THE MACROINVERTEBRATE COMMUNITIES OF HEADWATER REACHES

Understand factors which control the aquatic communities of headwater streams

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Table of contents

Table of figures and tables

Acronyms and abbreviations

EPA Environmental Protection Agency
EPT Ephemeroptera, Plecoptera, Trichoptera
SBES School of Biology and Environmental Science
SSNet Small Stream Network
UCD University College Dublin
WFD Water Framework Directive

Introduction

The European legislation called the Water Framework Directive, WFD, presents precise objectives to improve water quality, hydromorphology and biodiversity in order that a majority of freshwaters be, at least, in “good status”. To achieve these objectives, it is important to understand the functioning of a river ecosystem and the role of each component of this system.

In Ireland, because more than 75% of the river network is small streams corresponding to 1 and 2 Strahler order, these are important quantitatively. In fact, it is equivalent to 63,731 km of a total length of around 85,000 km of river channel (SSNet Project team 2019). Small streams are also important because they provide many ecosystems services that are essential for human well-being including economic development. They act as capillaries and collect water which is transported downstream to the larger rivers and reservoirs from which people can abstract water for domestic, agriculture and industry (Biggs, von Fumetti, and Kelly-Quinn 2017). This is one of the important ecosystem services provided by small streams. Small streams also provide habitats and nursery areas for biodiversity especially for salmonids and macroinvertebrates often typical of these ecosystems (SSNet Project team 2019). In general they are the origin of many elements like nutrient, organic matter and of course water that the rest of the river system depends on. In fact, they provide downstream waterbodies 90% of its water (Biggs, von Fumetti, and Kelly-Quinn 2017). Small streams control floods and exchange with groundwater and downstream. They also transport sediments and nutrients (SSNet Project team 2019) thus their quality can affect water quality further downstream. Therefore, understanding pollution inputs in the small streams is important for policies and management actions to limit impacts on the rest of the network; particularly as they are numerous, often unknown and more vulnerable (Biggs, von Fumetti, and Kelly-Quinn 2017). In fact small streams are highly vulnerable to impacts from human activities such as commercial forestry and agriculture which generate water pollution with nutrients, pesticides and other crop treatments. This is because they have low dilution capacity and high contact with the land due to their narrow width.

Thus, a programme of research called Small Stream Network project and funded by the Irish Environmental Protection Agency (EPA) is focusing its work on the study of the role of Irish small streams in the hydromorphological, chemical and ecological water quality. The overall objective of the SSNet research is to advance knowledge on the role of small streams in water quality, biodiversity and ecosystem services protection that will inform policy, measures and management options to meet water quality and other resources protection targets (Kelly-Quinn et al. 2019). Further details are given in Section I-B).

This study contributes to one of the objectives of the research project about biology of small streams and more precisely for macroinvertebrates. It examined and compared the macroinvertebrate community structure of headwater streams at sites close to the source and further downstream to understand factors which can control these aquatic communities. The sites for this work were located in Wicklow County and represent two geological and physiographical categories. They were selected because they were minimally impacted by human activities such forestry or agriculture according available data and aerial images (looking at land-use).

There were two hypotheses; the first is that the community structure is different between upstream (at the source) and downstream sites, and the second that the taxon richness will be higher in downstream sites.

The report details in the first section the context of the study, that is a presentation of the university, followed by details of the SSNet project and definition of small streams. Then, the study sites and methods used are developed following by results. Finally the last part presents a discussion of the results and the study prospects.

I – Presentation of the contexts

A) UCD and the School of Biology and Environmental Science

University College Dublin was founded in 1854 by John Henry Human and was named the Catholic University. Around fifty years later, in the 1910s, the Catholic University became University College Dublin and had had its first president. The college of science had opened its doors. The university at that time was located in the centre of Dublin and in St Stephen Greens (UCD website s. d.).

Natural sciences and other, but also arts, politics and society and medicine were taught in UCD. Because of the expansion of the university and a growing number of students, more place was needed and it moved to the current campus in Belfield, Dublin in 1960s (UCD website s. d.). Today, UCD is the biggest university in Ireland and counts more than 30,000 students (UCD website s. d.); it is managed by the current president since 2014, Professor Andrew J Deeks (UCD website s. d.). Currently, 27% of students are from countries outside of Ireland, and research is really important for the university in its vision for a "global society" (UCD website s. d.).

Nowadays, the university has 6 colleges and 37 schools. One of those colleges is the UCD College of Science composed of 7 schools among which the School of Biology and Environmental Science (SBES) which is the largest biological centre of Ireland (UCD website s. d.). A one hundred people staff of academic, technical, research funded and administrative people has an important role for expert assessment in management policies for instance.

Three main research themes are addressed in SBES and are linked together. There are 'genetics and evolution: from genome to biome', 'cellular and molecular biology: from genes to biotechnology' and 'environmental change and sustainability: informing policy and practice' (UCD website s. d.). The last thematic is attending to understand the functioning and the role of the freshwater ecosystems for example in order to avoid important human impacts on those ecosystems and anticipate climate change effects. The projects, like SSNet project, within this field of research investigate the current state in order to improve management actions and to change policies (UCD website s. d.) in the studied ecosystems.

B) The SSNet Project

This research project runs from 2018 to 2022 and as mentioned its overall objective is to advance knowledge on the role of small streams in water quality, biodiversity and ecosystem services protection that will inform policy, measures and management options to meet water quality and other resources protection targets. Addressing pressures in the small stream will help achieve WFD objectives for water quality and the achievement of good status further downstream. To achieve this important aim, the lack of knowledge about small streams has to be filled and the comprehension of the role of the small streams is very important in this case (UCD, http://www.ucd.ie/ssnand/about/).
Apart from SBES several other partners are engaged in this project such as the UCD Dooge Centre for Water Resources Research, Queen Mary University of London, the Centre for Ecology & Hydrology Wallingford and the Environment & Department of Zoology, School of Natural Science in Trinity College Dublin.
The project has a number of work packages. Firstly an inventory of the available data has been carried out and a short report on the importance of the small stream network was produced (www.ucd.ie/ssnand). Three main work packages are dealing hydrochemistry especially the study of water nutrients, hydromorphology and biodiversity (macrophytes, phytobenthos and macroinvertebrates). This last work package is defined as the fifth objective will "determine the contribution of small streams to catchment biodiversity and the role of hydromorphology and hydrochemistry in controlling their biodiversity potential" (Kelly-Quinn et al. 2019).

C) The small streams

The small streams are mainly first and second Strahler order reaches (Kelly-Quinn et al. 2019). This streams classification consists in giving an ascending numbering to each water segment starting at the source. The reach order resulting from the meeting between two same order tributaries leads to the next order for this union (Figure 1).

Figure 1. The Strahler classification of streams (Kelly-Quinn et al. 2019)

However, the small streams are not only in the uplands, although the majority are. There are also small lowland and coastal streams (Kelly-Quinn et al. 2019) and in Europe they represent 80% of the river network (Biggs, von Fumetti, and Kelly-Quinn 2017). In Ireland, as mentioned, small streams represent 75% for 63,731km (Table 1).

Table 1. Length and percentage of channel length in each Strahler stream order based on the EPA river map network (available at www.ucd.ie/ssnet)

Strahler Stream Order	Length (km)	Total length (%)	Cumulative length (%)
1	43,265	51.01	51.01
2	20,466	24.13	75.14
3	10,702	12.62	87.76
4	5,829	6.87	94.6
5	2,842	3.35	98.0
6	1,432	1.69	99.7
7	278	0.3	100

Some of the 7th river length runs into the estuarine (transitional) waters

However the small streams study could be difficult. In fact, they are very different each other and classified by their flows: they can be permanent or temporary (i.e intermittent or ephemeral) (Biggs, von Fumetti, and Kelly-quinn 2017). In this study only permanent headwater stream were concerned. Furthermore, small streams are badly listed and work with satellite images is difficult to determine a source for example. Moreover due to many origins of a headwater streams (groundwater and glaciers feed, seepage-fed or lack outlets), the task to identify this source can be difficult (Biggs, von Fumetti, and Kelly-quinn 2017). Therefore the small streams stay very important because the ignorance of many of their processes do not help reducing their vulnerability. So they have to be a central concern in future projects such as the SSNet project.

II – Material and methods

A) Study sites

Firstly, in the context of the SSNet project, researchers have defined possible types of small streams based on GIS files on the Irish geology, soil and physiography. After a cross-checking, 27 categories have been created (Appendix 1) based on 3 geology classes (igneous, limestone and non-calcareous sedimentary), 3 soil types (peat, poor and well drained) and 3 physiography types (mountains, hills and plains).

For my study, I had to find among those categories 5 sites which satisfied several criteria. They had to be in catchments with EPA high status monitoring stations which meant low intensity agriculture and no commercial forestry. They also had to be easily accessible, near roads. They were located using GIS files with all required information and satellite images (Figures 2 and 3). Some characteristics of each site are detailed below (Table 2).

Table 2. Characteristics of each site valid for both of location A and B

Site	Tributary of	Catchment	Strahler order	Category
1	Cloghoge	Avoca	1	Igneous mountain peat
2	Cloghoge River	Avoca	1	Igneous mountain peat
3	Avonmore	Avoca	2	Igneous mountain peat
4	Vartry	Vartry	1	Non-calcareous sedimentary hills peat
5	Raheen	Avoca	1	Non-calcareous sedimentary mountains poor

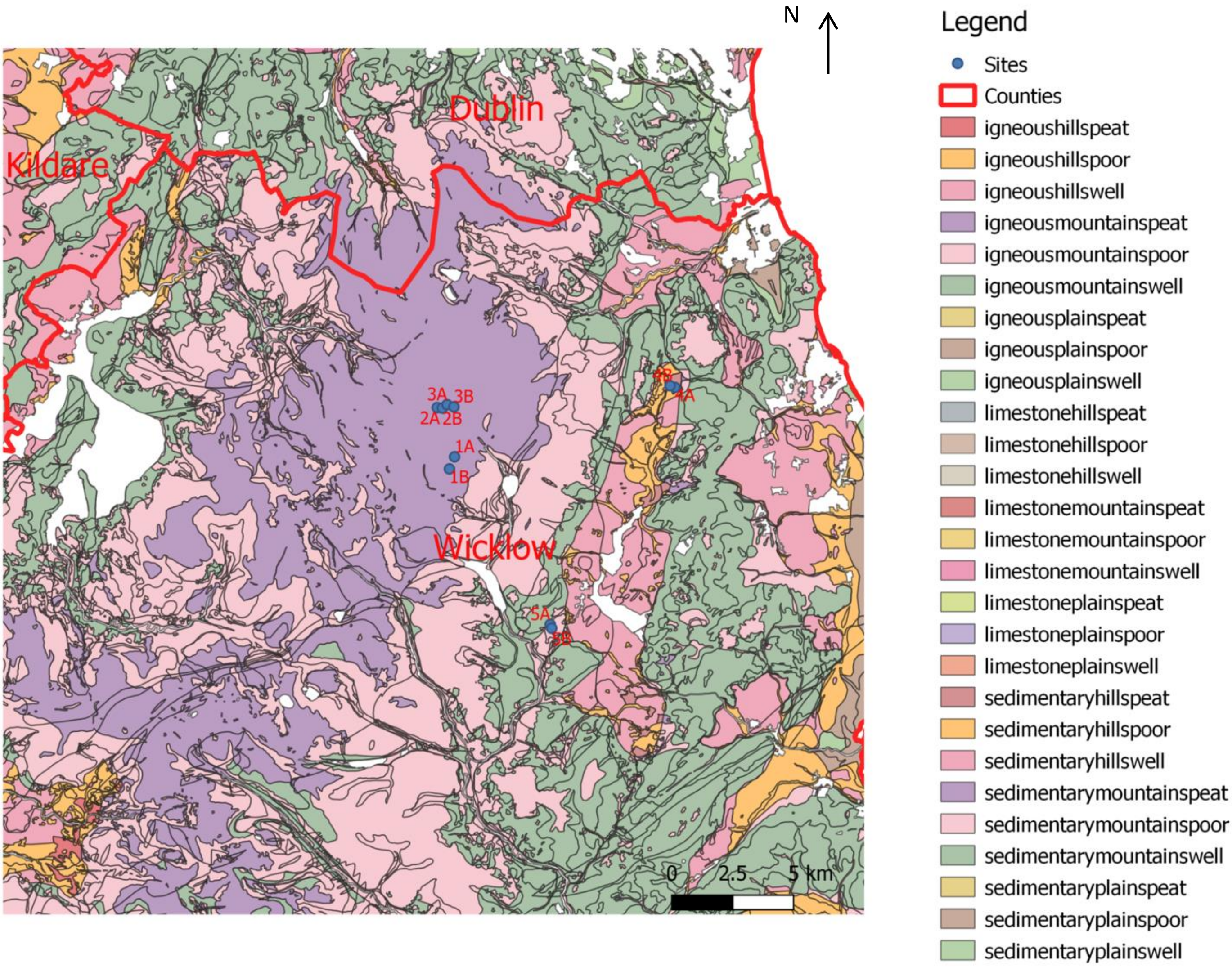

Figure 2. Sites Localisation on the map of the categories

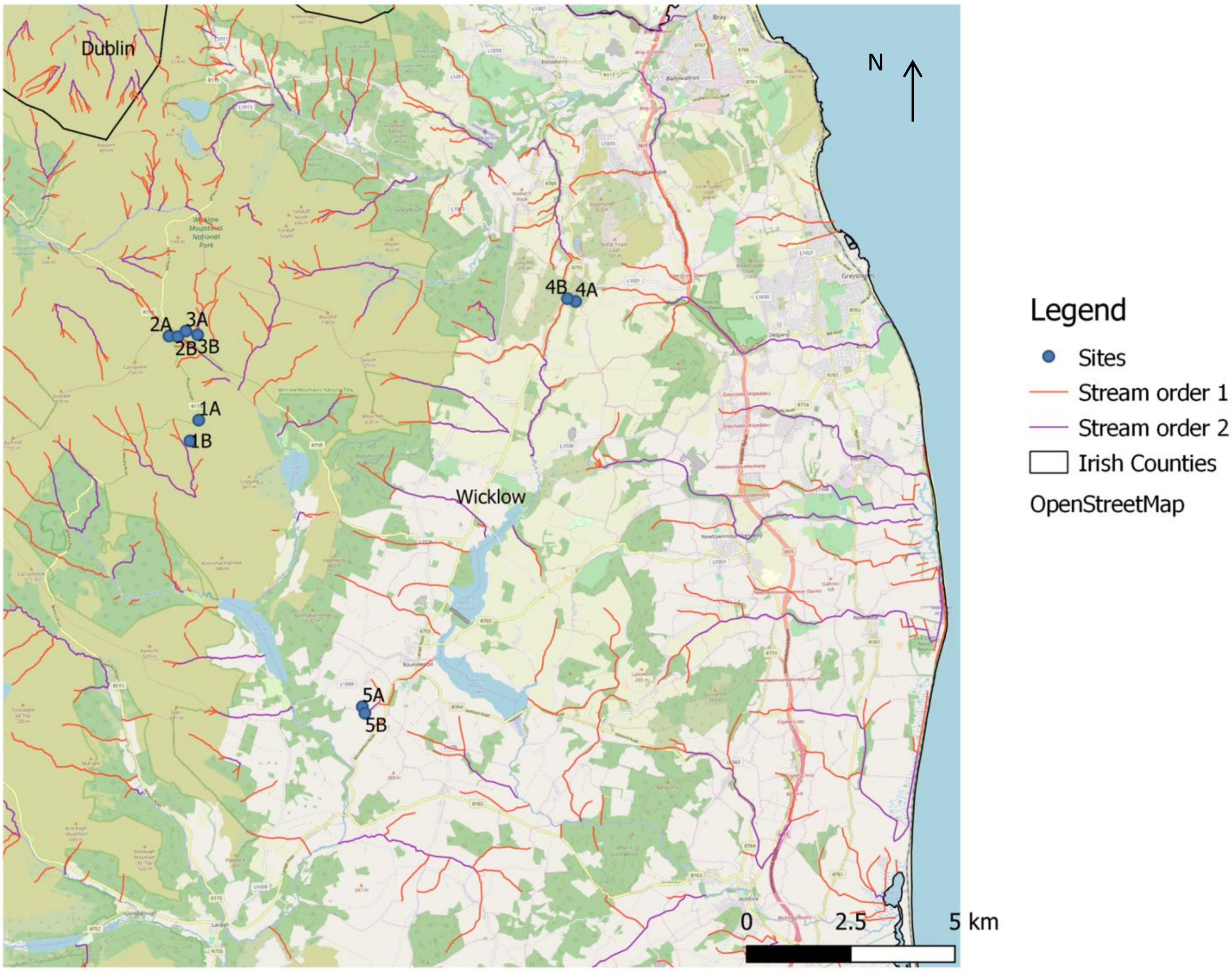

Figure 3. Sites localisation

B) On fieldwork

Figure 4. Kick net

The sampling was undertaken in two campaigns of one day each. For each site 2 locations were sampled: one close the reach source, Location A, and the other location, location B, at around 300 m from the source or more if the reach was not accessible and non-existent flows. The distances from the source for each site and locations are presented in more details in Table 3 below according to the place of sampling and maps. Six replications, 1-minute macroinvertebrate kick samples were taken at each location with a 25 cm-wide, 1 mm kick net (Figure 4) pond net over around 10 m length of stream. Furthermore, for each site and each location a fieldwork sheet was completed to describe the physical characteristics of the site but also to write down values of concentration of O2, temperature, pH, conductivity and dissolve oxygen using field probes (Appendix 2). Photographs were taken of each site to complete the characteristics of the study reaches. The macroinvertebrate samples were preserved in 80% IMS to be processed later in the laboratory.

Table 3. Distance from the source for each location

Site	1		2		3		4		5	
Location	A	B	A	B	A	B	A	B	A	B
Distance from the source (m)	60	620	Undetermined source on map	200 m from site 2A	Undetermined source on map	Undetermined source on map	200	650	100	400

C) Processing of samples

For the study, only 3 replicates (samples 1, 3 and 6) were processed for each site. Samples were washed through a 500µm sieve and sorted on an illuminated white tray. All invertebrates including of course mayflies (Ephemetoptera), stoneflies (Plecoptera) and caddis flies (Trichoptera) and also other individuals were sorted from the samples and preserved in IMS in labelled (site, date) tubes. After collection, each individual was identified using several specific identification keys (Table 4) to species level where possible. All other individuals have also been identified to phylum, order or class, or simply counted. After sampling and sorting 30 samples, invertebrates have been identified.

Table 4. Level of identification and used keys

Taxon	Level of Identification	Identification keys
Oligochaeta	Counted	Dobson et al. (2012)
Hirudinea	Counted	
Mollusca	Counted	
Amphipoda	Counted	
Isopoda	Counted	
Chironomidae	Counted	
Simuliidae	Counted	
Diptera larvae	Counted	
Coleoptera	Counted	
Collembola	Counted	
Acaris	Counted	
Ephemeroptera	Genus	Elliott and Humpesch (2012)
Plecoptera	Species	Hynes (1984); Feeley (2016)
Trichoptera	Genus	Wallace et al. (1990); Edington and Hildrew (2005)

The macroinvertebrate data were inputted into a spreadsheet in order to be used in the *Primer 6 & Permanova +* software. This software calculates a PERMANOVA analysis which is a multivariate variant of the ANOVA. It was used to test the differences in macroinvertebrate community structure and taxon richness between source and downstream sites. It is also a multi-factors analysis because rivers and locations are considered. This method of analysis tests hypotheses with permutation tests instead of from a distribution, moreover it calculates p-values with permutations rather than using tables. Before performing a PERMANOVA, a Permdisp test was run to test for Homogeneity of Variance. If the Permdisp was failed then the data were transformed, using either a Log x+1 or square root or fourth root transformation for community composition and presence-absence transformation for community structure. Permdisp was rerun on transformed data and if the p-value was still less than 0.05 then a stricter p-value of 0.01 was used on the untransformed data and to apply the PERMANOVA without transformation. A two-factor design was used with river as a random factor and location as a fixed factor. If there were less than 100 permutations the Monte Carlo p-value (p (MC)) was used. Where PERMANOVA analysis indicated a significant result pairwise comparisons were run to identify where the significant differences occurred.

III – Results

A) Fieldwork

The measurements taken with the field probes (Table 5) are obviously different between the two site 1 locations for the dissolve oxygen (D.O) and for conductivity between site 3 locations. Generally, conductivity was higher at Location B except in Site 3 and the concentration in oxygen is different between the two locations in Site 1 in location B and Site 2 in location A but the difference is just obvious for dissolve oxygen in Site 1 (Table 5).

Table 5. Chemical values for each site

Site	Location	pH	Dissolve Oxygen (%)	Temperature (°C)	Conductivity (µS)	[O2] (mg/L)
1	A	5.15	59.0	10.5	57.0	6.66
	B	5.4	91.4	11.1	44.7	10.28
2	A	4.07	92.0	10.5	63.8	16.44
	B	3.61	86.8	10.6	-	9.74
3	A	5.35	83.0	9.8	78.0	9.27
	B	4.21	88.6	9.0	43.5	9.99
4	A	6.7	90.1	16.9	124	8.78
	B	7.01	81.6	16.7	114.3	8.06
5	A	6.91	73.3	16.1	99.0	7.48
	B	7.01	88.9	15.7	90.0	9.14

The photographs taken for each site are presented on Appendix 3.

B) Identification

The identification of all individuals collected gave a total of 6 species of stoneflies, 2 genera of mayflies and 8 genera of caddis flies (Table 6), all the counts are presented in Appendix 4. For other invertebrates, many Chironomidae were added up (Appendix 4).

Table 6. Results of identification for EPT

Invertebrates	Species	Locations
Stoneflies	*Nemoura cinerea*	Site 1 LA, Site 1 LB, Site 2 L, Site 2 LB, Site 3 LA, Site 3 LB, Site 4 LA, Site 5 LA, Site 5 LB
	Nemurella pictetii	Site 1 LA, Site 2 LB, Site 3 LA, Site 3 LB
	Amphinemura sulcicollis	Site 1 LA, Site 1 LB
	Leuctra hippopus	Site 1 LB, Site 1 LB
	Leuctra fusca	Site 5 LB
	Brachyptera risi	Site 5 LA, Site 5 LB

Mayflies	*Leptophlebia sp.*	Site 1 LA, Site 1 LB, Site 2 LA, Site 2 LB, Site 3 LA, Site 3 LB, Site 4 LA, Site 4 LB, Site 5 LA, Site 5 LB
	Baetis sp.	Site 4 LB, Site 5 LA, Site 5 LB
Caddis flies	*Plectrocnemia sp.*	Site 1 LA, Site 1 LB, Site 2 LB, Site 3 LA, Site 3 LB, Site 4 LA, Site 4 LB, Site 5 LB
	Agapetus sp.	Site 5 LB
	Chaetopteryx sp.	Site 1 LA, Site 1 LB, Site 2 LB, Site 4 LA, Site 4 LB
	Micropterna sp.	Site 3 LB, Site 4 LB, Site 5 LA, Site 5 LB
	Sericostoma sp.	Site 5 LB
	Halesus sp.	Site 2 LB, Site 3 LA, Site 4 LB
	Stenophylax sp.	Site 1 LA
	Limnephilus sp.	Site 4 LA, Site 4 LB, Site 5 LA, Site 5 LB

C) PERMANOVA

1- Community composition

PERMANOVA results indicated that there was a significant interaction between river and location (F(4,20) = 3.5167, P < 0.01) for community composition (Table 7). Post-hoc pairwise analysis indicated that there was a significant difference between the upstream and downstream locations in the community composition of Cloghoge (t=2.748, p(MC) < 0.01). No significant differences in community composition were observed across the other rivers (Appendix 5).

Table 7. Results of PERMANOVA applied without transformation

PERMANOVA table of results

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)
Ri	4	43403	10851	7.3152	0.0002	4971	0.0002
Lo	1	6646.1	6646.1	1.2741	0.3362	3601	0.28
RixLo	4	20865	5216.3	3.5167	0.0002	4966	0.0002
Res	20	29666	1483.3				
Total	29	1.0058E5					

2- Community structure

In terms of community structure, PERMANOVA results indicated that there was a significant interaction between river and location (F(4,20) = 3.724, P < 0.01) (Table 8). Post-hoc pairwise analysis indicated that there was significant difference between the rivers rather than within the rivers (ie. upstream and downstream locations) (Appendix 5).

Table 8. Results of PERMANOVA applied with a presence-absence transformation

PERMANOVA table of results

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)
Ri	4	21537	5384.2	8.5242	0.0002	4980	0.0002
Lo	1	1526.6	1526.6	0.64901	0.5508	3552	0.6266
RixLo	4	9409.1	2352.3	3.724	0.0002	4986	0.0006
Res	20	12633	631.64				
Total	29	45106					

IV – Discussion and prospects

Currently factors influencing community structure and composition could only be assumed. More analysis has to be lead and calculations of abundance and richness are needed to complete the results and develop the discussion. Especially to explain, for example, the significant difference between the location A and B of Site 1. In fact, calculations of EPT abundance at each location could show more precisely the community composition which can explain this difference and orientate us to monitoring factors of macroinvertebrate communities.

Thus for the end of the study, abundances and richness will be calculated and the interpretations of the comparisons between upstream and downstream communities will be continued. Several papers, available data and fieldwork data can be used to understand community structures. The statistics work with *Primer 6 & Permanova +* will be completed using MDS plots and these will then be analysed. Several metrics will be calculated such as EPT abundance, specific abundance for mayflies, stoneflies and caddisflies and species richness will also be calculated. All results and conclusions will be developed during the final presentation. The objective is to complete data with the composition of these communities. These reflections would be used for the next study of another SBES intern who will work on the same site with other replications.
Moreover, the establishment of first data and the selection of best sites take time because the preparation for fieldwork is essential to not waste more time. The objective of the project is to provide data and not just take available data and analyse it directly. As a result, the work is long even for 5 sites due to the sorting and the identification of 30 samples.

Conclusion

Even if the analyses are not yet completed, the first results of statistics indicated that there was significant difference between locations in one site but not across rivers for the community composition. Then these indicated that, for community structure, it was the opposite. There was a difference between the rivers but not between locations.

The analyses are not yet sufficient to explain precisely which factors influence the community structure and composition between rivers and locations studied in this project. The objective is now to analyse all field data, photographs, results of statistics, calculated metrics and also available data about the sites to understand which factors influence the macroinvertebrate communities in headwater streams.

Even if, currently, no discussion is possible, the work is not yet finished. In fact, analyses will be continued. This work is part of a larger, more important project and was limited to just one area. Other studies will be conducted to increase the data and interpretation to achieve the goal of the SSNet project about macroinvertebrate biodiversity. The analyses will be completed by other projects for the remaining Irish river catchments. The time for this project was limited however and the production of data is exceedingly time consuming.

Therefore this research project and experimentation was a great opportunity to apply a research method and become aware that the establishment of data, sorting and identifying samples is an important task which takes time especially when you are not an expert.

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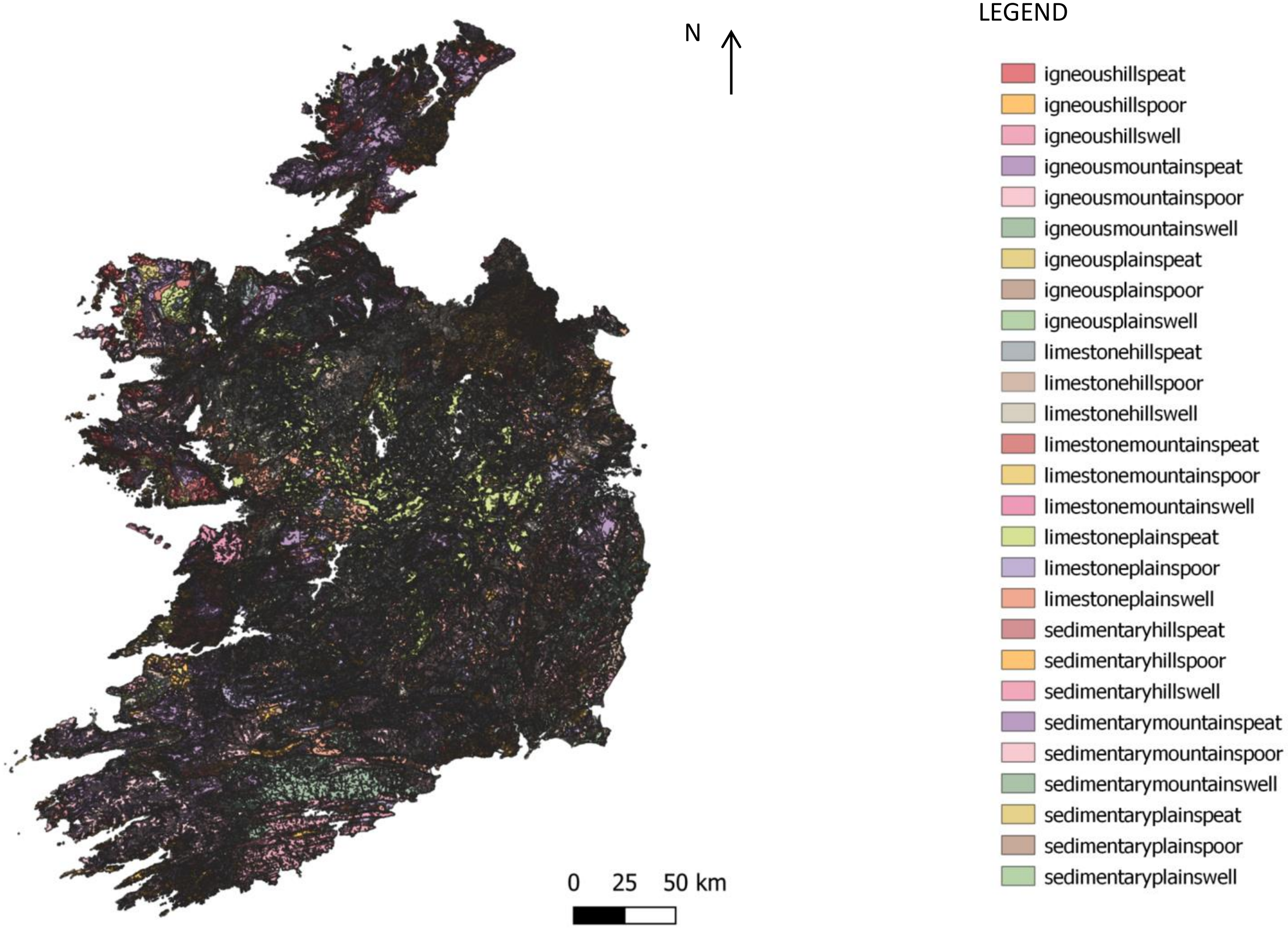
Appendix

Appendix 1. Categories for SSNet project



Appendix 2. One of the fieldwork sheets

Invertebrate Sampling Fieldsheet 2019

Site Information and Location

Site and Sample No. 1A	Surveyor: Rémy & Ed
Date: 2019/05/10	County: Wicklow
Locality:	Stream name and order: Tributary of Cloghoge; order 1
	EPA code:
River Category:	
GPS Coordinates: 53.116009, -6.032004	Photos : upstream 20190510_110535 Downstream 20190510_110640

General Site Description:

- Upland site
- peat bog

pH, D.O., Temp, Conductivity, O_2	5.15 ; 59% ; 10.5°C ; 57.0 µS ; 6.66 mg/L

Measurement	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Average Depth (cm)	0.1m					

Survey Site

Photos			
Stream Width 0.4m		Wetted:	Bankful:
Bank Composition	Vegetation	Right Bank:	Left Bank:
	Soil / Sediment		
	Rock Outcrop		
	Boulders		
Channel Structure	Run/Glide	20%	
	Pool	50%	
	Riffle	30%	
Channel Vegetation	Macrophytes	RB:	LB:
	Bryophytes	RB:	LB:
	Algae	RB:	LB:
	None	RB:	LB:
River Substrate (% cover)	Silt	Peat 70%	
	Sand	10%	
	Gravel	20%	
	Cobble		
	Boulder		

Appendix 3. Photographs of upstream and downstream of each site

Site	Location	Upstream	Downstream
1	A		
	B		
2	A		
	B		

3	A		
	B		
4	A		
	B		

5	A		
	B		

Appendix 4. Final results of identifications

Site	Nemoura cinerea	Nemurella pictetii	Amphinemura sulcicollis	Leuctra hippopus	Brachyptera risi	Leuctra fusca	Leptophlebia	Baetis sp.	Plectrocnemia sp.	Halesus sp.	Micropterna sp.	Limnephilus sp.	Chaetopteryx sp.	Stenophylax sp.
S1LAR1	94	386	1	0	0	0	3	0	22	0	0	0	1	0
S1LAR3	115	319	5	0	0	0	0	0	10	0	0	0	0	1
S1LAR6	66	186	0	0	0	0	0	0	14	0	0	0	1	0
S1LBR1	4	0	3	3	0	0	0	0	0	0	0	0	0	0
S1LBR3	2	0	1	2	0	0	1	0	1	0	0	0	2	0
S1LBR6	8	0	0	7	0	0	15	0	6	0	0	0	0	0
S2LAR1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S2LAR3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S2LAR6	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S2LBR1	26	2	0	0	0	0	2	0	3	6	0	0	10	0
S2LBR3	49	28	0	2	0	0	12	0	26	2	0	0	11	0
S2LBR6	0	4	0	0	0	0	54	0	17	0	0	0	1	0
S3LAR1	16	18	0	0	0	0	18	0	10	0	0	0	5	0
S3LAR3	11	17	0	0	0	0	5	0	1	2	0	0	6	0
S3LAR6	14	45	0	0	0	0	12	0	12	0	0	0	0	0
S3LBR1	0	10	0	0	0	0	1	0	11	0	0	0	0	0
S3LBR3	1	1	0	0	0	0	2	0	5	0	1	0	0	0
S3LBR6	16	11	0	0	0	0	1	0	16	0	0	0	0	0
S4LAR1	1	0	0	0	0	0	1	0	1	0	0	41	2	0
S4LAR3	0	0	0	0	0	0	0	0	25	0	0	26	4	0
S4LAR6	1	0	0	0	0	0	0	0	17	0	0	36	2	0
S4LBR1	0	0	0	0	0	0	1	0	62	2	0	19	2	0
S4LBR3	0	0	0	0	0	0	0	2	74	3	0	0	4	0
S4LBR6	0	0	0	0	0	0	0	0	41	1	1	2	3	0
S5LAR1	0	0	0	0	0	0	0	0	0	0	0	1	0	0
S5LAR3	2	0	0	0	1	0	3	0	0	0	12	5	0	0
S5LAR6	3	0	0	0	2	0	0	5	0	0	1	2	0	0
S5LBR1	15	0	0	0	12	3	9	64	14	0	0	0	0	0
S5LBR3	7	0	0	0	15	1	0	24	3	0	2	0	0	0
S5LBR6	0	0	0	0	1	0	5	38	42	0	1	1	0	0

Site	Sericostoma sp.	Agapetus sp.	Chironomidae	Simuliidae	Diptera	Terrestrial	Collembola	Coleoptera	Acaris	Oligochaeta	Isopoda	Hirudinea	Mollusca	Amphipoda
S1LAR1	0	0	394	15	18	3	1	32	0	2	0	0	0	0
S1LAR3	0	0	113	35	10	7	0	13	0	0	0	0	0	0
S1LAR6	0	0	70	71	10	3	0	17	0	0	0	0	0	0
S1LBR1	0	0	0	3	0	2	0	0	0	2	0	0	0	0
S1LBR3	0	0	0	0	2	8	0	2	0	0	0	0	0	0
S1LBR6	0	0	1	0	3	2	0	0	1	0	0	0	0	0
S2LAR1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S2LAR3	0	0	0	0	0	3	0	0	0	0	0	0	0	0
S2LAR6	0	0	0	0	0	4	2	0	0	0	0	0	0	0
S2LBR1	0	0	2	1	3	7	4	3	0	0	0	0	0	0
S2LBR3	0	0	5	1	3	3	2	9	1	0	0	0	0	0
S2LBR6	0	0	4	0	1	5	0	2	0	0	0	0	0	0
S3LAR1	0	0	51	0	2	4	0	8	1	0	0	0	0	0
S3LAR3	0	0	9	0	1	2	0	0	0	26	0	0	0	0
S3LAR6	0	0	36	0	2	5	0	0	0	0	0	0	0	0
S3LBR1	0	0	7	0	7	3	0	0	0	0	0	0	0	0
S3LBR3	0	0	1	2	7	5	0	2	0	30	0	0	0	0
S3LBR6	0	0	7	3	6	7	0	0	0	1	0	0	0	0
S4LAR1	0	0	298	1	152	39	0	32	0	18	420	28	58	28
S4LAR3	0	0	530	0	278	23	0	25	0	0	533	26	114	44
S4LAR6	0	0	350	0	67	18	0	37	0	0	330	4	18	54
S4LBR1	0	0	1717	0	152	21	0	88	0	0	158	11	444	197
S4LBR3	0	0	676	0	43	10	1	41	0	11	85	2	59	230
S4LBR6	0	0	427	26	33	59	0	45	0	6	72	2	26	86
S5LAR1	0	0	106	0	21	0	0	12	0	37	0	0	6	1
S5LAR3	0	0	73	33	14	1	0	29	1	27	0	1	20	0
S5LAR6	0	0	173	39	16	6	0	32	0	15	0	0	22	0
S5LBR1	0	11	114	28	30	9	0	28	0	2	0	0	4	44
S5LBR3	0	15	18	26	15	12	0	10	0	0	0	0	1	24
S5LBR6	2	7	747	17	48	30	4	71	0	7	1	0	131	59

Appendix 5. Results of PERMANOVA and Perdisp from the Primer 6 & Permanova +

ANALYSIS WITHOUT TRANSFORMATION FOR THE COMMUNITY COMPONSITION

PAIRWISE 1

Term 'RixLo' for pairs of levels of factor 'Rivers'

Within level 'upstream' of factor 'Location'

Groups	t	P(perm)	Unique perms	P(MC)
Cloghoge, Cloghoge River	2.279	0.0964	10	0.0234
Cloghoge, Avonmore	2.939	0.106	10	0.0112
Cloghoge, Vartry	4.313	0.098	10	0.0032
Cloghoge, Raheen	3.1259	0.0968	10	0.0086
Cloghoge River, Avonmore	2.0254	0.1004	10	0.042
Cloghoge River, Vartry	2.4102	0.104	10	0.0246
Cloghoge River, Raheen	2.2873	0.0986	10	0.0254
Avonmore, Vartry	4.0861	0.104	10	0.003
Avonmore, Raheen	2.7649	0.096	10	0.0114
Vartry, Raheen	4.1435	0.1064	10	0.0026

PAIRWISE 2

Term 'RixLo' for pairs of levels of factor 'Location'

Within level 'Cloghoge' of factor 'Rivers'

Groups	t	P(perm)	Unique perms	P(MC)
upstream, downstream	2.748	0.0964	10	0.0104

Within level 'Cloghoge River' of factor 'Rivers'

Groups	t	P(perm)	Unique perms	P(MC)
upstream, downstream	1.6846	0.102	10	0.0832

Within level 'Avonmore' of factor 'Rivers'

Groups	t	P(perm)	Unique perms	P(MC)
upstream, downstream	1.5812	0.1058	10	0.1098

Within level 'Vartry' of factor 'Rivers'

Groups	t	P(perm)	Unique perms	P(MC)
upstream, downstream	1.8534	0.0998	10	0.0668

Within level 'Raheen' of factor 'Rivers'

Groups	t	P(perm)	Unique perms	P(MC)
upstream, downstream	1.6273	0.0988	10	0.101

ANALYSIS WITH PRESENCE-ABSENCE TRANSFORMATION FOR COMMUNITY STRUCTURE

PAIR-WISE TESTS

Term 'RixLo' for pairs of levels of factor 'Rivers'

Within level 'upstream' of factor 'Location'

Groups	t	P(perm)	Unique perms	P(MC)
Cloghoge, Cloghoge River	2.0658	0.0956	10	0.0418
Cloghoge, Avonmore	2.1286	0.098	10	0.0476
Cloghoge, Vartry	4.4836	0.1012	10	0.0088
Cloghoge, Raheen	2.9491	0.0984	10	0.0108
Cloghoge River, Avonmore	2.0568	0.1012	7	0.0476
Cloghoge River, Vartry	2.2117	0.101	10	0.0326
Cloghoge River, Raheen	2.1196	0.0946	10	0.0358
Avonmore, Vartry	4.8599	0.0974	10	0.0044
Avonmore, Raheen	3.371	0.1026	10	0.0082
Vartry, Raheen	2.1873	0.1068	10	0.0442

PAIR-WISE TESTS

Term 'RixLo' for pairs of levels of factor 'Location'

Within level 'Cloghoge' of factor 'Rivers'

Groups	t	P(perm)	Unique perms	P(MC)
upstream, downstream	1.6237	0.0908	10	0.1114

Within level 'Cloghoge River' of factor 'Rivers'

Groups	t	P(perm)	Unique perms	P(MC)
upstream, downstream	2.0202	0.0984	10	0.0452

Within level 'Avonmore' of factor 'Rivers'

Groups	t	P(perm)	Unique perms	P(MC)
upstream, downstream	1.1745	0.3976	10	0.3173

Within level 'Vartry' of factor 'Rivers'

Groups	t	P(perm)	Unique perms	P(MC)
upstream, downstream	1.5486	0.0936	10	0.124

Within level 'Raheen' of factor 'Rivers'

Groups	t	P(perm)	Unique perms	P(MC)
upstream, downstream	1.7747	0.105	10	0.0782