum*
CP	- *Crangonyx pseudogarcilis*
CPOM	- Coarse particulate organic matter
DH	- *Dikerogammarus haemobaphes*
DOM	- Dissolved organic matter

FBA - Freshwater Biological Association
FFG - Functional feeding group
FPOM - Fine particulate organic matter
GB - Great Britain
GP - *Gammarus pulex*
IGP - Intraguild predation
IMS - Industrial methylated spirit
MANOVA - Multiple Analysis of Variance
MLR - Multiple linear regression
MoRPh - Modular River Physical survey
NOF - Number of flow types
NBM - Number of bed material types
ABMS - Average bed material size
EOBS - Extend of superficial bed siltation
CPHC - Channel physical habitat complexity
NAVM - Number of aquatic vegetation morphotype
RPHC - Riparian physical habitat complexity
RVC - Riparian vegetation complexity
HLUP - Human land use pressure
REINF - Channel reinforcement
ENNP - Extent of non-native plants
FLOW - River flow rate as measured with a flow meter
PCA - Principal component analysis
PiB - Pishiobury Brook
POM - Particulate organic matter
RDA - Redundancy analysis
RSPB - Royal Society for the Protection of Birds
SPA - Special Protections Area
SSSI - Site of Special Scientific interest
WHPT - Walley, Hawkes, Paisley and Trigg survey
WVDEP - West Virginia Department of Environmental Protection

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Chapter 1: Introduction and literature review

1.1 Invasive ecology

The world’s freshwater biome, facing unprecedented and growing threats from human activities, is often considered one of the most endangered ecosystems in the world. One of the major and perpetual threat often cited is the impact of invasion by exotic species (Abell, 2002; Dudgeon et al., 2006; Strayer & Dudgeon, 2010; Reid; 2019). In the UK, the Ponto-Caspian region is considered an invasive species ‘donor hotspot’. 23 Ponto-Caspian high-risk freshwater invaders have been confirmed as established in the UK and it is predicted that several others have also arrived (Gallardo & Aldridge, 2015). Gallardo & Aldridge (2015) warn of an invasion meltdown, whereby mutualistic or competitive interactions between invasive species facilitate each other’s establishment, potentially bringing about large ecological and economic impacts. it is therefore crucial to assess the full scope of threats posed by Ponto-Caspian invaders, and this study focuses on a relatively recent invader of the British Isles – *Dikerogammarus haemobaphes* (Eichwald, 1841), commonly known as the demon shrimp. For the purposes of this study, *D. haemobaphes* is defined as an invasive species that has propagated from its donor region, and has overcame transport, environmental suitability and reproductive barriers to become widespread (Colautti & MacIsaac, 2004). In contrast to its more infamous cousin, the killer shrimp (*Dikerogammarus villosus*, Sowinsky, 1894), much of the demon shrimp’s ecosystem impact is unconfirmed by scientific study. The information collected through this study hopes to provide insight into the demon shrimp’s ecology, particularly its impact on the benthic macroinvertebrate communities and their associated ecosystem functions.

1.2 *Dikerogammarus*

1.2.1 *D. haemobaphes*: invasion history

D. haemobaphes was first found outside of its native range in 1955 (Nesemann et al., 1995). From Hungary, it migrated up the Danube River using the southern corridor, which connects rivers Danube and Rhine (Figure 1). By 1976 it had reached the upper Danube (Tittizer 1996), and later the upper Danube Canal in 1993 (Schleuter et al., 1994). In 2000 there are records of *D. haemobaphes* in the

Netherlands, where it reached the North Sea basin via Rhine River (Scholl et al., 1995). Range expansion along the central corridor to Poland also occurred, and by 1999, it had become the dominant gammarid in Vistula River (bij de Vaate et al., 2002), even replacing a previous invader, *Chaetogammarus ischnus* (Stebbing, 1899) in the lowland freshwater deltas (Jazdzewski et al., 2004). Figure 2 illustrates the migration route *of D. haemobaphes*, possible due to three main transport vectors, namely: i) construction of canals that connect river basins, ii) water management strategies of canals, and iii) transportation to a new region via ballast water in vessels (bij de Vaate et al. 2002).

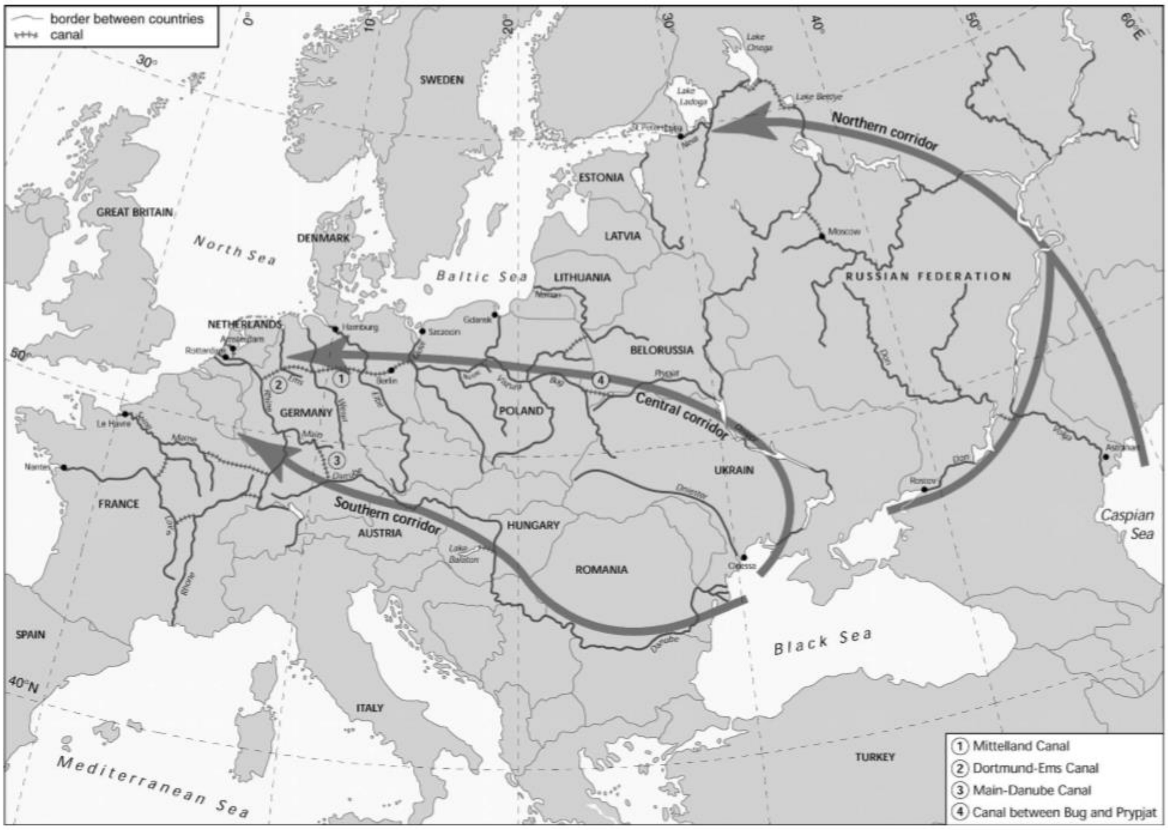

Figure 1: Migration corridors of Ponto-Caspian species in Europe. Source: bij de Vaate et al. (2002).

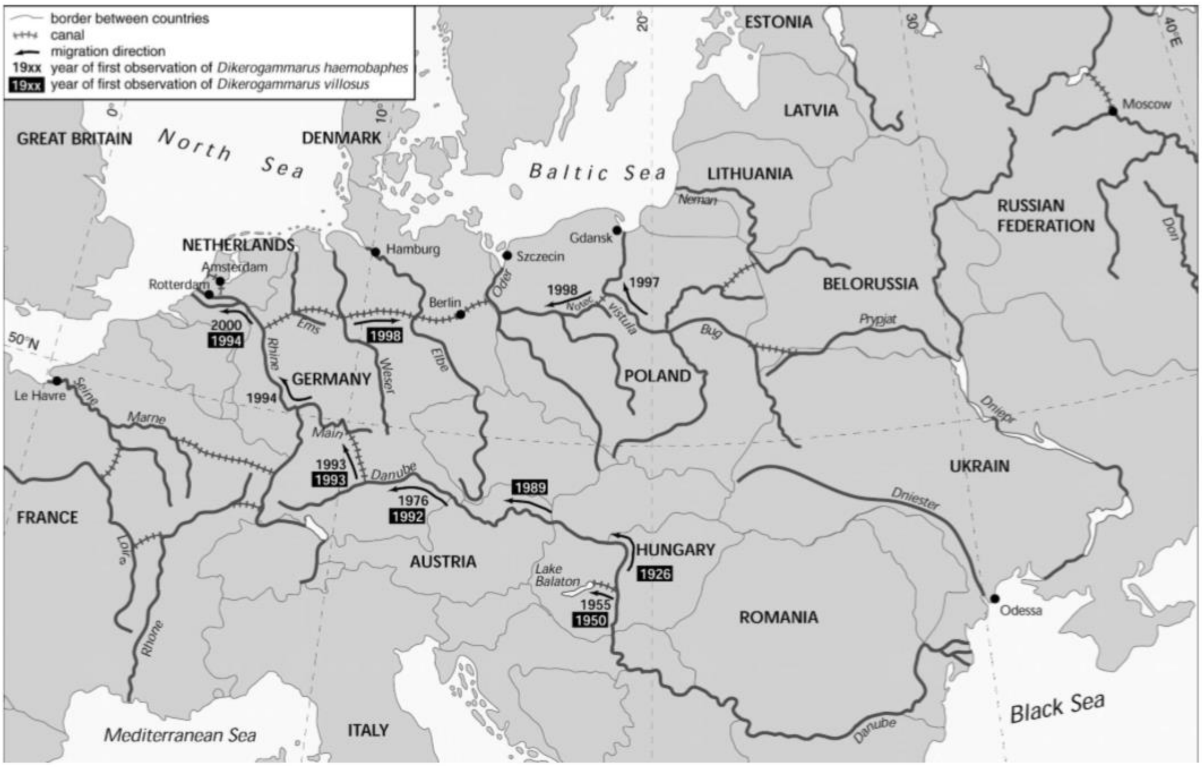

Figure 2: Migration patterns of *D. haemobaphes* and *D. villosus* in eastern Europe. Source: bij de Vatte et al. (2002).

While *D. bispinosus* have not yet been discovered in the UK (Dobson, 2013), the appearance of *D. villosus* in England in 2010 triggered the establishment of an alert system to report suspected sightings of *Dikerogammarus* species (Environmental Agency, 2012). It was through this system that *D. haemobaphes* was first recorded in the River Severn in 2012. Its initial entry date is thought to be years earlier, as subsequent surveys later in 2012 found established populations across the Midlands and Thames Valley (Gallardo & Aldridge, 2013; Aldridge, 2013). The westward spread from Netherlands to GB was not unexpected. 50% of invasive freshwater species recorded in GB since 1973 had come from the Netherlands, where they were previously established (Aldridge, 2013). In 2017 alone, Netherlands accounts for 13.35% of total tonnage loaded and unloaded at UK ports (Maritime Statistics, 2018). Netherlands vessels' ballast water, together with maritime activities involving recreational boat traffic, international watersports and angling events presented many opportunities for *D. haemobaphes* to 'hitchhike' and reach the shores of Great Britain (Aldridge, 2013).

Gallardo & Aldridge (2013) found much of GB to have similar bioclimatic conditions as *D. haemobaphes'*s native and invaded range in mainland Europe (Figure 3A). Their estimation was validated by Johns et al. (2018), who showed that the prediction map largely aligns with their current recorded distribution, except for the eastern and southern seaboard regions (Figure 3B). In the last seven years, *D. haemobaphes* has migrated far and wide, being found as far north as Ripon Canal in North Yorkshire. Meanwhile, *D. villosus*, who are known to replace and eliminate *D. haemobaphes* (Grabowski et al., 2007), has a sparse and patchy distribution. This is possibly due to the two gammarids preferring slightly different environmental conditions. For instance, Rachalewski et al. (2018) found *D. villosus* to prefer warmer temperatures above 30˚C, while D. haemobaphes tolerated temperature ranges between 6˚C and 30˚C (Table 1).

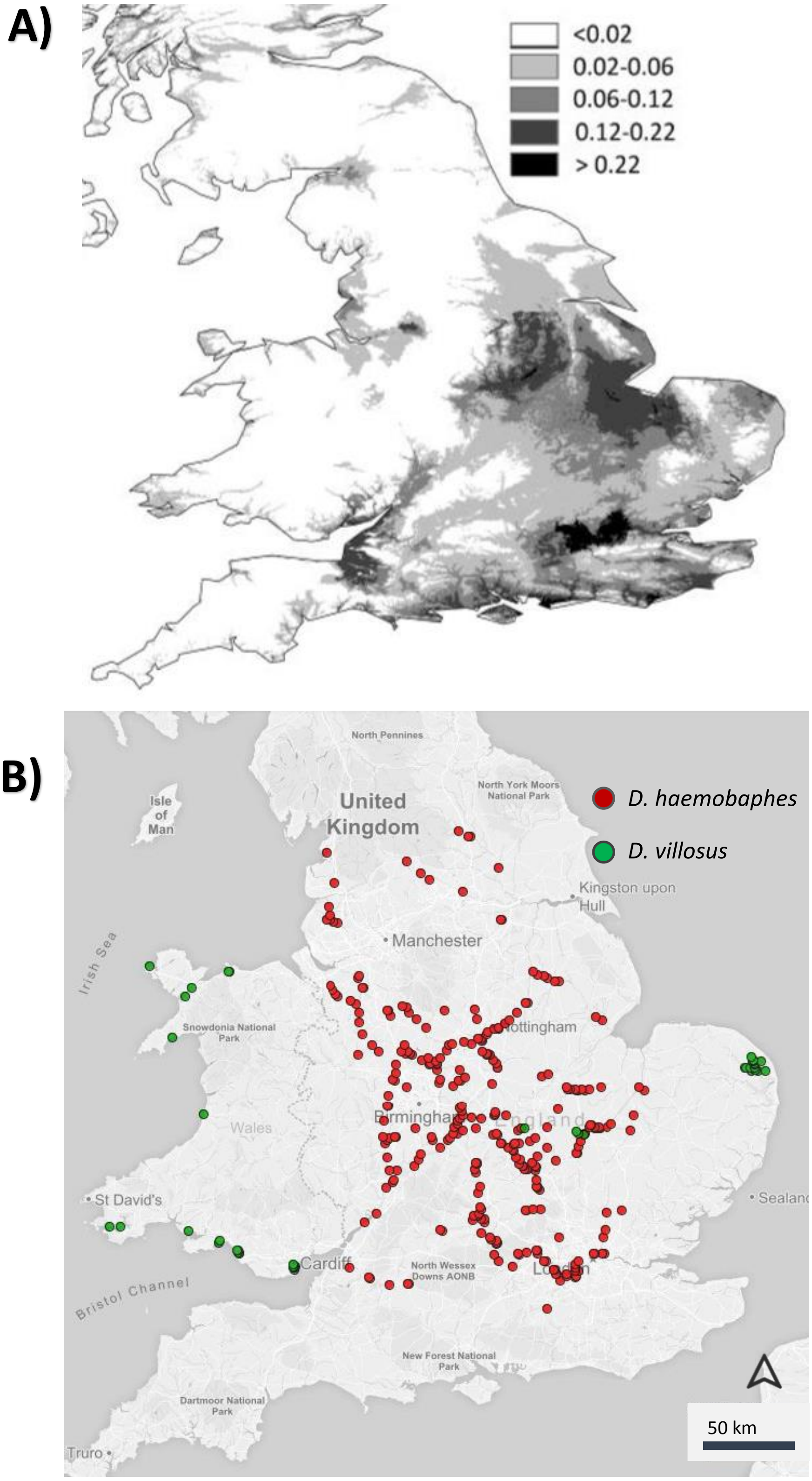

Figure 3: A) Habitat suitability for *D. haemobaphes* in Great Britain based on distributions in mainland Europe using a model built on climate variables and water chemistry. Source: Aldridge (2013). B) Distribution of *D. villosus* (317 records) and *D. haemobaphes* (646 records) in Great Britain. Map retrieved on 06 Aug 2019 from NBN Atlas.

1.2.2 *Dikerogammarus*: life-history and ecology

There are 10 known species under the genus *Dikerogammarus*, three of which are considered invasive across eastern and western Europe: *D. bispinosus* (Martynov, 1925), *D. villosus*, and *D. haemobaphes*. The rapid range expansion of *Dikerogammarus* may be attributed to several highly favourable life-history traits that allowed them to occupy new habitats and eliminate native amphipods. For instance, compared to the native *Gammarus pulex* (Linnaeus, 1758), *D. haemobaphes* reaches sexual maturity quicker, has longer reproductive period, larger brood size, higher partial fecundity, and shorter egg development time (Table 1). These reproductive traits led to *D. haemobaphes* turning over three generations every year, as opposed to one to two generations in *G. pulex*. Combined with their ability to tolerate a wide range of environmental conditions such as salinity, temperature, and anthropogenic pollution, *D. haemobaphes* expanded their range rapidly and eventually displaced many native and exotic species (bij de Vaate et al., 2002; Jazdzewski et al., 2004).

Within Europe, *D. haemobaphes* is often found to coexist with the zebra mussel *Dreissena polymorpha* (Pallas, 1771), another prevalent invasive species that originated from South Russia and Ukraine. *D. haemobaphes* were often abundant in sites covered in mussel shells (Wawrzyniak-Wydrowska & Gruszka, 2005; Zytkowicz et al., 2008). Controlled experiments by Kobak et al. (2009, 2013) further proves that they were attracted to the presence of living mussels, using cues associated with periostracum (the outermost layer of mollusc shell) and biofilm to locate them. It is likely that they are utilising them as shelters to avoid predation, much like the behaviours exhibited in *D. villosus* (Kobak et al., 2014). This symbiotic relationship between *D. haemobaphes* and Dreissenid corroborates with Simberloff and Von Holle’s invasion meltdown hypothesis (1999), in which groups of nonindigenous species facilitate each other’s invasion through “increasing the likelihood of survival and/or of ecological impact, and possibly the magnitude of impact.” (p.22). The widespread presence of Dreissenid across GB had therefore probably sped up the range expansion of *D. haemobaphes*.

Table 1: Life-history traits of *D. haemobaphes* and *G. pulex*

Life-history trait	***D. haemobaphes***	***G. pulex***
Sex ratio F:M	40% to 80%	50%
Gravid female size	7.0 to 14.5mm	7.3 to 8.2mm
Maturity index	0.67	0.88
Partial fecundity index	3.21 – 4.57	1.66
Brood size	Various numbers reported, between 20 to 100 eggs, depending on season. Positive correlation found between female size and clutch size.	6 to 9, also positively correlated to female size.
Egg development time	Three weeks from fertilisation to release of juvenile, in temperatures between 10˚C and 13˚C.	Three to five weeks from fertilisation to release of juvenile, in temperatures between 10 ˚C and 15 ˚C.
Breeding period in months	8 months, from autumn to summer, overwinters.	7 months, resting period in early winter
Generations year^{-1}	3	1-2
Temperature tolerance	Between 6˚C and 30˚C.	Best adapted to 10-20 ˚C.
Salinity tolerance	Up to 8PSU.	Up to 0.67 PSU
Human impacts	Tolerant to high level of pollution and habitat degradation.	Tolerant to medium level of pollution and habitat degradation
Sources	Ponomareva, 1975; Kititsyna, 1980; Kley & Maier, 2006; Grabowski et al., 2007; Pockl, 2009; Bacela et al., 2009	Beadle & Cragg, 1940; Hynes, 1955; Nilsson, 1977; Iversen & Jessen, 1977; Welton, 1979; Pockl, 2009; Maazouzi et al., 2011

1.2.3 *Dikerogammarus*: Predation

Although gammarids were typically classified as leaf litter shredders, behavioural (Dick et al., 2002; Kelly et al., 2002; Platvoet et al., 2009; Constable & Birkby, 2016), morphological (Mayer et al., 2008; van der Velde et al., 2009), and gut content studies (Bacela-Spychalska & van der Velde, 2013) have shown them to be omnivorous and very effective predators. Van der Velde et al. (2009) recorded chironomids, mayflies, oligochaetes amongst other macroinvertebrates in *Dikerogammarus* gut contents, with predation rates increasing throughout spring and summer months as temperature rises. This could be attributed to higher predation rate by juveniles (40% higher kill rate than adults), who required more protein intake to support rapid growth (Whiteledge & Rabeni, 1997). Within *Dikerogammarus*, *D. haemobaphes* and *D. villosus* are found to exert similar levels of intra-guild predation (IGP) pressure on each other, while both are stronger predators than *G. pulex* (Kinzler et al., 2009). There was also evidence of cannibalism within the native and invasive gammarids, with *D. haemobaphes* having the highest relative cannibalism rate (Kinzler et al., 2009).

1.2.4 *Dikerogammarus*: Leaf-litter processing

Despite being omnivores, gammarids still played an important role in leaf litter processing. For instance, *G. pulex* was found to consistently consume leaf materials even when a preferred prey is present, albeit at a lowered rate (Kelly et al., 2002). In this study, shredders are defined as animals that consume and convert relatively large particulate organic matter into animal tissue, respiratory CO2, and finer particulate organic matter in the form of ejected faeces (Cummins, 1974). A brief exploration of macroinvertebrate functional feeding groups can be found in Section 1.3.1.

At the time of writing, there is very limited study on the leaf shredding capabilities of *D. haemobaphes*. One notable experiment by Constable & Birkby (2016) found them to be 92.9% less efficient at detritus shredding compared to *G. pulex*, however some of the replicates did not achieve similar results, leading to the authors suggesting that differential rates of microbial decomposition could be at play. The experiment also finds a statistically insignificant decrease in leaf shredding efficiency of *G. pulex* when *D. haemobaphes* is present in the same enclosure. In-situ leaf litter shredding experiment by Graham (2014) in River Cherwell found that as the ratio of *D. haemobaphes* to *G. pulex* increases, leaf litter decomposition rate steadily decreases.

1.3 Freshwater macroinvertebrates as indicator of stream health

Kolkwitz and Marsson's (1902, 1909) work on saprobity, or degree of pollution in rivers inspired the method of monitoring ecosystem conditions using macroinvertebrate biodiversity as a proxy. By studying the associations between taxa and their preferred environmental conditions, a list of biological indicator organisms can be determined. And thus, freshwater scientists across Europe have developed a myriad of scoring systems based on the bioindicator concept (Cairns & Pratt, 1993). In the UK, the Biological Monitoring Working Party (BMWP) score was first introduced in 1980 (Hawkes, 1998) and was adopted by regulatory bodies in England and Wales until recently being superseded by the Walley, Hawkes, Paisley and Trigg score (WHPT, Paisley et al., 2014). The following sections detail a few other monitoring tools relevant to this study.

1.3.1 Functional feeding groups

In 1974, Cummins proposed an invertebrate functional feeding group (FFG) concept of monitoring water quality, using the linkages between invertebrates and their dependent food source as an indicator of nutrient availability in streams. As the abundance or biomass of respective group was linked to the amount of corresponding nutritional source categories, its analysis provided insights into the state of the stream, be it natural seasonal fluctuations or alterations following human impact (Merritt et al., 2017).

In place of taxonomic units, FFG distinguished macroinvertebrate taxa into five functional groups depending on their feeding activity. Shredders are detritivores that feed on coarse particulate organic matter (CPOM, >1mm diameter) such as whole leaf litter and other large organic materials. Collector-gatherers consume fine particulate organic matter (FPOM, 0.005-1mm diameter) gathered from the sediment, while collector-filterers feed on suspended FPOM from flowing water. Scrapers are invertebrates specially adapted to feed on periphyton or attached algae on exposed surfaces. Predators predominantly prey on smaller species or early instars of larger species, and are responsible for the majority of mortality in the other FFGs (Cummins 1974, Merritt et al., 2017).

The FFG concept recognised the importance of detritus decomposition rate in energy release into stream ecosystems. Shredders played a key role in breaking down larger detritus materials, initiating them into secondary production (Graca, 2001). 60% of what shredders consumed are converted into FPOM in the form of ejected faeces, ready to be consumed by collectors (Cummins, 1974). Amongst shredders, gammarids are considered keystone species in freshwater ecosystems that control litter breakdown (Piscart et al., 2009). Despite relatively lower per capita efficiency compared to other shredder families, their usually high abundance gives them control over energy input into the system, and thus have trickle-down effect on population dynamics (MacNiel et al., 1997).

1.3.2. ARMI and MoRPh: Citizen science biomonitoring tools

In recent years, academics working alongside citizen counterparts in scientific research is becoming more commonplace. From astronomy (Straub, 2016), taxonomy (Fontaine et al., 2012) to biodiversity monitoring (Chandler et al., 2017), everyday people curious about the natural world have made significant contributions towards the advancement of science in various fields. Due to its potential for high spatial and temporal coverage on biodiversity data, biomonitoring by citizen scientists is especially useful in conservation science, feeding knowledge into the agenda setting, formulation, and evaluation steps of the policy cycle (Figure 4). By mobilising volunteers, research that was previously unfeasible due to scale, manpower, funding or other practical reasons are now achievable. The public's involvement not only engages them in the decision-making process, but also provides scientists with a human perspective of environmental issues, to help build a holistic view of relationships between humans and their environment (McKinley et al., 2017). Doubts do exist about the quality of data collected through crowdsourcing (Kosmala et al., 2016), however by applying principles of usability inspection, such as heuristic evaluation and cognitive walkthrough (Nielson, 1994), coupled with in-field trials (England et al., 2017), an effective and intuitive workflow can be developed to collect relevant data that are accurate.

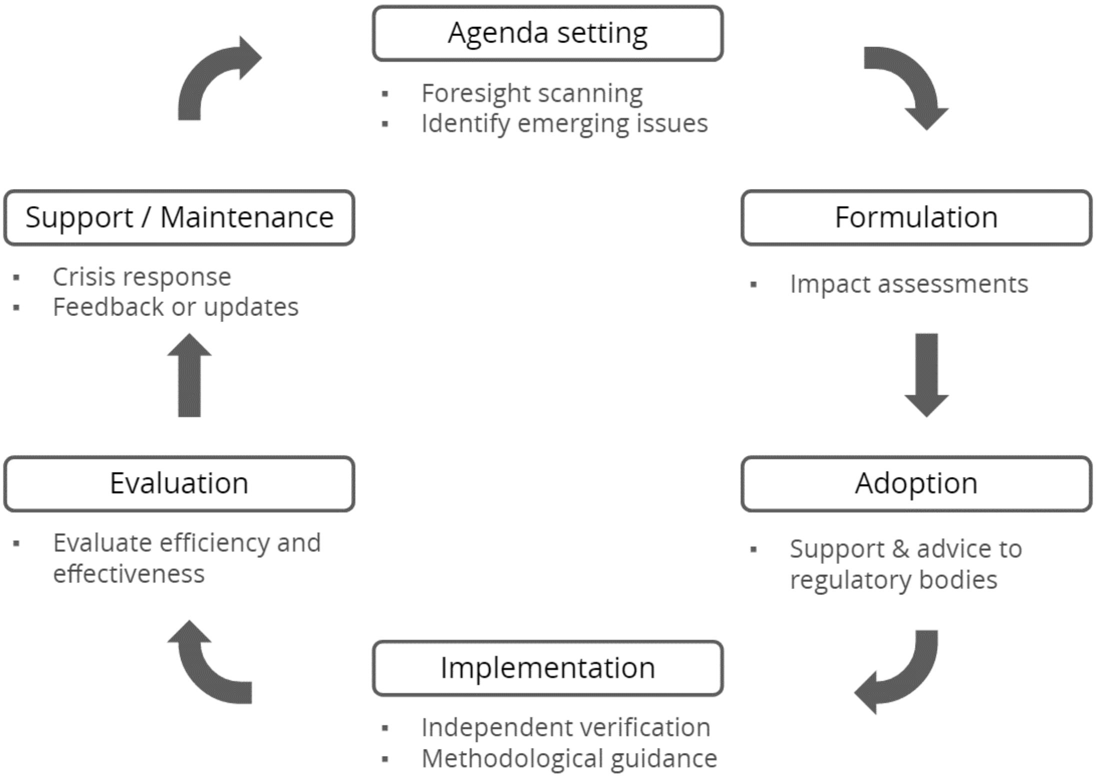

Figure 4: The six stages of the policy cycle. Source: European Geosciences Union, n.d., https://www.egu.eu/policy/basics/cycle/, on 19 August 2019.

One such example is the Angler's Riverfly Monitoring Initiative (ARMI). A UK-wide river monitoring project for water quality assessment that has been growing since its establishment in 2007, with over 2000 volunteers monitoring more than 1600 sites that are organised into 35 regional hubs across the UK (Brooks et al., 2019). Developed by the Riverfly Partnership, in conjunction with the Environmental Agency, it uses a simplified version of BWMP– a three-minute kick sampling to survey eight benthic macroinvertebrate taxa that reflect water conditions. By allowing self-organised local citizens to generate their own data using reliable methods, a meaningful partnership is generated between volunteers and regulatory authorities to communicate the state of their local stream, and its appropriate management strategy.

As with surveys conducted worldwide, it is now not unusual to record multiple invasive species during routine sampling. Currently, these observations are reported in the "observations space" by volunteers and have no weighing on the final scoring. Using scores generated from sites where invasives are present can raise doubt about its accuracy to reflect habitat health (Mathers et al., 2016). This highlights the need for biomonitoring tools to be flexible and allow for revisions, so that it can account for new findings in taxonomic or invasive ecology studies (Guareschi & Wood, 2009). In keeping with its goal to best monitor and conserve rivers, ARMI is currently being revised and expanded as experience build and new knowledge emerge. This study uses the Extended ARMI, which samples 33 invertebrate groups, as opposed to eight in the existing ARMI (Brooks et al., 2019). Both native and invasive taxa are included and assigned a positive or negative score depending on their pollution tolerance and ecosystem impact. Details of this more nuanced assessment is included in Section 2.3.

It is only logical to find that river biodiversity and its ‘riverscape’ shared linkages with the surrounding terrestrial landscape. As invertebrate taxa express their abiotic preference and tolerance, patterns of biodiversity distribution will reflect, and be influenced, by stream landscape and riverscape structures (Weins, 2002; Palmer et al., 2010). A review of UK’s existing hydromorphological assessment methods reveal a similar gap as identified by Belletti et al. (2015), that there is a disregard for key physical processes that dictate flow, sediment transport, and river channel adjustments (England & Gurnell, 2016). The authors also point out that there was no assessment process in place to survey riparian habitat, while the ad-hoc approach means that data is collected at varying spatial scales. The lack of a systematic survey method created gaps in data, which made it difficult to integrate knowledge that will best inform management decisions (England & Gurnell, 2016).

With the intention to create a multiscale framework, the Modular River Physical (MoRPh) survey is developed by academics from Queen Mary and University of London, with support from the Environment Agency (England et al., 2017). Launched in late Spring 2016 as a citizen science tool, it aims to monitor the, “physical habitat mosaic and human interventions and pressures within short river reaches” (Shuker et al., 2017, p.3) of between 10 and 40m in length. This fine scale survey characterises local physical habitat by mobilising volunteers to conduct field surveys, capturing fine scale information that cannot be obtained from remote sensing methods (Shuker et al., 2017). It is a useful tool not only to characterise stretches of river, but also complement other types of biomonitoring to create a holistic understanding of the riverine ecosystem.

1.4 Aims and objectives

In comparison to *D. villosus*, much of the environmental impacts of *D. haemobaphes* has been largely understudied. This study attempts to assess their impact in GB by evaluating river health in streams with low and high abundances of *D. haemobaphes*. River health is represented by the state of benthic macroinvertebrate assemblages inhabiting the streams, in relation to the streams hydromorphological conditions. To further understand *D. haemobaphes*’s impact on ecosystem functioning, patterns in decomposition rates will also be evaluated using colonisation traps. Using the Extended ARMI scores and MoRPh surveys, the study also tests if citizen science methodologies can produce robust datasets that are fit for use in scientific studies.

Chapter 2: Methodology

This section details the field, laboratory, experimental, and statistical analysis used throughout the study. All sites were sampled between May and mid-June to minimise seasonal fluctuations on invertebrate community composition and decomposition rate.

2.1 Study sites

Four study sites were chosen within Hertfordshire based on their number of *D. haemobaphes*, ease of access, and physical condition. Landowners' permission was requested before visiting private land, and a thorough risk assessment was conducted before any field work was carried out. Details of each sampling site are as follow:

Site 1) Colne – River Colne, Springwell Lane Downstream, TQ 04318 93061, 30m module

River Colne is a tributary of the River Thames located within the Colne Valley. It rises perennially from a subterranean river in North Mymms Park in Hertfordshire. The sample was collected from the Springwell Lane Riverfly monitoring site, with access permission from the North Harrow Waltonians Angling Society (*Pers. Comm.*, Booker T.). Two outfalls and a minor (<10% width) deflector were found on the left bank, but otherwise the channel was free flowing without backwaters or being choked by plants. Gravel-pebble (2-64mm diameter) dominated the riverbed, with scatterings of sand (0.0625-2mm diameter) and thin silt (0.00195-0.0625mm diameter) layers along the module. It was surrounded by private residential lands on both banks, and accessible from a road bridge approximately 20m upstream. To the best of knowledge*, D. haemobaphes* was first noted in April 2019 with the capture of two individuals in a Riverfly monitoring event.

Site 2) Gade – River Gade, Gade Avenue Railway Bridge, TQ 09064 96235, 30m module

River Gade is a chalk stream tributary of the River Colne and part of the Chilterns Area of Outstanding Natural Beauty (AONB). The sampling site was in Watford, below Cassiobury Park, under a railway bridge. The bridge provided artificial reinforcement and intermediate (10-25m) shading to the module, while a pool was created by fallen tree branch debris. Channel bed was dominated by gravel-pebble and littered with large trash such as car parts and traffic cones. Surrounding land-use was a matrix of public green space, residential, and private businesses including a farm and fishery. It is monitored regularly by the Environmental Agency and two instances of *D. haemobaphes* were recorded, in September 2017 and again in November 2018.

Site 3) BB – River Stort, Bailey Bridge, TL 39696 10177, 20m module
The 38km long River Stort is a tributary of the River Lee, where it meets in Hoddesdon. From Hoddesdon to Bishop’s Stortford, 22km of the river had been canalised to create the Stort Navigation, which has been in use since 1769 (Leeming, 2009). Sampling was done next to Bailey Bridge, a private footbridge going across the river. The channel bed substrate was a mix of cobble (64-256mm diameter), gravel-pebble, sand and silt, with some parts reinforced by builder’s waste. Surrounding land-use includes a sewage works by Thames Water, and RSPB Rye Meads – a 58.5-hectare Site of Special Scientific Interest (SSSI), Special Protection Area (SPA) and part of the Lea Valley RAMSAR site (The Royal Society for the Protection of Birds [RSPB], n.d.).

Site 4) PiB – River Stort, Pishiobury Brook, TL 47608 13132, 20m module
Pishiobury Brook is one of the backwater reaches of River Stort (Figure 2), and is publicly accessible via a footpath along Stort Navigation. Both banks appear natural with no artificial structures except for an inconspicuous outfall on the right bank. Parts of the channel are shaded by trees growing on the bank face and top. Channel substrate is a mixture of cobble, gravel-pebble, sand and silt, with continuous thin silt layers throughout the module.

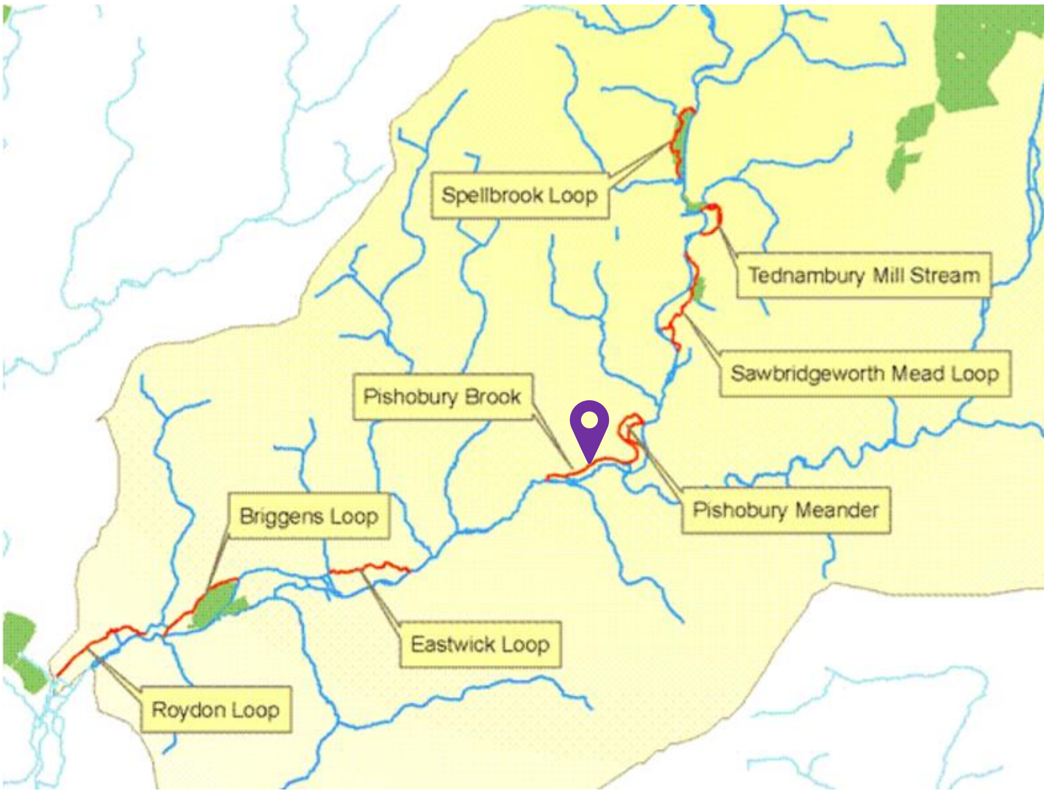

Figure 5: Back water loops and reaches of River Stort. Source: Leeming (2009). Main channels of Stort Navigation and tributaries of River Stort are lined in blue, approximate location of Pishiobury Brook sampling site is marked in purple.

2.2 Environmental parameters

After module lengths were determined based on river width, a MoRPh survey was conducted at each site to collect quantifiable observations on the rivers' hydromorphological conditions. Measurements of the bank top, bank face, channel margin and channel bed were taken based on what was observed on the day, and not by what was known about the site. 14 indices were estimated from the raw data, which summarised the river channel and riparian characteristics, as well as human pressures and impacts surrounding the module (Shuker et al., 2017). River flow speed was also taken at the modules' mid-point, using a Valeport BFM0012A-LCD open channel flow meter.

2.3 Macroinvertebrate survey

To survey the benthic macroinvertebrate community, a three-minute kick sampling was carried out at each site following the standard protocol as listed by the

Freshwater Biological Association (FBA, n.d.). A hand net with 0.25m frame, 0.5m deep net and 1mm mesh size was used, and total sampling time was divided equally across different habitats of the channel (Brooks et al., 2019). The samples collected were then stored in 100% Industrial Methylated Spirit (IMS) to be sorted in the laboratories at UCL. All invertebrates were identified to family level, while all freshwater shrimps were further identified to species level. Families and species were categorised into five functional feeding groups (FFG): collector-filterer, collector-gatherer, shredder, scraper, and predator, using Merritt et al. (2017) and a guide developed by West Virginia Department of Environmental Protection (WVDEP, n.d.). A comprehensive list of publications used for identification can be found in Appendix 1.0.

From the abundance data, an Extended ARMI score was calculated for each river, using a draft version of the Extended ARMI scheme currently being developed and trialled by The Riverfly Partnership (Brooks et al., 2019). Each group was given a positive (1 to 4) or negative (-4 to -1) score, depending on their abundance and tolerance to stressors, which included nutrient enrichment, slow flow, sedimentation, and acidification (Table 2). Table 3 compares riverfly groups included in the current and Extended ARMI, and their associated value.

Table 2: Scores given to Riverfly groups depending on abundance from a three-minute kick sample.

Riverfly abundance	Score
1 – 9	±1
10 – 99	±2
100 -999	±3
over 1000	±4

Table 3: Riverfly groups included in ARMI and Extended ARMI, and their associated scoring value. '+' means that the riverfly group is given a positive score based on their abundance, while '-' means a negative score is given based on abundance.

Riverfly group	ARMI	Extended ARMI
Flatworm		-
Freshwater snail		-
Limpet		+
Bivalve		-
Aquatic worm		-
Leech		-
Crayfish		+
Water hog-louse		-
Freshwater shrimp	+	+
Invasive non-native shrimp		-
Mayfly: Angler's curse	+	-
Mayfly: Olive	+	+
Mayfly: Prong gilled		+
Mayfly: Flat-bodied	+	+
Mayfly: Green drake		+
Mayfly: Blue-winged olive	+	+
Stonefly	+	+
Drangonfly & damselfly		-
Water boatmen		-
Other water bugs		-
Water beetles (adult & larva)		-
Alderfly		-
Caseless caddis: Green sedge	+	+
Caseless caddis: Net spinner	+	+
Caseless caddis: Non-gilled	+	+
Cased cadis: Hood case-maker	+	-
Cased cadis: Weighted case-maker	+	+
Cased cadis: Brush tailed caddis	+	+
Cased cadis: Others	+	+
Cranefly		+
Blackfly		+
Non-biting midges		-
Water snipe fly		+

2.4 Decomposition rate

Based on the leaf-litter bag method for measuring decomposition rate (Boulton & Boon, 1991), colonisation traps developed by Murray Thompson and Ian Patmore were used to capture decomposition rates in each river (Zhang, 2017; Keates, 2018).

Two mesh bags containing pre-weighted cloth papers were placed and secured in two ends of a trap, separated into two chambers by an insert. While microorganisms were allowed into both chambers, the fine mesh bags and corresponding lids prevented benthic macroinvertebrates from entering, and so captured microbial decomposition activity only (Boulton & Boon, 1991). The coarse mesh chamber captured both macroinvertebrate and microbial decomposition as they colonise and feed on the paper materials. Cloth paper was chosen over leaf litter assays as dry leaves tend to fracture into small pieces and fall out of the coarse mesh bags during transport. Cloth paper has also shown to reflect decomposition patterns as leaf litter assays, and its uniformity in material makes for a suitable tool for comparative studies such as this (Tiegs et al., 2007).

Eight traps were installed in each site and left in for four weeks to allow for measurable decomposition to occur. They were secured to the river bed using square downpipe brackets and metal tent pegs. The traps are positioned across the width of the river to capture activities in different river microhabitats; and perpendicular to flow direction to minimise sediment accumulation within the chambers, and the potential to lose lids or traps to flow pressure.

After four weeks, the traps were retrieved from the rivers and any macroinvertebrates occupying the coarse chamber were captured and identified following methods in kick sampling. The remaining cloth papers were then removed, cleaned, and dried on pre-weighted petri dishes at 40˚C in a dehydration chamber, over two nights or until completely dry.

2.5 Data analysis

This section details the statistical methods used in exploring datasets, and their purpose of use. Initial exploratory analyses were done using IBM SPSS Statistics Version 22.0 (IBM Corporation, New York). All regressions were performed in Rstudio Version 0.98.994 (RStudio Team, 2014). Gradient length calculation, gradient analyses, ordination calculations were performed in CANOCO for Windows 5.10 (ter Braak & Smilauer, 1998).

2.5.1 Environmental variables

14 indices were derived from the MoRPh surveys, however categorical indices were excluded from the analyses. Together with river flow rate, Table 3 lists all environmental indices used. As there were only four sites (n=4), non-parametric Spearman test was used to analyse for collinearity between different parameters. It was not possible to perform a Detrended Correspondence Analysis (DCA) to determine gradient length of the data set, due to the response data having 77% nonzero values. A Principal Components Analysis (PCA) was suggested by CANOCO to explore similarities in environmental conditions between the rivers. A one-way Analysis of Variance (ANOVA) and post-hoc Tukey tests was used to test for significant differences between sites.

Table 4: Environmental indices estimated from MoRPh and flow meter measurements.

Index type	Index name	
River channel characteristics	NOF	Number of flow types
	NBM	Number of bed material types
	ABMS	Average bed material size
	EOBS	Extend of bed siltation
	CPHC	Channel physical habitat complexity
	NAVM	Number of aquatic vegetation morphotypes
	FLOW	River flow speed (m/s)
Riparian (bank face and top) character	RPHC	Riparian physical habitat complexity
	RVC	Riparian vegetation complexity
Human pressures and impacts	HLUP	Degree of human pressure imposed by land cover on the bank tops
	REINF	Channel reinforcement
	ENNP	Extent of non-native invasive plants

2.5.2 Macroinvertebrate response to environmental parameters

DCA for kick sample invertebrate abundance shows that the gradient lengths are short (<2 s.d. units), therefore redundancy analysis (RDA) was used to explore patterns in macroinvertebrate communities and potentially detect distinctive communities associated with environmental variables. As Spearman test returns no significant correlations between environmental parameters, all indices were included, without transformation, in an exploratory RDA analysis. Simple linear regressions were used to explore relationships between biodiversity and individual parameters.

Finally, a Multiple Linear Regression (MLR) model was built using the 5 most important drivers identified.

2.5.3 Decomposition rate

Differences in paper weight before and after the experiment gives: microbial decomposition rate (R_{micro}, g/day), and microbial + invertebrate decomposition rate (R_{all}, g/day). True macroinvertebrate decomposition rate (R_{invert}, g/day) can be obtained from $R_{invert} = R_{all} – R_{micro}$. Normality and homogeneity of variance assumptions were tested using Levene test and Shapiro-Wilk test respectively. A one-way ANOVA and post-hoc Tukey tests were performed to detect difference in decomposition rates between rivers.

2.5.4 Impact of *D. haemobaphes*

To understand if the presence and abundance of *D. haemobaphes* would have an impact on river decomposition rates, an MLR was performed using relative abundances of invertebrates from the colonisation traps as independent variables, and decomposition rate as the dependent variable. To explore if demon shrimp impacts macroinvertebrate community assemblages, an RDA was performed with DH relative abundance as the single parameter. Only families that occur in both kick sampling and colonisation traps were included in the RDA, which meant that no predatory families were included, as colonisation traps did not attract many predators. Significant relationships between DH and individual families were then explored using linear regressions.

2.5.5 Comparing sampling methods

To test if kick sampling and colonisation traps capture different snapshots of the benthic macroinvertebrate community, a multiple ANOVA (MANOVA) was used to test for differences between sampling methods. For the analysis, the two sets of biodiversity data were classified according to their FFGs. Simple linear regressions of colonisation trap against kick sample were also plotted for taxon richness, extended ARMI scores, and effective number of species calculated from the exponential form of Shannon-Weiner diversity index (Jost, 2007).

Chapter 3: Results

3.1 Environmental variables

From the PCA, the first principal component corresponded to a measure of NBM and CPHC, while the second component corresponded to a measure of ENNP, NAVM, and RPHC (Figure 6). PiB appeared the most different compared to the other three sites, while Colne and Gade were the most similar in terms of river physical habitat conditions. PiB was different in NBM, CPHC, NOF, and FLOW compared to other streams. It had the slowest flow speed, and minimal physical habitat complexity within the channel (Table 5). BB was characterised by high human modification, with channel bed and bank side reinforcements, and was the only site where invasive Himalayan Balsam (*Impatiens glandulifera)* was found. River Gade was unique to have relatively high extend of superficial bed siltation, and subsequently the smallest average alluvial bed material particle size (ABMS is expressed as phi units, where the larger the number, the smaller the particle size). Table 5 details the value of individual indices used in the analysis. One-way ANOVA and subsequent multiple comparison tests returned no significant difference between the physical conditions of each river (Table 6).

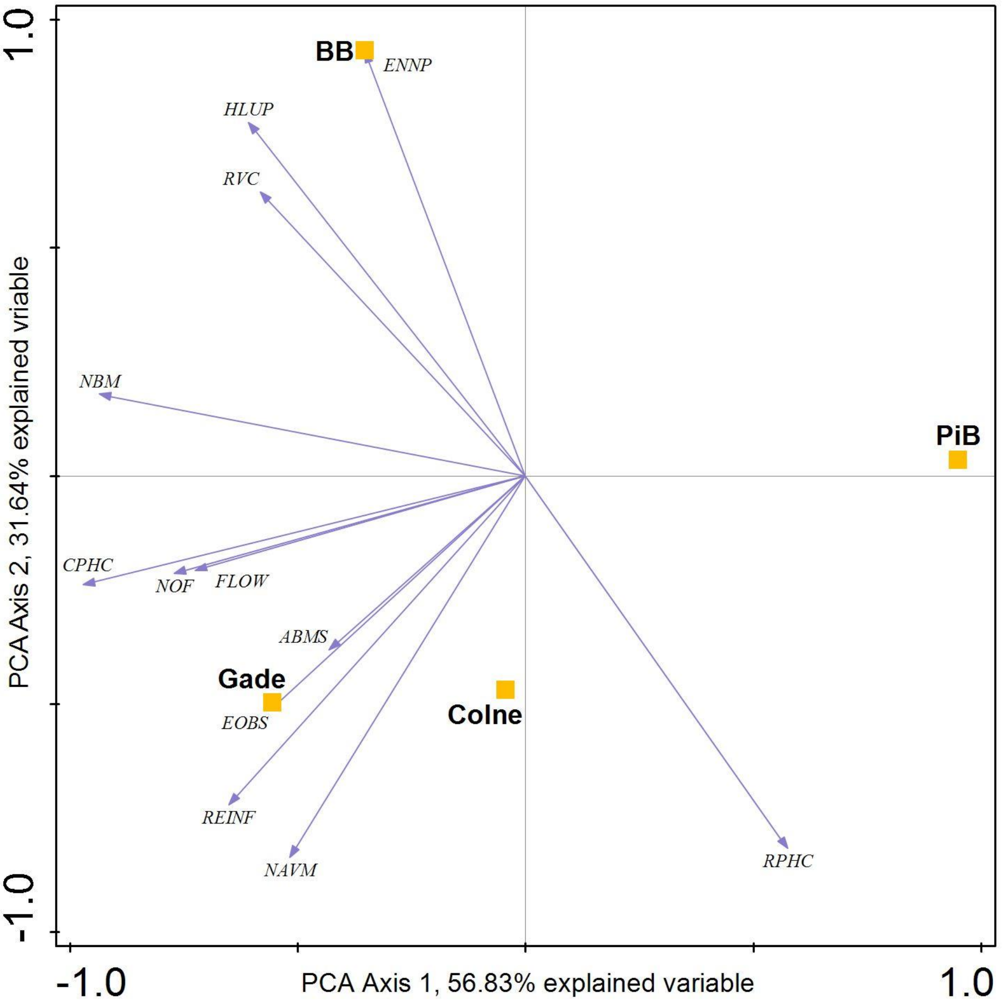

Figure 6: PCA ordination of environmental parameters drawn from MoRPh indices and river flow speed. Axis 1 and 2 explained 88.47% of observed variables. Index name are listed in Table 4. BB= Bailey Bridge, PiB= Pishiobury Brook/

Table 5: Value of MoRPh indices for sampling sites. Only one MoRPh survey was conducted for each site.

Index	Colne	Gade	BB	PiB
NOF	3.000	2.000	2.000	0.000
NBM	4.000	4.000	5.000	0.000
ABMS	-0.902	0.444	-0.687	-0.578
EOBS	0.100	3.550	0.000	0.000
CPHC	3.750	5.833	3.750	0.000
NAVM	4.000	4.000	1.000	1.000
RPHC	2.940	2.738	1.690	3.143
RVC	4.000	5.500	6.000	4.500
HLUP	0.000	2.000	5.000	0.000
REINF	0.950	1.900	0.133	0.000
ENNP	0.000	0.000	1.900	0.000
FLOW	0.540	0.387	0.402	0.156

Table 6: Multiple comparison tests for difference in environmental conditions between sites.

Site	**Estimates**	**Confidence Interval**	**p=value, CI=95%**
BB vs Colne	0.318	-1.774 -- 2.409	0.977
BB vs Gade	-0.514	-2.605 -- 1.578	0.913
BB vs PiB	1.497	-0.594 -- 3.589	0.238
Colne vs Gade	-0.831	-2.923 -- 1.260	0.715
Colne vs PiB	1.180	-0.912 -- 3.271	0.443
Gade vs PiB	2.011	-0.081 -- 4.102	0.063

3.2 Decomposition rate

Out of the 32 traps laid out, one trap was washed away by River Colne, 2 traps had missing lids, while four traps in BB had all cloth papers in the coarse mesh consumed after four weeks. Decomposition rates were thus analysed for the remaining 23 traps (Figure 7). After four weeks in the rivers, mean decomposition rate recorded at Bailey Bridge (n=4, 0.0361g/day) is markedly higher than Pishiobury (n=8, 0.0210g/day), Gade (n=7, 0.0103g/day), and Colne (n=6, 0.0074g/day).

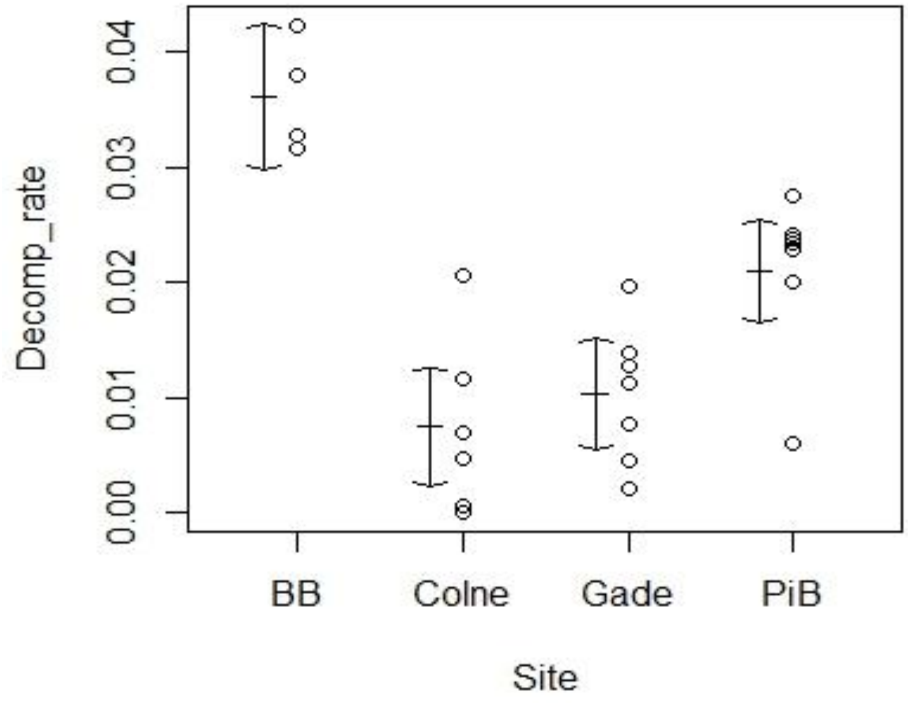

Figure 7: Plot of decomposition rate (g/day) against sampling site. Arrows represent 95% Tukey confidence interval.

Preliminary tests found an equal distribution of variance (Levene test p-value = 0.887), while the underlying error also fitted the normality assumption (Shapiro-Wilk test p-value = 0.958). One-way ANOVA found significant difference between sites ($F=19.5$, $p<0$), and multiple comparisons showed that all sites were significantly different from each other, except when comparing Colne and Gade (Table 7).

Table 7: Multiple comparison table of decomposition rates between two different sampling sites. P-value is given at a 95% confidence level. Comparisons showing significant difference between sites are highlighted in bold.

Site	Estimates	Confidence Interval			p-value
BB vs Colne	0.029	0.017	--	0.040	**<0.000**
BB vs Gade	0.026	0.015	--	0.037	**<0.000**
BB vs PiB	0.015	0.004	--	0.026	**0.005**
Colne vs Gade	-0.003	-0.013	--	0.007	0.858
Colne vs PiB	-0.014	-0.023	--	0.004	**0.005**
Gade vs PiB	-0.011	-0.020	--	0.001	**0.021**

To find out which taxa was influencing stream decomposition rates, multiple linear regressions of decomposition rates against macroinvertebrate abundance was used. It found that a model built with *D. haemobaphes*, Baetidae, and Simuliidae had a significant influence on decomposition rates (Table 8, adjusted $R^2=63.05$, p-value<0).

Table 8: Models built using stepwise MLR, with decomposition rates as the dependent variable, and colonisation trap taxa as independent variables. P-value is given at 95% confidence level. DH= D. haemobaphes.

Model	Adjusted R^2 (%)	β-value	F value	p-value
DH	28.92	0.57	10.36	<0
DH + Baetidae	46.69	-0.45	11.07	<0
DH + Baetidae + Simuliidae	63.05	-0.41	14.08	<0

3.3 Benthic macroinvertebrate community

A total of 51 families were recorded from all four sites, of which three species of non-native shrimps were found: *D. haemobaphes; Chelicorophium curvispinum* (G.O. Sars, 1895); and *Crangonyx pseudogarcilis* (Bousefield, 1958). One species of invasive Tateidae, *Potamopyrgus antipodarum* (Gray, 1843) was also recorded predominantly in BB and PiB, and in very low numbers (2 individuals) in Gade. Juveniles of the invasive signal crayfish, *Pacifastacus leniusculus* (Dana, 1852), from the family Astacidae, was also recorded in Colne and PiB. The freshwater shrimps *G. pulex* (GP)*, D. haemobaphes* (DH)*, C. curvispinum* (CC; G.O. Sars, 1895), and *C. pseudogarcilis* (CP, Bousefield, 1958) were considered separately as individual species in the analysis. Complete lists of macroinvertebrate taxa, abundance, and FFG allocations are listed in Appendix 2.0.

From the kick samples, Colne recorded the highest number of taxa, but the two sites on River Stort (BB and PiB) achieved higher alpha diversity (Table 4). Overall, kick sample managed to capture a larger number of invertebrates per sample, compared to colonisation traps, as reflected by the lower biodiversity and taxon richness. Extended ARMI scores from kick sampling ranked Gade to have the highest water quality, followed by Colne, BB and PiB. Ranking obtained from colonisation traps follow a similar pattern, except Colne and Gade achieved the same scores.

Table 9: Summary of benthic macroinvertebrate community surveyed in this study.

	Kick sample (n=4)				**Colonisation trap (n=29)**			
	Colne	Gade	BB	PiB	Colne	Gade	BB	PiB
Abundance	1773	2122	1135	1306	394	652	472	737
Taxon richness	35	31	26	24	20	20	15	15
Shannon-Weiner	2.02	2.02	2.31	2.27	1.68	1.43	1.63	1.29
Effective no. of species	7.51	7.54	10.09	9.64	5.37	4.16	5.10	3.62
Extended ARMI	4	8	1	0	-1	-1	-5	-7

Freshwater amphipods, mayflies (Ephemeroptera), fly larvae (Diptera), and freshwater bivalves and snails represented the largest groups of benthic macroinvertebrates in the rivers. *D. haemobaphes* was found in large numbers in BB, accounting for 20.79% of all recorded taxa (Figure 8) in the kick sample, while no native gammarids was recorded from either of the sampling methods. From kick sampling, *D. haemobaphes* is present at very low numbers (<1%) in other streams.

In comparison, colonisation traps recorded much higher proportion of *D. haemobaphes*, ranging from 15.1% to 72.9% in BB, and 2.0% to 7.0% in other streams (Figure 9). It is interesting to note that in all traps where no *D. haemobaphes* were found, *G. pulex* abundance increases markedly, while the opposite is also true.

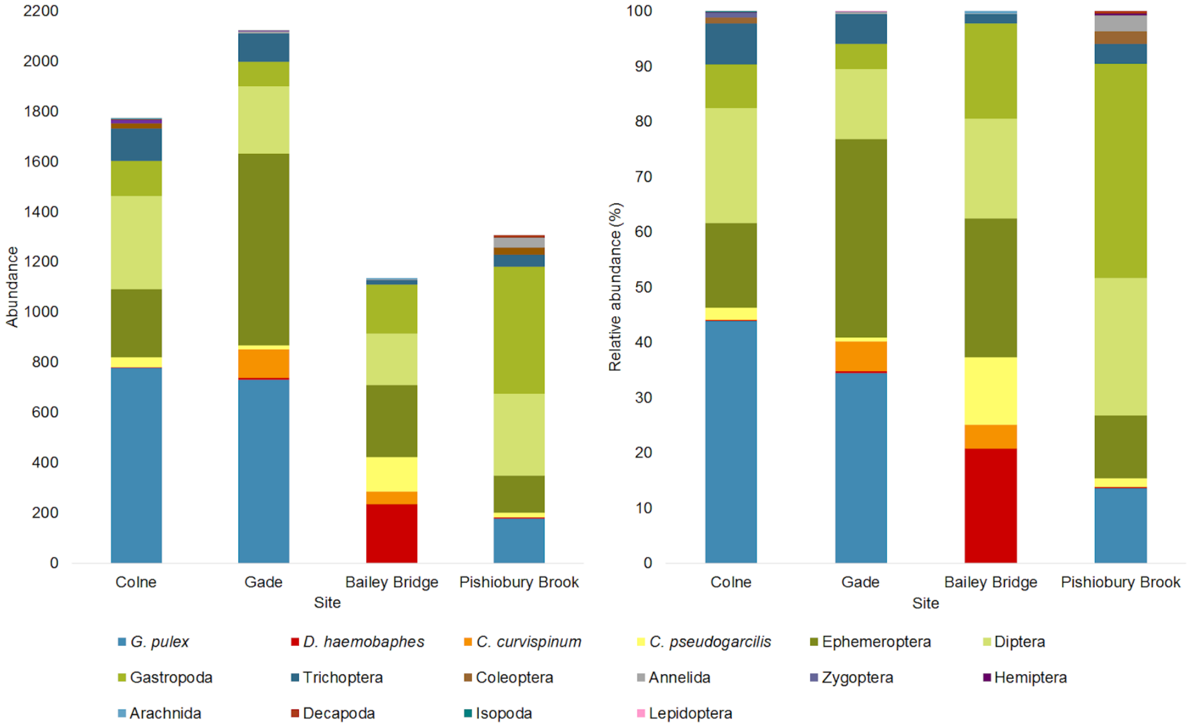

Figure 8: Absolute and percentage relative abundance of benthic macroinvertebrate obtained from three-minute kick sampling. Freshwater shrimps are displayed as species while all other families were grouped to order level.

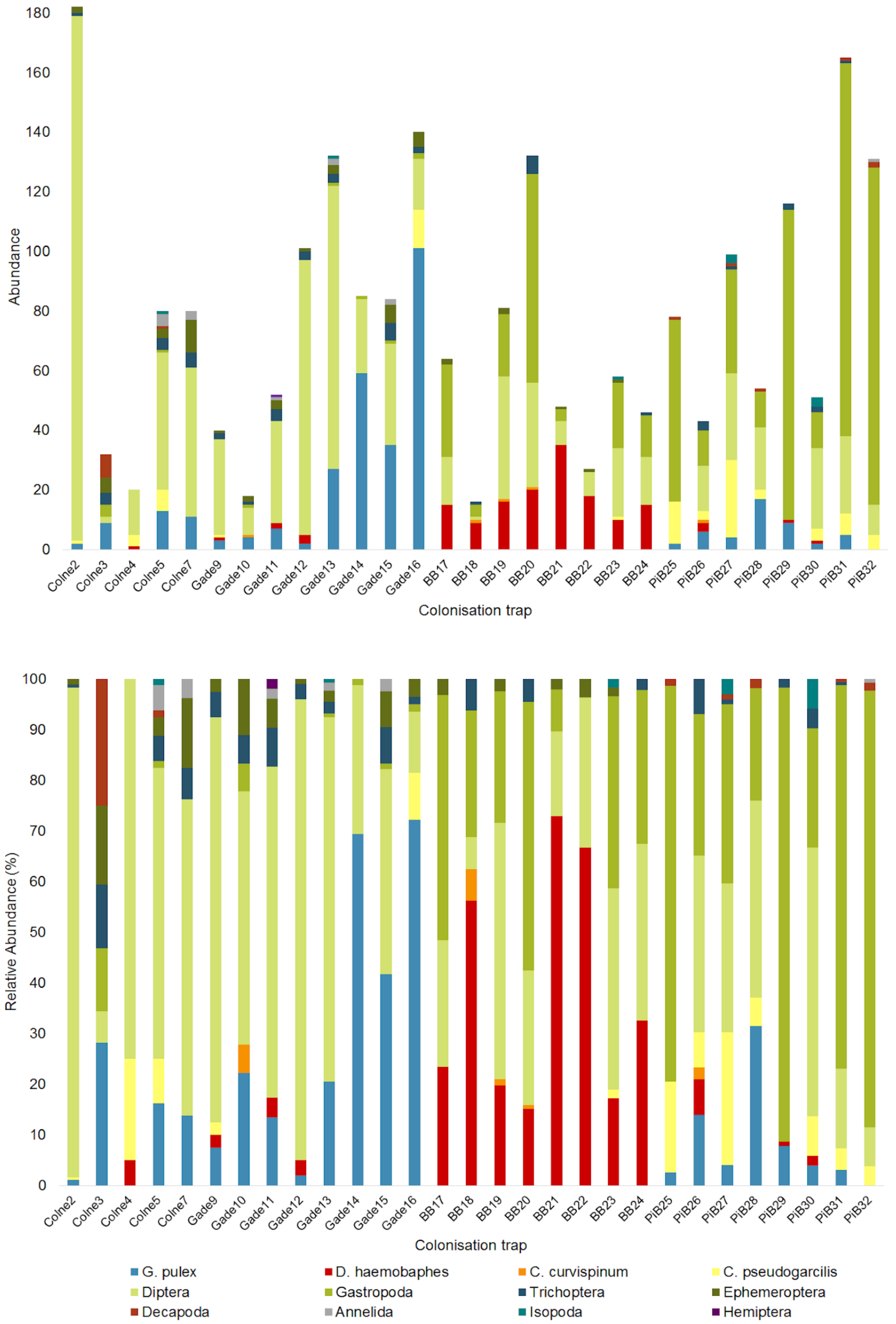

Figure 9: Absolute and percentage relative abundance of benthic macroinvertebrate found in colonisation traps. Freshwater shrimps are displayed as species while all other families were grouped to order level.

Figure 10 shows the distribution of FFGs in each site. While collector-gatherer and shredders are the biggest groups in all sites, shredder is the dominant group only in Colne (46.8% in kick sampling). In other streams, collector-gatherer has a higher relative abundance. Scraper is exceptionally abundant in PiB, between 70% and 92% higher than Colne, Gade and BB, due to the high abundance of invasive Tateidae.

In Colne, Gade and PiB, *G. pulex* is the most abundant of shredder group, and in the case of BB, *D. haemobaphes* dominates the shredder group (98.5% of all shredders in kick samples, 62.3% in colonisation traps). Scrapers are exceptionally high in PiB, between 70% and 92% higher than other streams in kick samples. This is due to its large population of invasive *P. antipodarum*, making up 27% of the entire macroinvertebrate community in kick sampling, and 62% in colonisation traps. Collector-gatherers are best represented by Caenidae, Baetidae and Chironomidae in kick samples, while Chironomidae dominate the group in colonisation traps. Compared to the ten predator families recorded in kick samples, only two families of predators are recorded in colonisation traps, Glossiphonidae and Veliidae, both of which were not found in kick samples.

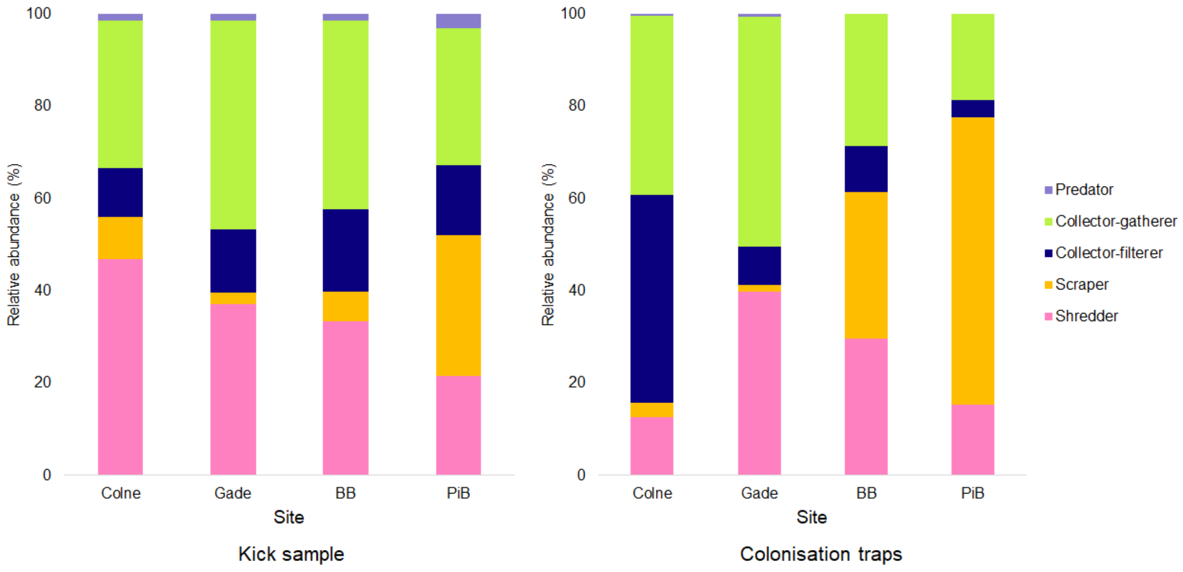

Figure 10: Relative abundance of functional feeding groups at each site, collected from kick sampling and colonisation traps.

Colne and Gade were associated with similar invertebrate groups, including many caddisflies (Trichoptera), mayflies and true flies. Two streams on River Stort, PiB and BB showed association with many non-native species. PiB is characterised by high numbers of Tateidae and Astacidae, while BB has a high numbers of *C. pseudogarcilis* (CP), and *D. haemobaphes* (DH). From the ordination plots PiB appeared to be the most dissimilar in terms of benthic macroinvertebrate community composition, while Colne and Gade had very similar macroinvertebrate assemblages (Figure 11).

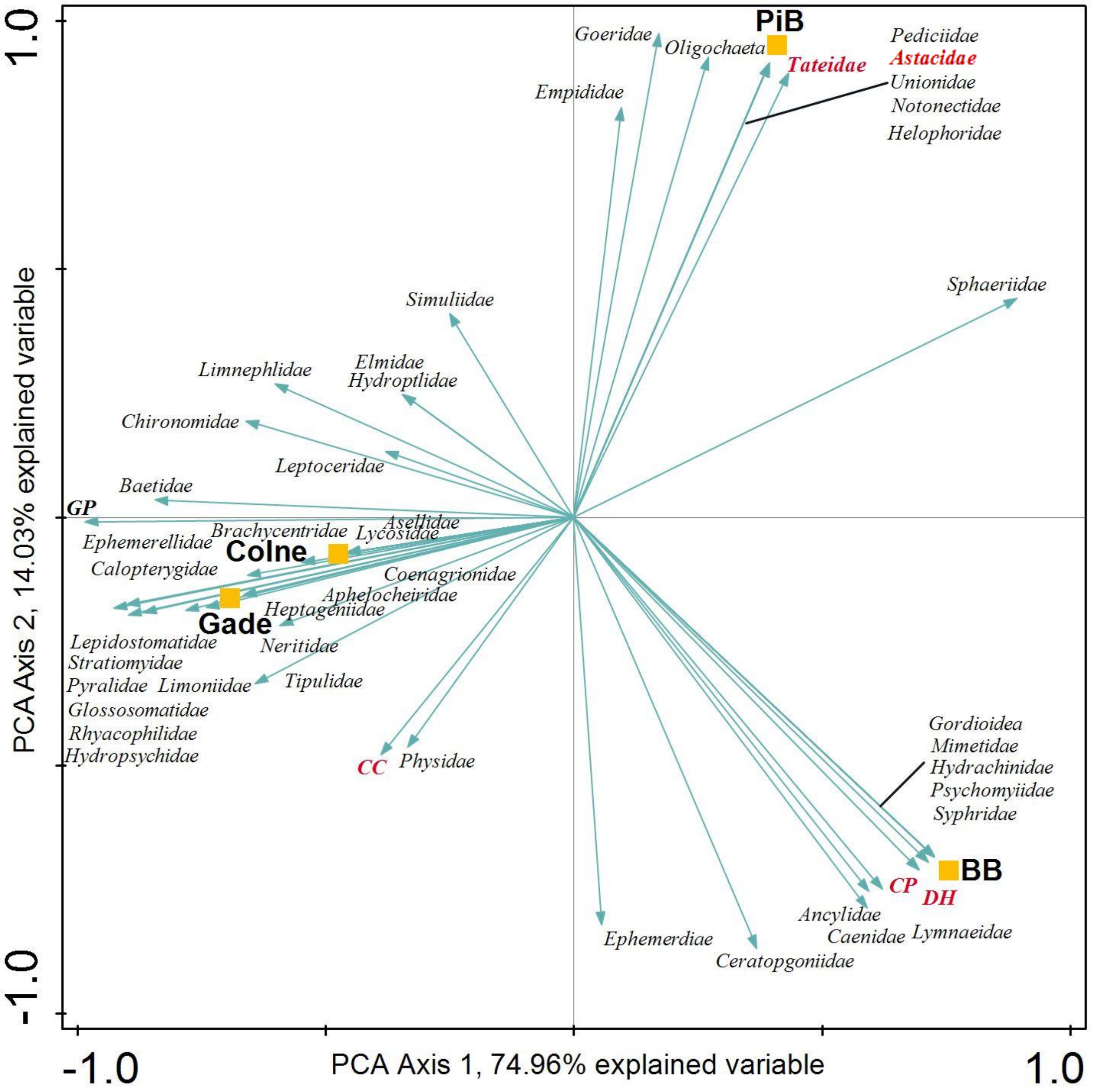

Figure 11: PCA ordination of macroinvertebrate showing association to the four sampling sites. Axis 1 and 2 explained 88.99% observed variance. Non-native species are marked in red while yellow squares show position of sampling sites. GP= *G. pulex*, CC= *C. curvispinum*, CP= *C. pseudogarcilis*, DH= *D. haemobaphes*.

PCA using colonisation trap biodiversity data showed more dissimilarities between Gade and Colne, although River Stort sites still displayed affinity for invasive species, resembling associations shown in kick samples (Figure 12). The only two families of predators recorded, Glossiphonidae and Veliidae, were associated with Gade, although one individual of Glossiphonidae was also recorded in Colne.

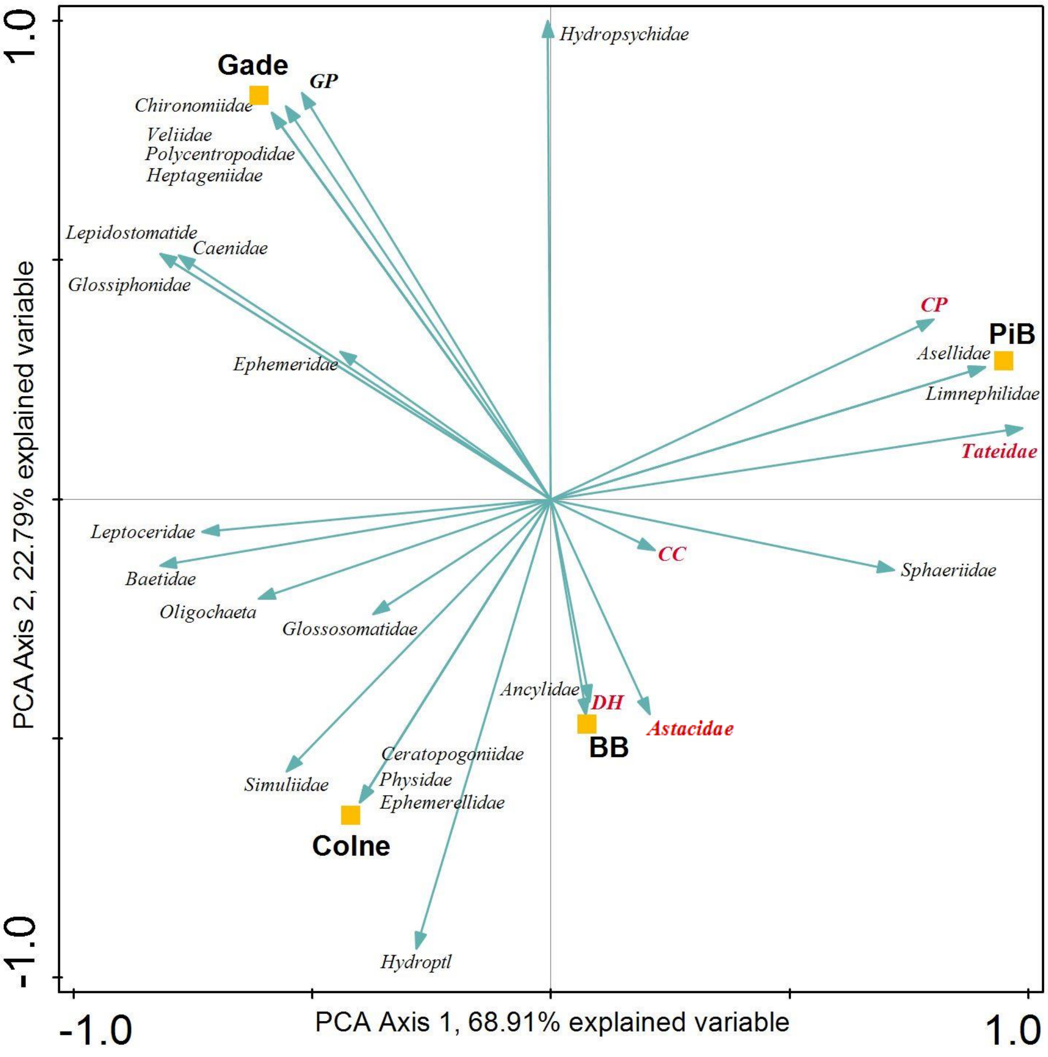

Figure 12: PCA ordination plot of macroinvertebrate from colonisation traps showing association to sampling sites. Axis 1 and 2 explained 91.7% observed variance. Non-native species are marked in red while yellow squares show position of sampling sites.

3.4 Macroinvertebrate response to environmental parameters

As the sample size is low (n=4), a non-parametric Spearman test for collinearity returned no significant correlations between environmental indices. An exploratory RDA with all indices returned Figure 13, which visualises close correlation of many parameters, particularly between: ABMS, EOBS, NAVM and REINF; NOF, FLOW and CPHC; and RVC, HLUP, and ENNP. RPHC was also very negatively correlated to RVC and HLUP.

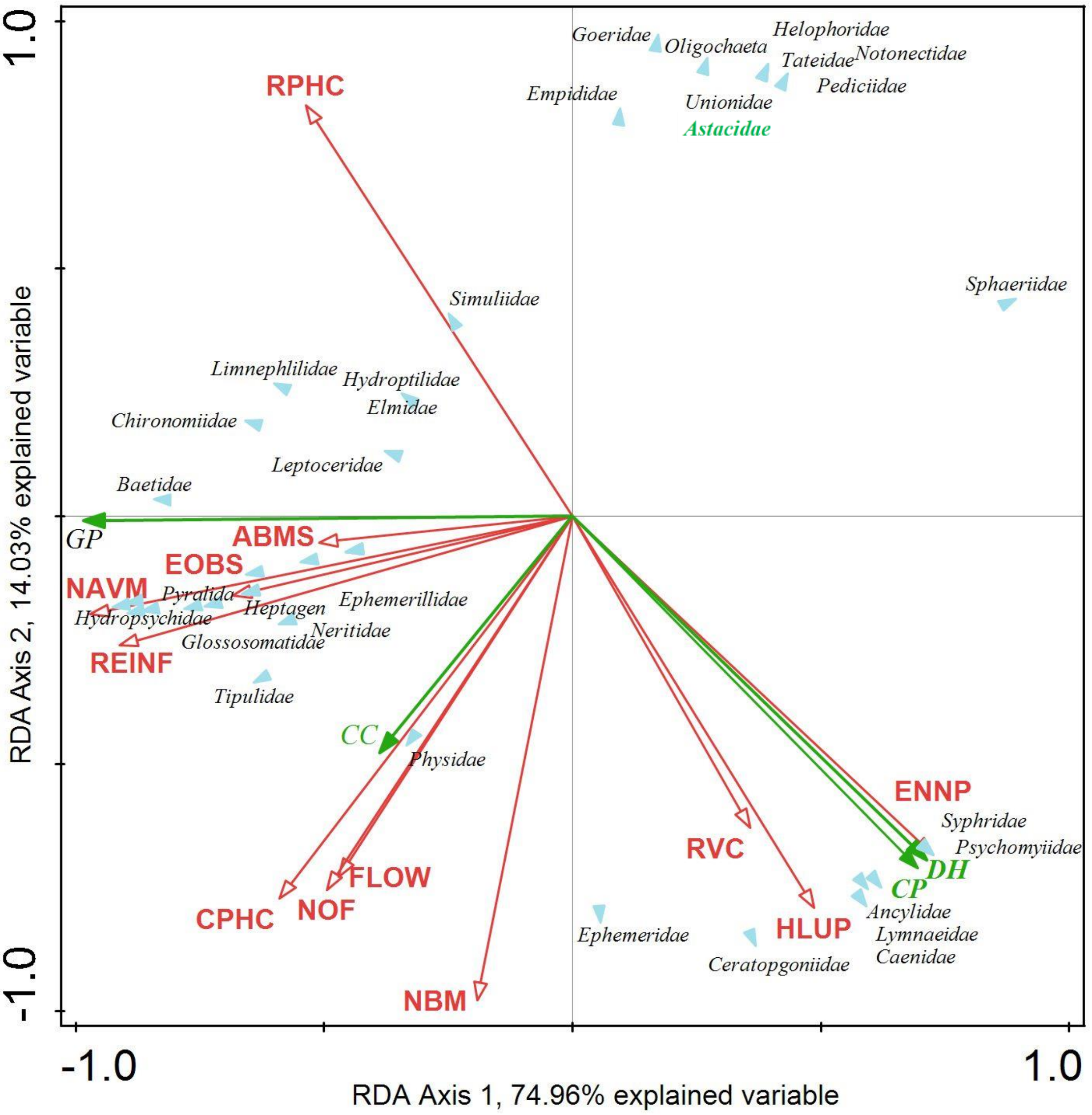

Figure 13: RDA ordination plot using macroinvertebrate data and all environmental indices. Axis 1 and 2 explained 88.99% of observed variance, although the high percentage is due to inclusion of all parameters.

Using individual simple linear regressions, it was discovered that the number of aquatic vegetation morphotype (NAVM) is the determinant environmental parameter influencing patterns in macroinvertebrate biodiversity (Table 10). Using logic and the Spearman collinearity matrix, an MLR built on the five potential drivers returns a model with NAVM and riparian physical habitat complexity (RPHC) as the two key drivers (Table 11). Figure14 shows the revised RDA analysis, while Figure 15 expresses the analysis in terms of FFGs. NAVM as a first component measures 73.43% of explained variable in macroinvertebrate assemblages, while RPHC as the second component provides an additional 14.00% explained variable.

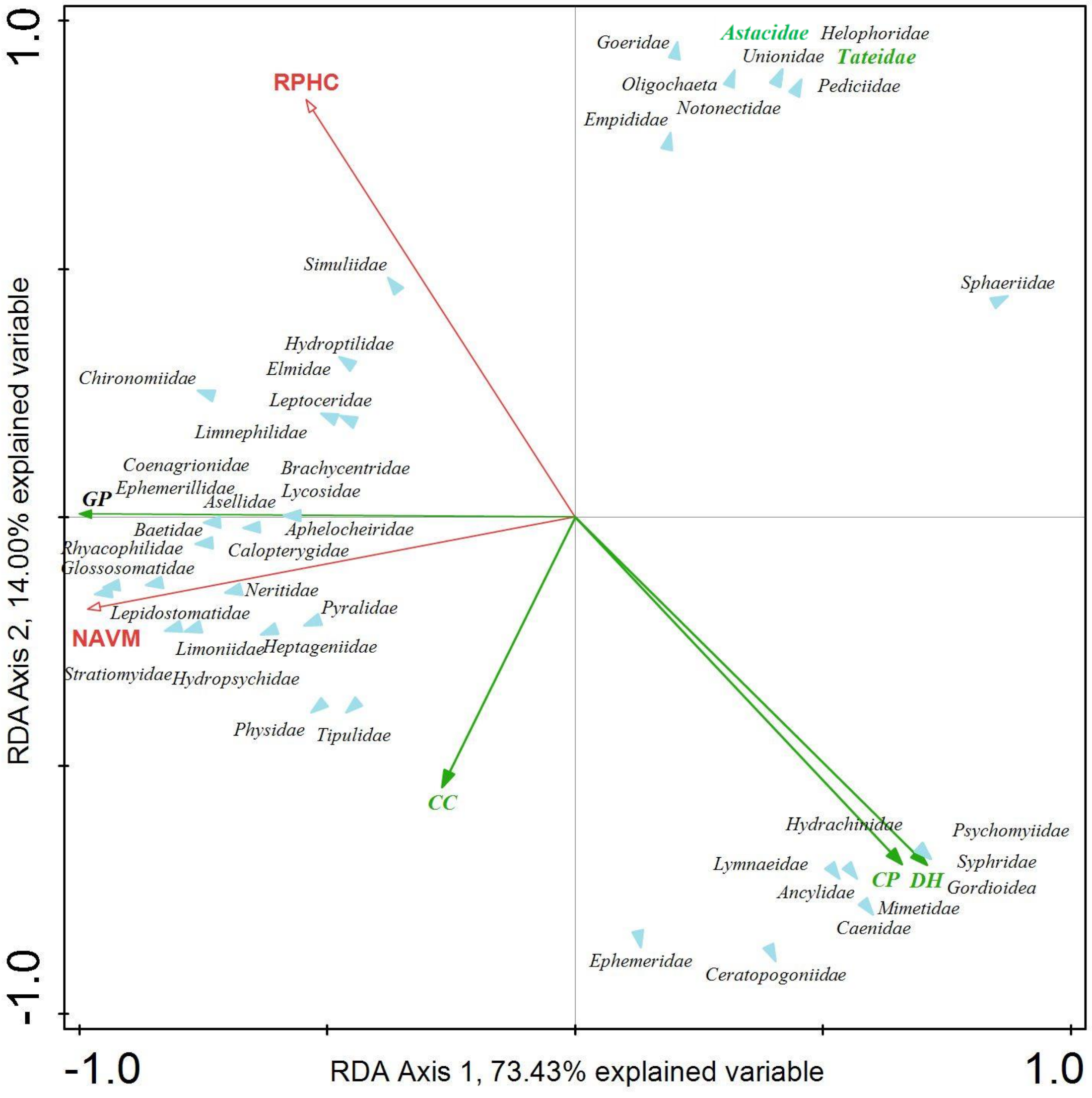

Figure 14: Revised RDA ordination plot of biodiversity pattern influenced by environmental parameters. Axis 1 and 2 explained 87.43% of observed variable.

NAVM = number of aquatic vegetation morphotype; RHPC = riparian habitat physical complexity. Invasive species are highlighted in green.

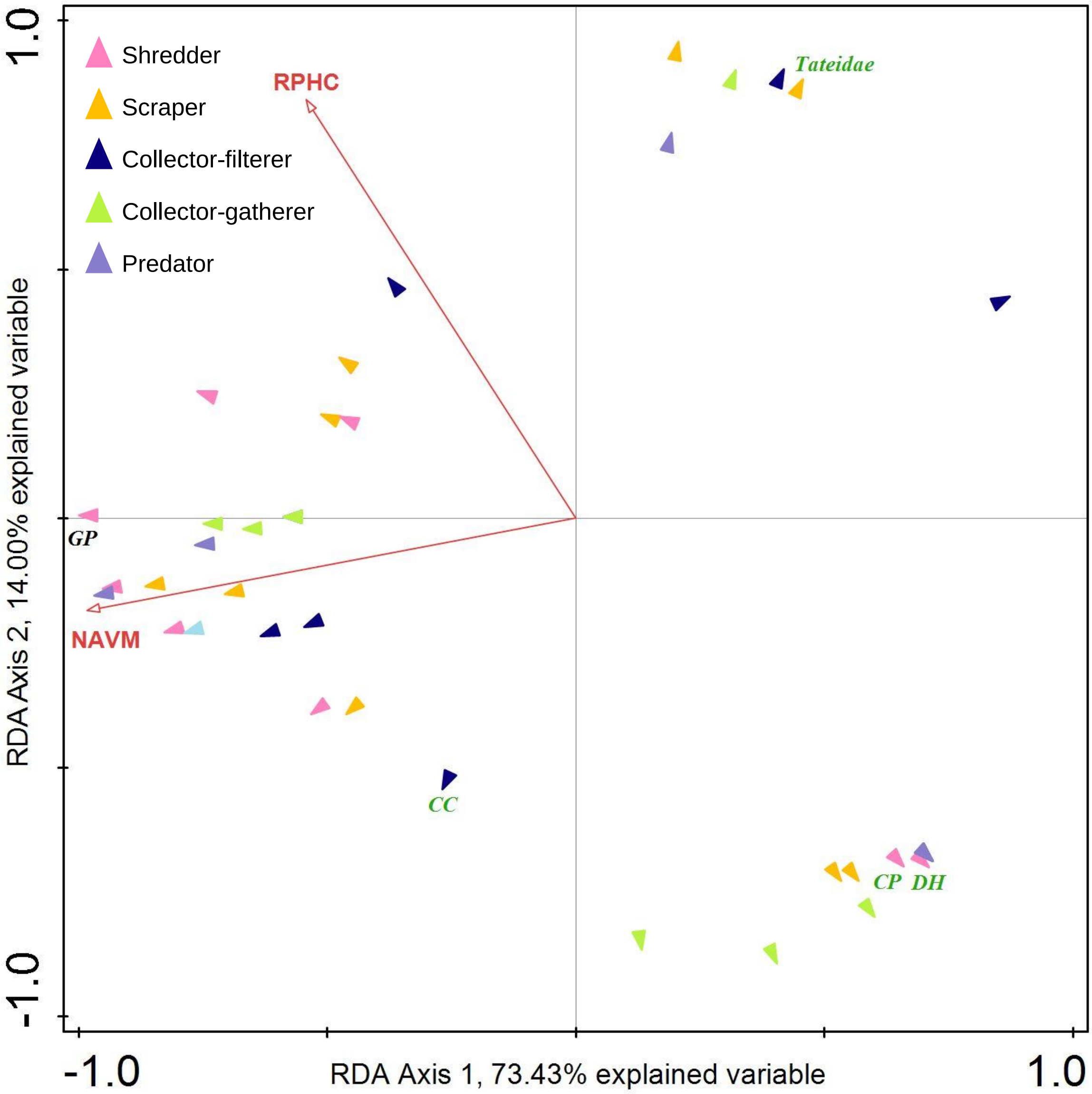

Figure 15: Revised RDA plot expressed as functional feeding groups. NAVM = number of aquatic vegetation morphotype; RHPC = riparian habitat physical complexity. Invasive species are highlighted in green.

Table 10: Linear regression outputs using individual environmental parameter as predictor for observed biodiversity patterns. Significant results at $p<0.05$ level are highlighted in bold.

MoRPh index	R^2	Adjusted R^2	β-value	t-value	p-value
NAVM	0.982	0.973	-0.991	-10.406	**0.009**
REINF	0.763	0.645	-0.874	-2.539	0.126
ENNP	0.456	0.197	0.682	1.318	0.318
NOF	0.358	0.037	-0.598	-1.056	0.402
CPHC	0.358	0.037	-0.599	-1.057	0.401
EOBS	0.343	0.014	-0.585	-1.021	0.415
FLOW	0.339	0.009	-0.583	-1.014	0.417
RPHC	0.248	-0.127	-0.498	-0.813	0.502
HLUP	0.233	-0.150	0.483	0.780	0.517
RVC	0.168	-0.247	0.410	0.636	0.590
ABMS	0.149	-0.276	-0.387	-0.593	0.613
NBM	0.069	-0.396	-0.263	-0.386	0.737

Table 11: Stepwise MLR using selected environmental parameters. Successful models with significant results at $p<0.05$ level are highlighted in bold.

Model	R^2	Adjusted R^2	Std. Error	p-value
NAVM	0.982	0.973	0.225	**0.009**
NAVM + RPHC	1.000	1.000	0.020	**0.040**
NAVM + RPHC + CPHC	1.000	-	-	-

3.5 Macroinvertebrate response to DH abundance

Only families that occurred in both kick sampling and colonisation traps were included in the ordination analysis, which meant no predatory families were included, as they recorded completely different predator taxa. *D. haemobaphes* alone as the first component explained 8.66% of variations observed in the invertebrate community (Figure 16A). Hydroptilidae, Glossosomatidae, Caenidae, and Ancylidae were likely to occur with increasing abundance of *D. haemobaphes*, while *G. pulex* was very negatively impacted by *D. haemobaphes* abundance. Individual linear regressions return *G. pulex*, *C. pseudogarcilis*, Chironomidae, Simuliidae and Tateidae to have negative, significant relationships with increasing *D. haemobaphes* abundance, with *G. pulex* and Tateidae showing the highest evidence (Table 12, $p<0.000$). Its detrimental impact spans all FFG: *G. pulex* and *C. pseudogarcilis* are shredders, Chironomidae is a collector-gatherer, Simuliidae is collector-filterer, while Tateidae is a scraper (Figure 16B). However, as Tateidae was mainly present only in PiB, care should be taken when interpreting its relationship with *D. haemobaphes*, as there may be underlying abiotic or biotic factors influencing their spread and abundance.

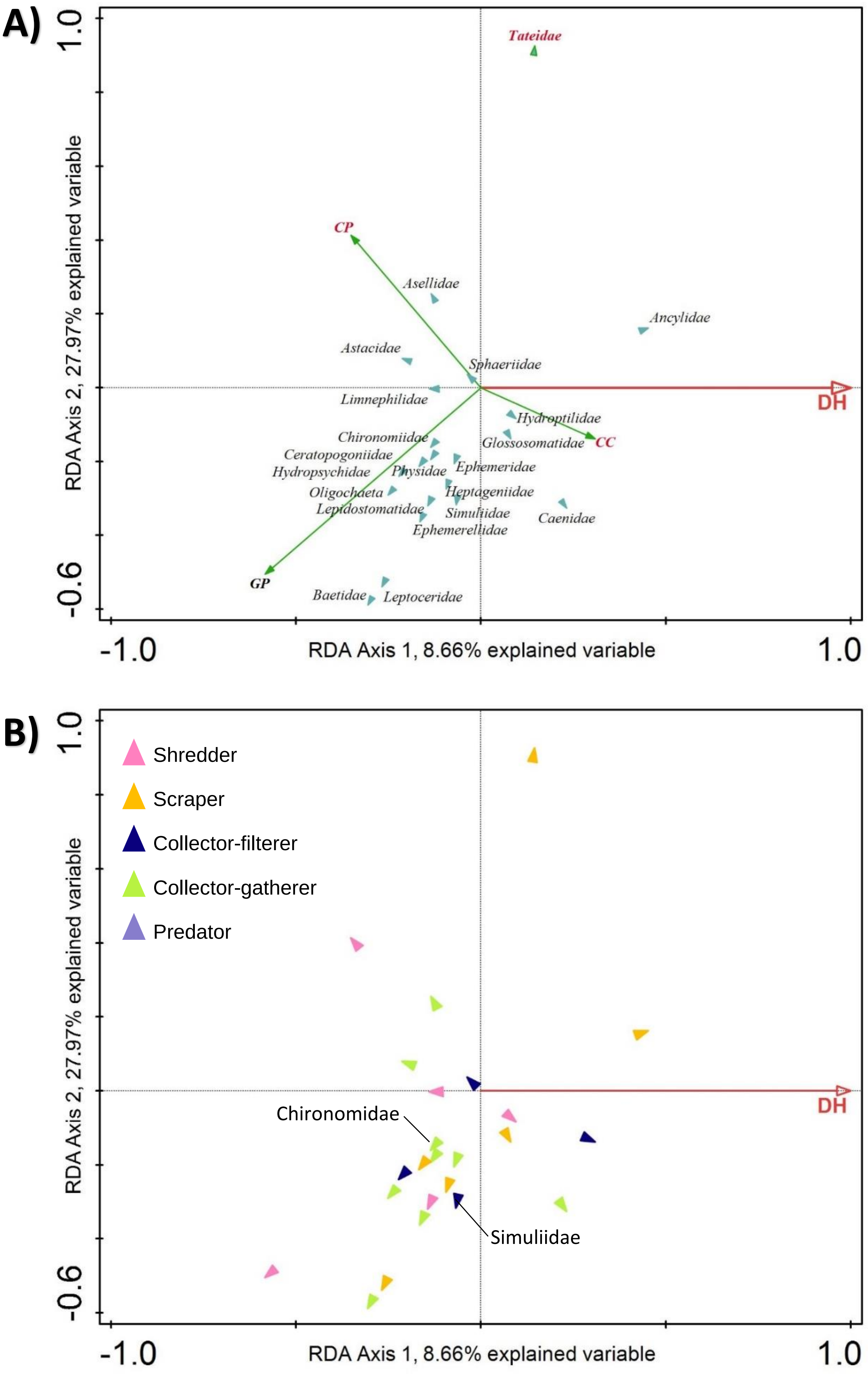

Figure 16: RDA ordination with DH relative abundance as the single predictor, with A) families as response, and B) functional groups as response. Axis 1 and 2 explained 36.63% of all observed variables.

Table 12: Results of linear regression between DH and individual families or species. Significant results at p<0.05 level are highlighted in bold.

ID	Estimate	Std. Error	t-value	p-value, CI=0.95
GP	-0.028	0.005	-5.964	**<0.000**
CC	-0.072	0.041	-1.740	0.107
CP	-0.028	0.011	-2.541	**0.026**
Astacidae	-0.029	0.025	-1.129	0.281
Ephemeridae	0.097	0.150	0.648	0.529
Caenidae	0.016	0.066	0.241	0.814
Baetidae	-0.032	0.023	-1.364	0.198
Ephemerellidae	-0.045	0.081	-0.556	0.588
Heptageniidae	-0.036	0.232	-0.156	0.088
Leptoceridae	-0.046	0.104	-0.439	0.506
Glossosomatidea	-0.038	0.055	-0.685	0.506
Limnephilidae	0.163	0.349	0.466	0.650
Lepidostomatidae	-0.089	0.200	-0.443	0.666
Hydroptilidae	0.156	0.171	0.912	0.380
Hydropsychidae	0.044	0.044	0.994	0.340
Chironomidae	-0.019	±0.005	-4.031	**0.002**
Simuliidae	-0.021	±0.006	-3.416	**0.005**
Ceratopogoniidae	-0.619	0.503	-1.231	0.242
Asellidae	-0.015	0.056	-0.258	0.801
Oligochaeta	-0.087	0.049	-1.779	0.100
Ancylidae	0.119	0.084	1.410	0.184
Physidae	0.026	0.312	0.082	0.936
Sphaeridae	-0.007	0.024	-0.285	0.780
Tateidae	-0.024	±0.005	-5.106	**<0.000**

3.6 Comparing sampling methods

Comparing the two methods, kick sampling managed to capture more individuals, and more families than colonisation traps (Table 9). Figure 10 visualises the fact that cloth paper as feeding materials in the traps did not attract many predators, while the boxplot in Figure 17 shows that invertebrate abundance sampled from the traps also have higher variances compared to those from kick samples. ANOVA and post-hoc Tukey tests returned no significant difference between trap types, except for predators (Table 10).

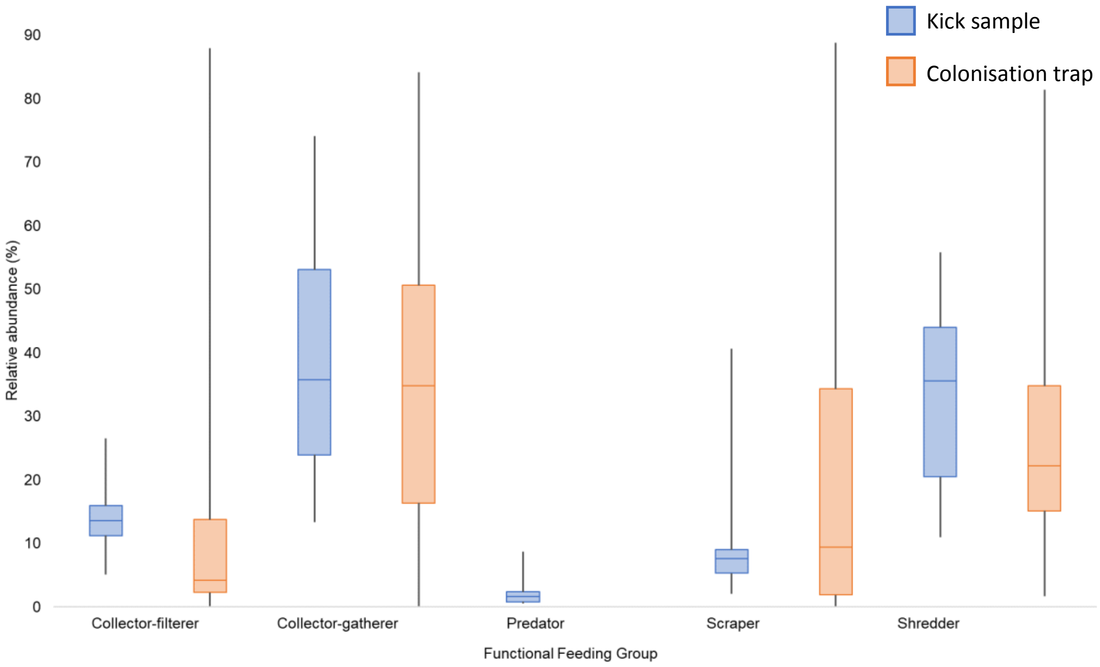

Figure 17: Boxplot of relative macroinvertebrate abundance captured using kick sampling and colonisation traps. Invertebrates were classified according to their functional feeding groups.

Table 13: MANOVA outputs comparing functional feeding groups captured using different sampling methods. Significant results at p<0.05 level are highlighted in bold.

Comparison between sampling methods		
FFG	F-value	p-value
Collector-filterer	3.3641	0.07514
Collector-gatherer	0.08	0.7789
Predator	38.083	**4.60E-07**
Scraper	0.0374	0.8478
Shredder	0.9495	0.3365

Plotting linear regression of colonisation traps and kick sample for different types of indices showed positive relationships when using taxon richness and extended ARMI scores (Figure 12). However as sample sizes are very small (n=4), this is not indicative of a correlative relationship between the two sampling methods. Thus, results from one sampling method should not be used to predict that of another, until more robust relationships are shown.

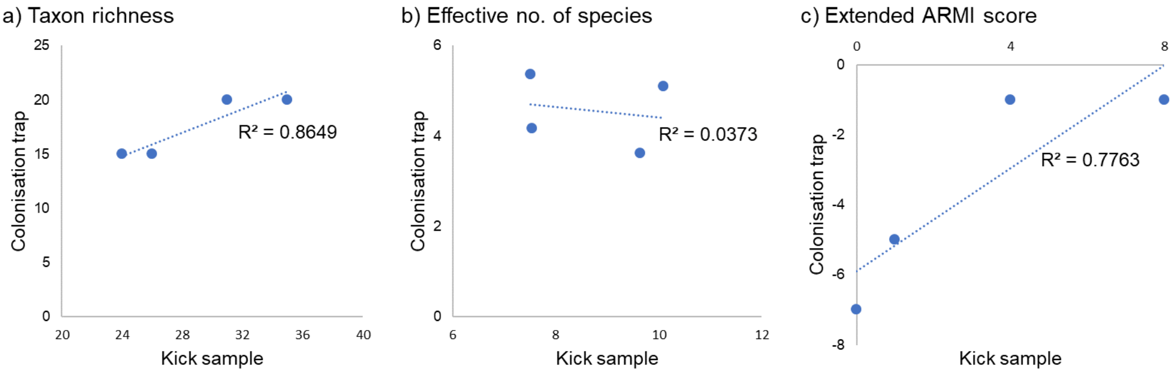

Figure 18: Regressions of colonisation traps against kick samples for different indices: a) taxon richness, b) effective number of species, and c) extended Riverfly ARMI scores.

Chapter 4: Discussion

4.1 Physical and hydrological conditions of streams

In the four sites, physical and hydrological characteristics of the bank top, bank face, and channel are quantified using MoRPh indices and measurements of water flow speed. Colne is a high energy stream, having the fastest flow and largest substrate size. Within the channel, it also has the highest macrophyte complexity, supporting many vegetation morphotypes. Gade is characterised by high channel physical complexity caused by patches of large organic matter accumulations, which also results in relatively high levels of channel bed siltation. It is a modified stream, its banks reinforced with concrete to support the railway bridge overhead. BB is considered to have the highest level of human modification. Reinforcements come in forms of a foot bridge over its width, and builder's waste stabilising the channel bed. It is also located near a Thames water treatment plant, and approximately 1.3km away from the River Lee Navigation. PiB on the other hand, had no reinforcements and minimal land-use pressure in its immediate surroundings. Between the four sites, it has the highest riparian physical complexity and the slowest flow. None of the streams had weirs in place, nor are the channels choked by overgrown plants. More importantly, although there are observed variations between the sites, their differences from each other were not statistically significant. This makes them streams with relatively similar hydrological and physical conditions that are suitable for comparison.

4.2 Abiotic factors influencing macroinvertebrate community composition

Ordination analysis found that overall, channel vegetation complexity and riparian physical habitat complexity are the two most influential environmental parameters that influence macroinvertebrate assemblages in the streams. Most taxa in the study fall within associations with these two parameters, comprising members from all FFGs. Vegetation complexity influences the number of microhabitat pockets with differentiating ecotones that can provide food and shelter, while riparian heterogeneity determines energy input into the streams for shredder consumption and secondary production.

The riparian zone is well known as an important determinant of stream ecology, being the interface between terrestrial and aquatic systems. The concept model of riparian zones is detailed in Gregory et al. (1991), dissecting its definition, classification and position in ecosystem functioning from a comprehensive suite of perspectives. Riparian physical heterogeneity and riparian vegetation complexity engages in a two-way dynamic process. While the spatial dimension of riparian vegetation reflects heterogeneity in geomorphology, riparian plant communities can also influence the evolution of geomorphic surfaces, through the root network's ability to reduce bank side erosion rate and increase channel roughness during flood events (Gregory et al., 1991). Riparian health and heterogeneity control energy input into streams by determining detritus quantity and quality. Leaf litter may become a limiting resource for shredders (Giller & Malmqvist, 1998), showing that conditions of riparian habitat and vegetation can have trickle down impacts on aquatic invertebrate assemblages (Graca, 2001).

Human land use is often a source of habitat modification or degradation, and this is no exception in freshwater systems. A modified stream usually has less riparian physical habitat heterogeneity compared to more "natural" streams. This could mean reduced organic material input into the streams and lowered secondary production by macroinvertebrates. The results are in accordance with Fu et al. (2016), where shredder abundance on the River Stort and its backwater streams are lower than in Colne and Gade, while the more modified Gade site has lower shredder abundance compared to Colne. Shredders are present at lower numbers than collector-gatherers in BB, and lower than scrappers and collector-gatherers in PiB. While the invasive gammarid coexists with *G. pulex* in Colne, Gade and PiB at similarly low levels (<0.5%), *G. pulex* abundance is 61% to 69% lower than Colne and Gade respectively. It is possible that other than exotic species invasion, human land use is influencing the abundance of *G. pulex* in River Stort.

On the other hand, aquatic macrophytes can indicate water quality and influence channel flow regime (Manolaki & Papastergiadou, 2013), both factors which have consequences on macroinvertebrate assemblages. Less diversity in plant assemblages could mean high pollution in the stream, as it only allows pollutant-tolerant species such as *Ranunculus* and *Cladophora* to survive (Hughs et al., 2008).

Shoot density, shoot and root structure, and shoot flexibility can also influence sediment transport. As the plants grow, channel width reduces over time, leading to increased flow speed that can remobilise fine sediment from coarse bed materials, creating a more heterogeneous river bed. Alternatively, overgrown plants choking the channel can drastically reduce flow, retaining fine sediments and so transforming the channel bed into a homogeneous habitat (Wilkes et al., 2019). Generally, more complex macrophyte stands can harbour a disproportionately greater number of small species, resulting in a higher overall biomass in the stream (McAbendroth et al., 2005).

Many caddisfly families, such as Rhyacophilidae, Glossosomatidae, Lepidostomatidae, and Limnephilidae are more likely to occur with increasing NAVM. This is because Trichopterans select their habitat based on a particular flow speed, depending on their feeding methods. For instance, Glossosomatidae species are scrappers that prefer fast riffles, possibly seeking feeding surfaces that are silt-free. Meanwhile, Hydropsychidae have adapted to fast flows by building anchored shelters out of silk, with flow directors that allow them to filter-feed efficiently (Mackay & Wiggins, 1979).

From an FFG perspective, while shredder abundance is mostly accredited to high riparian habitat heterogeneity, collectors and scrapers are especially benefited by macrophyte complexity. For scrapers such as Neritidae that feed on live plant materials, higher complexity means a higher variety of food source (Jahnig et al., 2009). Fine sediment trapped by macrophytes usually retains high organic content (Wilkes et al., 2019), which then becomes available as resources for gatherers and filterers, such as Baetidae, Ephemerellidae, and Asellidae.

Although it is not included in the final ordination, human land use and disturbances show close associations with invasive species, indicated by the position of *D. haemobaphes* and *C. pseudogarcilis* in Figure 13. Canal networks built for industrial and economical purposes, such as the Stort and Lee navigation, create connections between channels and river basins that were previously unconnected. *D. haemobaphes* is known to have spread throughout eastern Europe using the Danube-Rhine canal network, and then towards the Baltic Sea using canals connecting the Rhine, Weser, Elbe, and Oder River in the central corridor (bij de

Vaate et al., 2002). Human modification may have also induced changes in habitat conditions that can weaken native species (Piscart et al., 2009), further facilitating range expansion by non-native species.

4.3 Impacts of *D. haemobaphes* on individual families and FFGs

RDA ordination of macroinvertebrate assemblages in colonisation traps show that most taxa respond negatively to increasing *D. haemobaphes* abundance, except for Ancylidae, Hydroptilidae, Glossosomatidae and Caenidae. Of these taxa, only Hydroptilidae is a shredder, which shows a probability of occurring at low *D. haemobaphes* abundance. A series of linear regressions using taxa that occur in both kick samples and colonisation traps reveals significant, negative relationships with five taxa.

Given the history of *D. haemobaphes* successfully replacing previously dominant species (bij de Vaate et al., 2002), it is possible that when established, *D. haemobaphes* can replace native *G. pulex*. Johns et al. (2018) showed that in the River Trent and Thames, where *D. haemobaphes* was first discovered, *G. pulex* numbers dropped by 77.3% and 85.6% respectively between 2013 and 2016. Mechanisms driving this decline may be intraguild predation (Kinzler et al., 2009), resource competition, or reduced fitness of *G. pulex* in the presence of *D. haemobaphes*. An exposure experiment by MacNiel & Briffa (2019) identified non-consumptive effects of *D. villosus*. Despite not being predated on, *G. pulex* shredding efficiency continued to decrease, showing no habituation but rather increasing sensitivity to predator presence. Likewise, reduced feeding and increased energy expenditure on avoiding *D. haemobaphes* may also decrease *G. pulex* fitness, making them more susceptible to being displaced. Although a pattern of avoidance is observed in colonisation traps (Figure 9), more empirical data should be collected to confirm the significance of this behaviour.

Negative impacts of *D. haemobaphes* is not limited to amphipods. Larval stages of Chironomidae and Simuliidae are small, soft-bodied organisms, fitting the profile for *Dikerogammarus*’ known prey type (Bovy et al., 2014). The findings are supported by van der Velde et al. (2009) who found Chironomidae, Oligochaeta, and smaller members of Ephemeroptera in their gut contents. It is therefore apparent that *D.*

haemobaphes has a detrimental impact that extends beyond the shredder FFG. As well as reducing shredder numbers and displacing *G. pulex*, they may also be altering POM processing at subsequent stages of breakdown, compounding their pressure on the nutrient cycling process in streams.

One species of Tateidae was recorded in this study, the invasive Jenkin's spire shell or New Zealand mud snail *Potamopyrgus antipodarum.* Although a negative association was found with *D. haemobaphes*, a very large population is only found in PiB, while a much smaller population is present in BB. Their exclusion from Colne and Gade could mean that there are other abiotic influences or physical barriers to their distribution and abundance, and further investigation is required to draw a credible conclusion.

4.4 Impacts of *D. haemobaphes* on decomposition rates

Given the negative impact on many shredder families, it is counterintuitive to find a much higher decomposition rate in BB, especially when none of the keystone decomposer, *G. pulex,* is present. This study shows that *D. haemobaphes* might have higher detritus shredding capabilities than previously measured. Assumptions about *D. haemobaphes* impact are largely based on studies on *D. villosus* where isotope analysis found that the two species occupy the same trophic position (Bacela-Spychalska & van der Velde, 2013). Ex-situ experiments with *D. villosus* show they have a lower shredding rate than *G. pulex* (Piscart et al., 2011; MacNiel, 2013). Conversely, *D. villosus* are also found to be very efficient shredders (Worischka et al., 2018), and can be better at detritus processing than *G. pulex* at elevated temperatures. This is possibly due to their superior tolerance for extreme high temperatures compared to the native gammarid (Truhlar et al, 2014). Graham's (2014) in-situ leaf shredding experiments found decomposition rates decrease with increasing *D. haemobaphes* proportion in the traps. However, this can be a result of reduced shredding activity by *G. pulex* when *D. haemobaphes* is present, as previously discussed (MacNiel & Briffa, 2019).

Results of this study are more inclined to agree with Worischka et al. (2018) and Truhlar et al. (2014). It is possible that under the conditions where their preferred prey taxa are less abundant (no *G. pulex*, less chironomids and oligochaetes*), D.*

haemobaphes turns to cannibalism (Kinzler et al., 2009), or detritus as their main energy source. Repeated sampling at BB and laboratory experiments are needed to further explore this hypothesis.

4.5 Application of citizen science tools in scientific research

The Extended ARMI appears to have worked well, generally assigning lower scores to less healthy rivers. As its scores are derived from absolute abundances, and itsapplication depends on the trigger level assigned to individual sites, Extended ARMI should not be directly interpreted as a biodiversity index. The colonisation trap has potential as an affordable citizen science monitoring tool to generate data on decomposition rates as a supplementary proxy for river health (Young et al., 2008). The regression correlation between Extended ARMI scores obtained from kick sampling and colonisation traps shows potential for consolidating data to produce a more nuanced assessment of macroinvertebrate assemblages and river health.

A higher decomposition rate indicates a higher secondary production, where shredders are receiving ample organic material input from the surrounding terrestrial system, converting them into FPOM and DOM for use by other FFGs (Cummins, 1974). Just like the ARMI system, there needs to be a ‘trigger level’ for each site, so that when a lower-than-normal decomposition rate is recorded a hierarchical reporting system is in place to double-check, and to inform the national regulatory bodies (Brooks et al., 2019). One technical issue is the need for high sensitivity weighing scales to capture measurable decomposition rate, up to 4 decimal points. One way to achieve this is by having pre-weighted papers in mesh bags distributed to citizen scientists that are subsequently sent back to labs for processing and analysis (also suggested by Keates, 2018); in which case diligent record keeping would be critical to keep track of samples and ensure accurate data. A more efficient way of attaching the lids should also be explored, as the current system runs the risk of lids being washed away by strong flow.

In the field, traps are easy to install, but volunteers should take care not to lose invertebrates colonising the traps when removing them. They should be left submerged in the river for at least two weeks to allow for conditioning before shredding can take place. Detritus conditioning is the process of leaching to release soluble organic matter, and colonisation by fungi and protozoans (Cummins, 1974). Shredders prefer well leached, softened leaves with a dense microbial growth, a state that takes at least two weeks of conditioning to achieve (Cummins, 1974; Sutcliffe et al., 1981). Seasonal variations should also be considered, as the decomposition rate in summer is much higher, and all papers could be digested. If that occurs, no data can be retrieved from the cloth papers. Suggested total submerged time should be no longer than four weeks.

For environmental parameters, MoRPh indices appear to capture the hydromorphological characteristics well. For instance, flow velocity (FLOW) measured with a water flow meter and NOF were highly correlated. By using the survey, it eliminates need for specialised, expensive equipment in citizen science, and the simple survey can be scaled up quickly to help survey more stretches of river across the world.

This effective method for capturing the nuances of physical and hydrological features can have large potential in river restoration and freshwater conservation. Scientists are familiar with the positive relationship between habitat heterogeneity and species richness, but their understanding of its underlying mechanism is still largely under-researched (Ortega et al., 2018). Data gathered by MoRPh provides means to systematically quantify the hydromorphological characteristics of not just the streams, but also its surrounding terrestrial and riparian habitat. This high-resolution information allows in-depth analysis that might improve the understanding of interactions between macroinvertebrate assemblages and river hydromorphology. Knowing these parameters would make it easier for conservationists to increase the effectiveness and efficiency of their activity by deploying human resources such as planning, funding and manpower in the river restoration projects that will have the most impact.

As volunteers are familiarising themselves with the system, survey times can be long. However, with practice the process can be expected to speed up. Keen observation skills are required to spot different features of the physical habitat, while volunteers need to be reminded that they should record what they see at the time of survey, not what they know of its conditions. A mobile application is also being developed (*Pers. Comm*., Gurnell A.) so that volunteers can immediately upload recordings onto the database, making the process less time consuming and reducing margin for error.

4.6 Limitations

All sites used in this study recorded *D. haemobaphes*; the study therefore lacks a true control site. In the last few years, they have invaded rivers Colne and Gade, with a low number of individuals being regularly recorded during ARMI surveys. Searching for sites further upstream in the River Stort may find a control site with similar environmental parameters and allow a comprehensive assessment of *D. haemobaphes'* impact there.

Due to time constraints, macroinvertebrates other than amphipods are identified to family level. Species-level identification may unravel more species-specific interactions between invasive and native species. The FFG concept classifies invertebrates according to their feeding activity but does not account for diet plasticity. For instance, *G. pulex* and *D. haemobaphes* are both omnivorous. Regarding them as shredders alone may obscure other life-history or ecology trait that could influence their abundance and distribution (MacNiel et al., 1997).

Statistical analysis of environmental parameters is limited due to a singular MoRPh survey being conducted for each site. Repeated sampling close to the time of macroinvertebrate survey could perhaps generate more accurate readings of environmental indices. Concerning colonisation traps, Chironomid larvae are found to occupy some of the fine mesh bags meant for measuring microbial decomposition. Future studies using colonisation traps should make sure that the lids are well secured, perhaps using a smaller mesh size.

4.7 Further research

As the result shows contradictions with various studies, repeated sampling would be necessary to validate or disprove it. Future research should repeat sampling efforts at the study sites to gather more empirical data and account for seasonal variations.

Currently, aside from the risk assessment by Aldridge (2013), Bovy et al. (2014) is the only study predicting the impact of *D. haemobaphes* in the UK. While their invasion route is well tracked, the definitive trophic position and function of *D. haemobaphes* in UK inland waters is still largely unknown. Rather than making assumptions about their ecology and behaviour based on *D. villosus* studies, species-specific studies of the impact of *D. haemobaphes* on the UK freshwater ecosystem should be pursued using mesocosm or laboratory experiments. Research on *D. haemobaphes* metabolic rate, diet, interaction with freshwater vertebrates and other invasive species are all topics worth investigating. For instance, the non-consumptive effect of *D. haemobaphes* on *G. pulex* fitness should be investigated, in an experiment similar to MacNiel & Briffa (2019). Such studies will hopefully help paint a more complete picture of the underlying mechanisms supporting D. haemobaphes' invasion success, to better inform natural resource management and conservation planning decisions.

Chapter 5: Concluding thoughts

5.1 Conclusion

Surveys of macroinvertebrate assemblages and environmental parameters in streams Colne, Gade, Bailey Bridge and Pishiobury Brook, and decomposition rates measured in-situ have been conducted to identify potential impacts of the invasive gammarid, *Dikerogammarus haemobaphes* on UK's freshwater ecosystem. Main conclusions from this study are as follows:

i) River Stort has been successfully invaded by *D. haemobaphes*, evident in the complete wipe out of *G. pulex* in BB, and their presence found in the upstream backwater PiB. *D. haemobaphes* are also found to be present at very low numbers (<1% relative abundance) in rivers Colne and Gade.

ii) Native shredders, particularly *G. pulex*, may already have reduced abundance in River Stort before the invasion, due to degraded channel and riparian habitat, and water chemistry change caused by surrounding land use.

iii) Increase in *D. haemobaphes* abundance can potentially have a negative impact on most freshwater taxa. Linear regressions found negative, significant associations with *G. pulex*, *C. pseudogarcilis*, Chironomidae and *Simuliidae*. Although Tateidae also shows a negative significant interaction, their disproportionate abundance in River Stort sites alone makes it difficult to draw any reliable conclusions about the relationship.

iv) Key environmental drivers of macroinvertebrate assemblages are aquatic vegetation complexity, expressed in the number of aquatic vegetation morphotype, and riparian physical habitat complexity. Scrapers, collected-filterers and collector-gatherers are more closely associated with NAVM, while shredders depend on riparian organic material input as their food source.

v) Close associations between the extent of invasive plant species, human land use pressure and invasive species *D. haemobaphes* and *C. pseudogarcilis* suggest that habitat degradation by human activities may have assisted invasion.

vi) Extended ARMI scores determined that Colne is the healthiest stream, however analysis of MoRPh indices finds no significant difference between the sites.

vii) Given the rather similar environmental conditions and negative impact of *D. haemobaphes* on invertebrate assemblages, it is rather surprising to find the highest decomposition rate in Bailey Bridge, where *D. haemobaphes* have completely displaced *G. pulex*. It is therefore possible that *D. haemobaphes* is better than native gammarids at leaf litter processing, resulting in the higher decomposition rate. While studies have shown *D. haemobaphes* to be omnivorous and occupying a similar trophic position to *Dikerogammarus villosus*, in the absence of high energy, ubiquitous prey, the demon shrimps could turn to detritus as their main food source.

viii) The results infer that *D. haemobaphes* may have a two-fold impact on ecosystem functions in their invaded regions. In the early stages of invasion, they affect decomposition rate through predation pressure on shredder and collector species; and when G. pulex is completely displaced and other prey species become less abundant, they can increase decomposition rate due to their higher detritus processing efficiency.

ix) This study demonstrates that Extended ARMI and MoRPh can be robust, cost-effective methods for conducting biomonitoring and environmental surveys. Their simplistic, standardised methodologies mean surveys can be easily replicated and scaled up. This generates vast amounts of quality data that are useful in scientific studies and can inform resource management decisions. An evaluation of colonisation traps as a citizen science tool reveals high potential, with few technical or practical issues to be solved.

5.2 Auto-critique

I had always been fascinated by invertebrates and their ecology, however freshwater macroinvertebrate was something I was not familiar with and keen to learn about. The project piqued my interest as it allowed me to explore the dynamics of freshwater ecosystem further, and it was especially exciting to study a species whose ecosystem impact was largely unknown. I was also interested in understanding the strengths of citizen science tools in biomonitoring and generating robust datasets.

The biggest strength of this study was its comprehensive use of multiple techniques to capture changes in macroinvertebrate assemblages, environmental parameters and ecosystem functions. I was pleasantly surprised by the detailed dataset generated from these techniques that allowed for a comprehensive analysis.

The analysis process would be aided with more environmental parameter measurements. Due to time constraints only four MoRPh surveys were conducted. Given the high number of indices calculated, more samples would have been useful in detecting more variations between sites. In hindsight, I would probably attempt to locate all sampling sites from River Stort, to account for inherent differences between rivers in different catchments. Should time allow, more site visits upstream of Stort would perhaps yield a control site for comparison. An additional decomposition rate experiment that replicates the methods in Hugh (2014) will also generate comparable results.

Personal communications

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Gurnell, Angela. Professor of Physical Geography at Queen Mary University of London, founder and collaborator of the Urban River Survey, June – July 2019.

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Appendix

1.0 List of literature used in identification

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2.0 Abundance data

Table 14: Invertebrate abundance recorded in kick samples, classified into functional feeding groups (FFGs). Sh= shredder, Sc= scraper, Cf= collector-filterer, Cg= Collector-gatherer, Pr= predators.

FFG	Family	Colne	Gade	BB	PiB
Sh	DH	2	10	236	3
Sh	GP	777	729	0	178
Sh	CP	39	16	139	20
Sh	Limnephilidae	1	9	0	4
Sh	Lepidostomatidae	3	2	0	0
Sh	Hydroptilidae	4	1	1	2
Sh	Tipulidae	2	15	3	0
Sh	Limoniidae	2	5	0	0
Sh	Pediciidae	0	0	0	54
Sh	Helophoridae	0	0	0	22
Sc	Heptageniidae	0	21	0	0
Sc	Leptoceridae	83	12	13	28
Sc	Glossosomatidae	22	10	1	0
Sc	Goeridae	2	3	0	12
Sc	Elmidae	21	0	0	7
Sc	Ancylidae	4	0	13	0
Sc	Lymnaeidae	8	0	22	0
Sc	Physidae	14	3	6	0
Sc	Neritidae	8	2	1	0
Sc	Tateidae	0	2	17	350
Cf	CC	2	113	48	0
Cf	Brachycentridae	1	0	0	0
Cf	Psychomyiidae	0	0	1	0
Cf	Hydropsychidae	10	74	2	1
Cf	Simuliidae	68	14	15	42
Cf	Pyralidae	0	1	0	0
Cf	Sphaeriidae	106	89	137	155
Cf	Unionidae	0	0	0	1
Cg	Astacidae	0	0	0	6
Cg	Ephemeridae	18	86	95	4
Cg	Caenidae	16	45	189	3
Cg	Baetidae	201	606	2	141
Cg	Ephemerellidae	36	4	0	0
Cg	Chironomidae	288	195	148	192
Cg	Syphridae	0	0	2	0
Cg	Ceratopogoniidae	5	15	29	1
Cg	Asellidae	3	0	0	0
Cg	Oligochaeta	0	8	0	39
Pr	Rhyacophilidae	4	3	0	0
Pr	Calopterygidae	12	3	0	0
Pr	Coenagrionidae	1	0	0	0

Pr	Empididae	5	23	8	37
Pr	Aphelocheiridae	3	0	0	0
Pr	Notonectidae	0	0	0	4
Pr	Hydrachinidae	0	0	3	0
Pr	Lycosidae	1	0	0	0
Pr	Mimetidae	0	0	2	0
Pr	Gordioidea	0	0	2	0
	TOTAL	1772	2119	1135	1306

Table 15: Invertebrate abundance recorded in 29 colonisation traps, classified into functional feeding groups (FFGs). Abundance for each trap is combined by site. Sh= shredder, Sc= scraper, Cf= collector-filterer, Cg= Collector-gatherer, Pr= predators.

FFG	**Family**	**Colne**	**Gade**	**BB**	**PiB**
Sh	DH	1	6	138	5
Sh	GP	35	238	0	45
Sh	CP	12	14	1	62
Sh	Lepidostomatidae	1	2	0	0
Sh	Hydroptilidae	1	0	1	0
Sh	Limnephilidae	0	0	0	1
Sc	Tateidae	0	0	138	457
Sc	Leptoceridae	9	6	0	1
Sc	Glossosomatidae	2	3	5	1
Sc	Ancylidae	0	0	7	0
Sc	Heptageniidae	0	1	0	0
Sc	Physidae	1	0	0	0
Cf	CC	0	1	3	1
Cf	Simuliidae	173	37	21	4
Cf	Sphaeriidae	4	6	21	17
Cf	Hydropsychidae	1	9	2	6
Cf	Polycentropodidae	0	1	0	0
Cg	Chironomidae	115	301	127	124
Cg	Baetidae	12	8	1	0
Cg	Caenidae	3	11	5	0
Cg	Astacidae	9	0	0	6
Cg	Oligochaeta	6	3	0	1
Cg	Asellidae	1	1	1	6
Cg	Ephemerellidae	6	0	0	0
Cg	Ephemeridae	0	1	1	0
Cg	Ceratopogonidae	1	0	0	0
Pr	Glossiphonidae	1	2	0	0
Pr	Veliidae	0	1	0	0
	TOTAL	394	652	472	737

Table 16: Relative abundance (%) of FFGs in each site, captured by kick sampling and colonisation traps.

	Kick sampling (n=4)				**Colonisation trap (n=29)**			
	Colne	Gade	BB	PiB	Colne	Gade	BB	PiB
Shredder	46.8	37.1	33.4	21.7	12.69	39.88	29.66	15.33
Scraper	9.1	2.5	6.4	30.4	3.05	1.53	31.78	62.28
Collector-filterer	10.6	13.7	17.9	15.2	45.18	8.28	9.96	3.80
Collector-gatherer	32.0	45.3	41.0	29.6	38.83	49.85	28.60	18.59
Predator	1.5	1.4	1.3	3.1	0.25	0.46	0.00	0.00