Spatial and seasonal variation of peatland-fed riverine macroinvertebrate and benthic diatom assemblages and implications for assessment: a case study from Ireland

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Abstract

Blanket peat catchments are important biodiversity refugia. Key pressures on peatland catchment water bodies include artificial drainage, forestry, over-grazing, wind farm development and climate change, and assessment of these pressures requires sensitive monitoring programmes. This study, undertaken in two neighbouring blanket peat catchments, examined the variability in macroinvertebrate and diatom assemblages and related indices in response to spatial and seasonal variability. Multivariate analysis revealed significant trends in the taxa distribution of both groups and the indices downstream and away from the constraining influence of the peat. However, the ecological quality ratios and status assessments for the associated water bodies were consistent irrespective of spatial variability in assemblages and raw indices. Significant seasonal trends emerged only in the macroinvertebrate assemblages and indices. This study contributes to the understanding of sources of uncertainty in ecological assessment and thus provides valuable information for the calibration of assessment protocols for sensitive peatland catchments.

Keywords: Peatland catchments; Diatoms; Macroinvertebrates; Variation; WFD.

Introduction

Peatlands cover approximately 3% of the total landmass of the world (Bain et al., 2011), produce 10% of the global freshwater supply and contain one-third of the world's soil carbon (Joosten and Clarke, 2002). Peatland rivers play a significant role in the global carbon cycle and thus climate change, both by sequestering carbon and releasing it to the atmosphere (Mayorga et al., 2005; Holmes et al., 2008; Battin et al., 2009). The global area of peatlands has been reduced significantly since the 1800s through climate change and human activities, particularly by drainage for agriculture and forestry (Joosten and Clarke, 2002). Peatland forestry operations in temperate oceanic climatic conditions are particularly environmentally challenging due to high annual precipitation (Müller, 2000; Rodgers et al., 2010), high soil water content (Long and Jennings, 2006), low ground-bearing capacity (Owende et al., 2002) and the typically ecologically sensitive nature of the receiving water (O'Driscoll et al., 2012). Impacts of peatland forestry operations on the receiving water quality have been well documented. Afforestation operations such as road construction, peat drainage and fertilisation can cause increased sedimentation and eutrophication (Heikurainen et al., 1978; Joensuu et al., 1999; Cummins and Farrell 2003; Dalton et al., 2010) and closed canopy plantations are reported to increase the risk of acidification in streams (Fowler et al., 1989; Ormerod et al., 1989; Kelly-Quinn et al., 1996). More recently, harvesting activities have been shown to increase suspended sediment and nutrient export associated with the degradation of logging residues and mechanical disturbance on the ground (Cummins and Farrell, 2003; Nieminen, 2003; Rodgers et al., 2010). Excess nutrient and suspended sediment (SS) export can lead to eutrophication, deteriorating water quality and decreasing the ecological status of the receiving water (Paavilainen and Päivänen, 1995; Greig et al., 2007). Timber production is set to increase across Europe, supported by governments for its controversial potential to mitigate climate change through carbon sequestration and fossil fuel substitution (Forest Service, 2008; Riipinen & Dobson, 2010; Laudon et al., 2011). In addition, many of the peatland forests planted during the 20th century are now reaching harvestable age (Paavilainen and Päivänen, 1995) and concerns have been raised regarding the environmental effects of further peatland

afforestation and second rotation peatland forestry. A more complete understanding of the organisms inhabiting these systems, and their relationship with physical and chemical variability is needed to underpin evidence-based sustainable forestry policy developments (European Union, 2000; Laudon et al., 2011; Coillte, 2012).

Water bodies draining afforested peat catchments can be important biodiversity refugia (O'Driscoll et al., 2012) with headwaters draining blanket peat accommodating species such as *Salmo salar* L., *Lutra lutra* L., and *Margaritifera margaritifera* L., protected under the European Habitats Directive (EC, 2003). Ecological assessment (incorporating biotic indicators with physical and chemical variables) has become an integral component of new environmental legislation in Europe (EU, 2000), the United States (Gibson et al., 1996), Australia and New Zealand (ANZECC, 2000), with observed conditions being compared to expected conditions, in order to determine the level of anthropogenic change. The European Water Framework Directive (WFD) requires all member states to assess, monitor and, where necessary improve the ecological status class of all its water bodies by 2015 (EU, 2000). Macroinvertebrates and diatoms have been adopted world-wide as efficient indicators of water quality (Carter et al., 2007; Kelly et al., 2008). Uncertainty in estimating the true status class for a water body for a period arises from issues such as spatial variability, temporal variation, differing sampling methods and personnel, and errors in setting appropriate expected condition values due to limitations in the available reference sites' data (Kelly et al., 2009; Bennett et al., 2011; Clarke, 2013). Peat-fed rivers are particularly underrepresented in the derivation of methods for ecological status assessment (Kelly-Quinn et al., 2004; Kelly et al., 2008; O'Driscoll et al., 2012).

Physical and chemical variables driven by spatial and temporal factors have been shown to promote variation in macroinvertebrate and diatom assemblages for many systems (Potapova and Charles 2002; Šporka et al., 2006; Passy, 2007; Kelly et al., 2009). However recent studies have highlighted the limited considerations given to the spatial and seasonal variability of macroinvertebrate (Ramchunder et al., 2011) and diatom (O'Driscoll et al., 2012) assemblages in peatland systems. Peatland streams have been considered harsh environments (Ramchunder et al., 2011) due to the spate nature of the catchments (Müller, 2000) resulting in either frequent flooding (Holden & Burt, 2003) or extremely low flows owing to the low hydraulic conductivity of the peat

(Holden & Burt 2003; Lewis et al., 2011). Peatland catchments can be easily eroded resulting in high organically derived suspended sediment input into receiving waters (Evans et al., 2006; Long and Jennings, 2006). Decreased dissolved organic carbon has been reported downstream and away from the constraining influence of the peat and, while associations have yet to be made for the residing biota (Ramchunder et al., 2011; Pound et al., 2013), Feeley et al. (2013) reported forestry related stream acidification was primarily driven by organic acids. Peatland stream water pH can range from acidic to moderately alkaline depending on catchment geology (Miller et al., 2001; O'Driscoll et al., 2012). There is a general conception that peatland streams (Eyre et al., 2005; Ramchunder et al., 2011) accommodate impoverished macroinvertebrate assemblages and forested peatland streams are somewhat impaired (Feeley et al., 2011).

Based on the findings of earlier studies, this study hypothesises that: (H_1) changes in physical and chemical conditions downstream and away from the constraining influence of the peat would alter macroinvertebrate and diatom assemblages; (H_2) seasonality would result in a temporal trend in macroinvertebrate and diatom assemblages; and (H_3) corresponding changes would be observed in the raw biotic indices calculated from macroinvertebrate and diatom assemblages within a water body. From an EU WFD perspective, there is an expectation that all sections within a water body should behave similarly, and so, finally, this study hypothesises that (H_4) the EQRs (ecological quality ratios) derived from the raw biotic indices would account for the variation, and sections within a water body would show consistent ecological statuses. While broad surveys with a large number of rivers are important for providing overall context, prioritising, and assigning status class, more intensive catchment-based studies are needed to unpick natural and anthropogenic pressures within water bodies in order to locate uncertainty and design appropriate restoration strategies.

Methods

Study sites

This study was undertaken in the Burrishoole Catchment, Mayo, NW Ireland (Fig. 1). The Burrishoole has served as a significant catchment for fisheries research (*Salmo salar* L., *Salmo trutta*

L., and *Anguilla anguilla* L.) since the mid-1950s (Whelan et al., 1998). The majority of the catchment is overlain with blanket peat and receives an average precipitation of about 2,000 mm per year (Rodgers et al., 2010).The Burrishoole catchment is geologically split, with the western half comprising bedrock of quartzite and schist, acidic soils and water with poor buffering capacity whereas the east is much more complex with veins of volcanic rock, dolomite, wacke and pure schist (Kiely et al., 1974). Two rivers in the Burrishoole catchment were chosen for the study, the Glennamong, draining a sub-catchment on the western side of the catchment, and the Srahrevagh, draining a sub-catchment on the east. The main land uses are commercial coniferous forestry and sheep grazing.

Field sampling

Four sections were identified within each river, the upper section, located above all anthropogenic stressors, which drained open landscapes dominated by *Molinia caerulea* (L.), *Calluna vulgaris* (L.) and *Eriophorum vaginatum* (L.). The mid-upper and middle sections drained afforested peat with trees planted to the river's edge, whereas the lower section was located below the forestry and supported sheep grazing (Fig. 1). Three sites ([10 m apart) were sampled within each section. All four sections within the Srahrevagh were contained within one water body (Irish water body code: WE 32 781). The Glennamong sections were contained within two water bodies (WE32 2767 and WE 32 2441). WE 32 2767 is the upper tributary of WE 32 2441. The water body is the basic compliance reporting and management unit for the WFD and in Ireland rivers are divided based on hydro-morphological characteristics such as altitude, depth, size and flow and catchment rock type (EPA, 2006). Catchment area information was extracted from GIS maps. Sites were sampled quarterly (January, April, June, and September) for 2 years (2009–2010) with samples collected across 2 concurrent days. Water samples were taken and analysed on the same day for alkalinity and colour using standard procedures (APHA, 1998). Nutrient analysis included soluble reactive P (SRP), ammonium-N (NH_4-N) and nitrate-N (NO_3-N) using a Konelab 20 Analyser (Konelab Ltd., Finland). Water temperature, pH, and electrical conductivity (EC) were recorded in the field using a WTW Multiline P4 field meter. In addition, temperature was considered as a seasonal variable and so to

separate diurnal and site variation, temperature was also measured at one location in each river using high frequency (every 5 min) data logging (TidbiT v2 Water Temperature Data Logger: UTBI-001 Onset computer Corporation) and seasonal averages were calculated. High frequency water level data were collected (every 5 min) with an Orpheus mini water level recorder (OTT-hydrometry UK) at one gauging station in each river (Fig. 1). The number of days since the last flood (DSF) was calculated by counting the number of days since the last flood event (Biggs, 2000). The DSF variable was designed for high flow spells and relates to flows above a defined threshold. Variables were calculated for 3, 7, and 9 times the median average daily flows (Olden & Poff, 2003). Preliminary statistical analysis determined that 9 times of the median was the most appropriate variable to use due to the spatey nature of the catchment (Müller, 2000).

Macroinvertebrates were collected using a 1-min kick sample with a standard Freshwater Biological Association (FBA) kick-net (230 mm 9 225 mm frame, with 0.9 mm mesh) (Toner et al., 2005). All samples were immediately preserved in 70% Industrial Methylated Spirits and transported back to the laboratory for sorting and identification. Where possible, macroinvertebrates were identified to species level. The number of grazers was calculated by summing the total number of individuals that could be classified as grazers. All authors of macroinvertebrate taxonomic names can be found in supplementary material: Appendix A.

At each site, periphyton was scraped from five cobble surfaces with 100 ml of stream water in accordance with Kelly et al. (1998) and stone surface area was calculated (Dall, 1979). Laboratory analysis was carried out later the same day for periphyton chlorophyll a (Chl a g/m^2), ash free dry mass (AFDM g/m^2) and total phosphorus (TP) (APHA, 1998). Periphyton samples for diatom analysis were cleaned using the cold acid permanganate method (Hendley, 1974). Permanent slides were prepared using Naphrax (refractive index. = 1.74) as a mountant and at least 300 valves were identified and counted per slide using a 1,000x microscope equipped with a 100 phase contrast objective (CEN, 2004). The primary floras used were Krammer & Lange-Bertalot (1986, 1997, 2000, 2004). Certain taxa were difficult to identify and the approaches adopted for these species were as follows. *Achnanthidium minutissimum* varieties split into types based on Potapova and Charles (2002). Three types were found in these samples: the ‘‘capitates’’ morph which corresponds to ‘‘type

a'' in these samples; ''linear'' corresponds to ''type b'' and ''narrow-linear'' corresponding to ''type c''. These three *Achnanthidium* groups were largely present in girdle view and so were enumerated separately in girdle view and then divided between the three morphological groups based in proportion to their relative abundance. *Eunotia exigua*, also present in high numbers in girdle view, was difficult to distinguish from *Eunotia tenella* and *Eunotia meisteri* and so the three were combined and considered as *Eunotia exigua* complex. *Gomphonema parvulum* has been described with a number of varieties and attributed environmental preferences; however, populations in these samples had high morphological variability, and so have been termed *Gomphonema parvulum* complex. All authors of diatom taxonomic names can be found in supplementary material: Appendix B. All the samples were identified by one person, and the analyst took part in a diatom ring test to insure consistency.

During the sampling period of this study, an extreme flood event occurred in the Srahrevagh study river, where 52 mm of rain fell in 2 h (Dalton et al., 2010). Such events are considered to occur once in 250 years (Fealy et al., 2010), and there were, as a consequence, significant changes in the biota (O'Driscoll et al., 2012). Such events give rise to natural uncertainty, and so the impacted lower section was sampled again in summer 2011, following the major spate, for diatom assemblages.

Data analysis

Significance of factors site and season for all environmental variables was tested using Kruskal–Wallis tests. All biological variables were expressed as proportional abundances and fourth-root transformed to reduce the influence of dominant species. Species with an occurrence in less than 5% of samples were excluded. PERMANOVA (permutational analysis of variance: Anderson, 2005, version 1.6) was carried out on the macroinvertebrate and diatom assemblage datasets to determine the main sources of spatial and temporal variation in the data, with three explanatory variables (year, season, and site). 4,999 permutations, using raw data were carried out in all cases. All interactions were included, and pairwise posthoc comparisons, were used to further elucidate the significant patterns. Posthoc Bonferroni correction was applied after every test, and statistical significance was set at a 5% level. A similarity matrix (Bray–Curtis coefficient) was created to assess the similarity in

patterns among sites and sampling occasions (PRIMER v6, Clarke & Gorley, 2006). The data were ordinated by non-metric multidimensional scaling to form a resemblance matrix between the sampling events. The relationship between abiotic parameters and the macroinvertebrate and diatom assemblages was examined using a BVSTEP routine, the stepwise alternative to the BIO-ENV routine (Clarke & Gorley, 2006). The BIOENV procedure (Clarke & Ainsworth, 1993) implemented in PRIMER v6 (Clarke & Gorley, 2006) is a rank correlation procedure that provides a measure of the correlation between two rank similarity matrices, one based on biological data and one based on environmental variables. This procedure was applied to the abundance data to identify the environmental variables that provide the best explanation of the multivariate species composition patterns. The abiotic data set included the following attributes: altitude, upstream catchment area, temperature, conductivity, pH, colour, alkalinity, SRP, NH_4-N, NO_3-N, AFDM, number of grazers and DSF. A Spearman's rank correlation method (P) between abiotic and biological matrices was tested using a permutation procedure, under the null hypothesis that there is no relation between the two matrices. A similarity of percentage analysis (SIMPER, Clarke & Gorley, 2006) was used to determine those species that were responsible for the dissimilarity between groups identified from the MDS and PERMANOVA (i.e., site and season).

Commonly used indices such as the proportion of EPT (Ephemeroptera, Plecoptera, and Trichoptera) taxa and TDI (Trophic diatom index, Kelly et al., 2008) were calculated for each site (for species sensitivities see supplementary material: Appendix B). The SI (Medin's acid index for macroinvertebrates, Henrikson and Medin, 1986) and ACID (Acidity index for diatoms, Andrén & Jarlman, 2008) were chosen as suitable biotic indices of acidity in acid sensitive peatfed streams. The SI is a multi-metric composed of five criteria: presence of certain taxa with different acid sensitivities, presence of *Gammarus* spp., presence of certain acid sensitive macroinvertebrate groups, the ratio of Baetis spp./ Plecoptera (no. of individuals), and total number of taxa. ACID is based on the ratio of acid sensitive and acid tolerant diatom taxa (for calculations, see Andrén & Jarlman (2008) and for species sensitivities see supplementary material: Appendix B). The Irish Q Index (McGarrigle et al., 2002) and the TDI-EQR (Kelly et al., 2008) were used as measures of ecological status based on

macroinvertebrates and diatoms, respectively and can be reported in terms of status classes: bad, poor, moderate, good, and high. The TDI-EQR was calculated using the algorithm in Kelly et al. (2008) which uses alkalinity and season to predict the expected value of the metric in the absence of significant anthropogenic pressure. The alkalinity was set at 6 for low alkalinity rivers (\6.8 mg L^{-1} $CaCO_3$) (Kelly et al., 2008). The EQR has a scale of 0–1, with high values indicating high status. The EQR scale was then divided into five ecological status classes (high, good, moderate, poor, and bad) following the rationale described in Kelly et al. (2008). Univariate indices were analysed using ANOVA (analysis of variance) and pairwise comparisons were made using LSD post hoc tests with site and season as explanatory variables (Datadesk version 6.1). Model assumptions were checked using validation plots. The biotic indices (EPT, SI, ACID, and TDI) and EQRs (Q Index and TDI-EQR) were examined in terms of the variation that exists within a WFD water body (WE 32 781-Srahrevagh and WE 32 2441 main Glennamong river).

Results

River characterisations

Both rivers were spatey in nature and prone to flash floods (Fig. 2). However, alkalinity values were different between the two rivers. The Glennamong had very low values ranging from 0.1 to 10.7 mg $L^{-1}CaCO_3$, whereas in the Srahrevagh values ranged from 8.2 to 68.8 mg L^{-1} $CaCO_3$. In the Glennamong, site was a significant factor (Kruskal–Wallis tests, $P<0.001$ for all comparisons, Table 1) in determining colour, alkalinity and nitrogen with alkalinity increasing downstream and colour and nitrogen values decreasing downstream (supplementary material: Appendix C). Similarly, season was a significant factor in determining temperature, conductivity, colour, alkalinity, and nitrogen (Kruskal–Wallis tests, $P<0.001$ for all comparisons, Table 1). Temperature and colour increased from spring to winter, while conductivity and nitrogen were highest in spring (supplementary material: Appendix C). Alkalinity was highest in the summer (supplementary material: Appendix C). The Srahrevagh showed an increase in conductivity, colour and alkalinity downstream, with values ranging from 75 to 140 µS cm^{-1}, 30–71 Ptco and 14–69 mg l^{-1}, respectively

(supplementary material: Appendix C). In the Srahrevagh, site was also a significant factor (Kruskal–Wallis tests, $P<0.001$ for all comparisons, Table 1) in determining conductivity, colour, alkalinity, and nitrogen with conductivity, colour, and alkalinity increasing downstream and nitrogen values decreasing downstream (supplementary material: Appendix C). Similarly, season was a significant factor in determining temperature, conductivity, colour, alkalinity and nitrogen (Kruskal–Wallis tests, $P<0.001$ for all comparisons, Table 1). Conductivity decreased from spring to winter, while colour increased from spring to winter (supplementary material: Appendix C). Highest alkalinity and NH_4-N values were observed in summer and spring, respectively (supplementary material: Appendix C).

Spatial and temporal variation of macroinvertebrate and diatom assemblages

PERMANOVA indicated that all the main explanatory variables (year, site, and season) were significant sources of variation ($p_{mc}<0.05$) in the macroinvertebrate assemblages. The interaction terms year x season, site x season, year x site x season were also significant (Table 2). The interaction term year x site was not significant in the Glennamong but was significant in the Srahrevagh (Table 2). Detailed pairwise tests revealed that the three way interaction year x site x season was significant in the Glennamong owing to the fact that assemblages at the lower site varied significantly with season in 2009 but not in 2010 (Table 2). Overall, the post hoc pairwise comparisons indicated that the primary source of variation in the Glennamong and Srahrevagh invertebrate assemblages was due to the summer samples being different to all other seasons ($p_{mc}<0.05$), and the upper site being quite distinct from the lower sites ($p_{mc}<0.05$). The BVSTEP routine indicated seasonal temperature and altitude best explained the assemblage patterns in the Glennamong macroinvertebrates (Spearman's = 0.556), with temperature splitting the biological data along the y axis (with season) and altitude splitting the samples according to site (x axis) (Fig. 3a). SIMPER analysis indicated that site differences were attributed to increased abundances of *Nemoura cinerea* and *Amphinemura sulcicollis* at the upper site and increasing abundances of *Baetis rhodani* further downstream (Fig. 3b). SIMPER analysis indicated that the main seasonal differences in species were due to increased abundances of *Baetis rhodani* in summer and increased *Amphinemura sulcicollis*, *Chloroperla torrentium*, and *Brachyptera risi* out of the summer season (Fig. 3c).

In the Srahrevagh, the BVSTEP procedure indicated that temperature, altitude and phosphorus best explained the assemblage patterns (Spearman's = 0.358) with temperature splitting the biological data along the y axis (with season) and altitude splitting the samples according to site (x axis) (Fig. 4a). It should be noted that the stress value for the MDS in Fig. 4a was 0.23, which is rather high, and would warrant the inclusion of more dimensions in the MDS routine for clearer separation of samples (e.g., the stress for the 3D plot was 0.15). However, the 2D plot is included here for clarity. SIMPER analysis indicated that site differences were attributed to relatively higher abundances of *Gammarus dubenii* at the upper sites (Fig. 4b) and seasonal differences were due to increased abundances of simulid species and *Emphemerella ignita* in summer (Fig. 4c).

PERMANOVA indicated that all the main explanatory variables (year, site, and season) were significant sources of variation ($p_{mc}<0.05$) in the diatom assemblages (Table 2) in the Glennamong. In the Srahrevagh, however, PERMANOVA indicated that only site and season were significant sources of variation ($p_{mc}<0.05$) (Table 2). The majority of the interaction terms were also significant. The interaction terms year x season, site x season, year x site x season were also significant (Table 2). The interaction term year x site was not significant in the Glennamong but was significant in the Srahrevagh (Table 1). Detailed pairwise tests revealed that the three way interaction year x site x season was significant in the Glennamong owing to the fact that assemblages at the upper and mid-upper sites varied significantly with season in 2009 but not in 2010(Table 2). The post hoc pairwise comparisons indicated that year was not significant ($p_{mc} = 0.767$), nor was season ($p_{mc}<0.05$in all comparisons). Overall, the post hoc pairwise comparisons indicated that the primary source of variation in the diatom assemblages was site with the upper group being significantly different from the groups further down the river ($p_{mc}<0.001$in all cases). The BVSTEP routine indicated that diatom assemblages in the Glennamong were best correlated with altitude, upstream catchment area and alkalinity (Spearman's = 0.786) (Fig. 5a). SIMPER analysis revealed the split in the MDS plot was a result of the dominance of *Eunotia rhomboidea* at the upper group (Fig. 5b).

In the Srahrevagh, the BVSTEP routine indicated that altitude and DSF were best correlated (Spearman's = 0.588) with the assemblage patterns (Fig. 6a). SIMPER analysis revealed a dominance of *Achnanthes oblongella* at the upstream sites progressing to increased abundances of *Gomphonema*

olivaceoides and *Reimeria sinuata* at the downstream group was driving this trend (Fig. 6b). One year after a rainfall event with an expected return time of 250 years, *Epithemia adnata* and *Ctenophora pulchella*, which were not observed from the spring and summer samples in previous study (O'Driscoll et al., 2012), had reappeared (Fig. 7).

Variation of raw biotic indices and ecological classifications within water bodies

Within water bodies in the Glennamong and Srahrevagh year x site x season, year x site, year x season and year were not significant. In the Glennamong downstream water body (WE32 2441), the interaction term site 9 season was not significant in the EPT, SI, and TDI indices but was significant in the ACID index. This was due to differences between seasonal values at the middle and lower sites and not at the upper and mid-upper sites. In addition, season was a significant source of variation in EPT scores in the Glennamong with a 73% increase from summer to winter values ($P<0.0001$, Table 3). Similarly, in the Srahrevagh water body (WE32 2781), the interaction term site x season was not significant in the EPT, SI, and ACID indices but was significant in the TDI index. This was due to differences between seasonal values at the mid-upper sites and not at the other three. In addition, season was a significant source of variation in EPT numbers in the Srahrevagh (Table 3), with a 38% increase in values observed from summer to winter ($P<0.05$, Table 3). Season was also a significant source of variation in SI scores within the Srahrevagh water body ($P<0.0003$, Table 3), with higher values of SI in summer and lower values of SI in winter. The Q index varied between season only 0.8 in winter, spring and autumn and 0.6 in summer (supplementary material: Appendix D).

Periphyton Chl a ranged from 1.85 ± 1.14 to 24.09 ± 9.47 g m^{-2} in the Glennamong and 1.23 ± 0.46 to 6.79 ± 4.64 g m^{-2} in the Srahrevagh. Ash-free dry mass ranged from 1237.98 ± 345.58 to 5269.23 ± 3036.16 g m^{-2} in the Glennamong and 910.57 ± 412 to 6257.68 ± 5306.65 g m^{-2} in the Srahrevagh. Periphyton TP ranged from 2.91 ± 14.10to 18.72 ± 2.95 g m^{-2} in the Glennamong and 2.56 ± 2.16 to 37.77 ± 13.90 g m^{-2} in the Srahrevagh. In the Glennamong downstream water body (WE32 2441), site and season were significant sources of variation in ACID scores (Table 3; Fig. 8). ACID scores increased downstream ($P<0.01$), and higher ACID values were observed in summer ($P<0.0001$) indicating lower acidity. Season was a significant source of variation in TDI scores

(P<0.0007) (Table 3; Fig. 8), with values increasing from autumn to spring by 109% in the Glennamong and by 22% from summer to winter in the Srahrevagh). In the Srahrevagh water body (WE32 2781), site was a significant source of variation in ACID scores (Table 3; Fig. 8), with significantly higher values of ACID at the lower (L) site (P<0.0001). ACID values within this water body did not vary significantly with season. Site was also a significant source of variation in TDI scores (P<0.0001) (Table 3; Fig. 8), with significantly higher values of TDI at the lower (L) site and lower values at the upper (UP) site. TDI-EQR values were consistent across site and season and demonstrated high status for all samples.

Discussion

General spatial and temporal trends in the macroinvertebrate assemblages

This study confirmed significant spatial and temporal trends in the macroinvertebrate assemblages for rivers draining afforested peatland as predicted by H_1 andH_2. The upper reaches of the Glennamong drained unforested peat and were dominated by Plecoptera shredders. Organic matter inputs from catchments have been found to impact ecosystem functioning, with allochthonous additions being as important for supporting community respiration as primary production (Dawson et al., 2002). The dead leaf detritus from surrounding perennial *Molinia caerulea* L. and *Eriophorum vaginatum* L. grasslands can be readily made available to the shredder communities of these upper reaches of rivers. A distinct shift from a dominance of acid-tolerant Plecoptera species at the upper reaches of the Glennamong to increased abundances of circumneutral represented species such as *Baetis rhodani* further downstream coincided with increased alkalinity and decreased colour. Increased exposure of water to underlying bedrock and greater influence of groundwater contributions as catchment size increased has previously been related to increased pH, alkalinity, and EC downstream (Worrall et al., 2006; Ramchunder et al., 2011). Similarly, decreased colour in the Glennamong downstream and away from the constraining influence of the peat is likely caused by dilution of humic substances (Dawson et al., 2004). Baetis rhodani was the only Ephemeroptera species recorded in the acid sensitive Glennamong throughout all seasons and years highlighting a localised adaptation of this

reportedly acid-sensitive species (Feeley et al., 2011). Seasonality has been well established for many macroinvertebrates assemblages (Ramchunder et al., 2011; Johnson et al., 2012) and largely reflects life-histories (Giller &Twomey, 1993). Higher temperatures in the summer coincided with the appearance of *Emphemerella ignita* in the Srahrevagh and lower temperatures in the autumn, winter and spring samples corresponded with its disappearance. Lower temperatures were also associated with increased abundances of *Brachyptera risi* and *Rhithrogena semicolorata*. Bivoltine *Baetis rhodani* appeared in samples throughout the year. Evidence of spatial and seasonal trends in macroinvertebrate assemblages have been shown for many systems (Giller &Twomey, 1993; S˘porka et al., 2006) and more recently for blanket peat systems (Ramchunder et al., 2011), and these findings substantiate these trends for rivers draining afforested peatland.

General spatial and temporal patterns and driving factors of diatom assemblages

Spatial variation was also apparent in the diatom assemblages in support of H_1. The upper reaches of the Glennamong with slightly lower pH had greater abundances of acid tolerant *Eunotia rhomboidea* whilst lower reaches were dominated by more circumneutral *Achnanthes oblongella* and *Gomphonema parvulum*. It may well be significant however, that the more acid tolerant *Eunotia exigua* only appeared in mid-upper, middle and lower sites with pH of 5.8–6.2 and alkalinity of 6.7–10.7, but not in the upper sites with pH of 4.9 and alkalinity of 0.1. Possible explanations for this could be that the mid-upper and middle sections drain coniferous plantations and as a result may be subjected to episodic acidification. Forests are considered to be efficient sinks for atmospheric pollutants attributed to the interception of sulphates and nitrates (Fowler et al., 1989) and sea salts (Allott & Brennan, 2000) by the forest canopy coupled with the inability of the peatland soil and geology to buffer the acidity (Jenkins et al., 1990; Ormerod et al., 1991). In addition, dissolved organic carbon and inorganic aluminium concentrations have been shown to be higher in forested catchments (Ormerod et al., 1989; Feeley et al., 2013) and are recognised contributors to episodic acidification (Passy, 2006). However, further work would need to be carried out to ascertain this. A shift from *Achnanthes oblongella* to *Achnanthidium* types did occur between the upper and mid-upper sites in the Srahrevagh where grazers were less dominant and collector/ gatherers and predators were

most abundant. *Achnanthidium* types are associated with increased grazing pressure (Biggs et al., 1998) and the replacement of *Achnanthes oblongella* with *Achnanthidium* types downstream suggest a possible competitive association between these two taxa, however, further work using controlled experiments would need to be carried out to ascertain this.

Whereas both rivers in this study can be defined as oligotrophic (<30 µg l^{-1}, EPA, 2005) and measured concentrations were in line with the P concentration reported for water draining pristine peatlands (rarely exceeding 20 µg l^{-1}, Kenttämies, 1981), increased abundances of more P-tolerant *Reimeria sinuata* and *Planothidium lanceolatum* at the lower sites in the Srahrevagh would suggest some level of nutrient response. A combined effect of phased clearfelling activities upstream and sheep grazing on the areas draining the lower sections could explain observed patterns; however, nutrient pulses were not detected in the base flow nutrient sampling carried out in this study or by the storm flow P sampling conducted by Rodgers et al. (2010). Furthermore, O'Driscoll et al. (2013) demonstrated that owing to the dilution effect of the larger rivers on the receiving first order streams receiving forestry clearfelled runoff a corresponding rise in nutrients was not observed. The presence of nitrogen fixing taxa such as *Epithemia adnata* (Lowe et al., 1984; De Yoe et al. 1992; Scott et al., 2005) could suggest higher P concentrations contributed to a lowered N: P ratio (Kelly, 2003) and is further supported by the decreased availability of nitrogen from the top of the catchment to the lower reaches.

The expectation that diatom assemblages would demonstrate seasonal variation was not substantiated, thus H_2 was rejected for the diatom assemblages. This may be owing to the regular river spates which are not seasonally predictable and can occur at any time of the year (Müller, 2000), highlighting that the DSF variable, should not be classed as a 'seasonal' factor, but rather considered as a hydro-morphological feature of Atlantic blanket peat catchments. During periods of low flows, the lower sites in the Srahrevagh were characterised by larger diatom species such as *Ctenophora pulchella*, *Synedra ulna* and *Gomphonema truncatum* capable of forming tall structures that overtop the base layer of colonist taxa (Biggs et al., 1998). The upper reaches of the same river were dominated with r-strategist diatom species such *Achnanthes oblongella* and *Achnanthidium minutissimum* types. Although it has been shown that diatom communities can form stable colonies on

artificial substrates in only a few weeks (Cairns et al., 1983; Oemke & Burton, 1986), our results suggest that this is not the case for blanket peat-fed catchments. The steep-sided nature of the catchment, large amounts of rainfall and the saturated condition of blanket peat lead to rapid peaks in flow after rainfall (Holden & Burt, 2003). The temperate oceanic climate that predominates in the west of Ireland is relatively mild with snow occurring rarely in winters, and summer temperatures being generally less than 20°C. Indeed, so adapted are these rivers to flood events that 2 years after a rainfall event with an expected return time of 250 years, the long stalked loosely attached diatom species namely *Epithemia adnata* and *Ctenophora pulchella* which had disappeared from the spring and summer samples in previous study (O'Driscoll et al., 2012) had reappeared. While Mykrä et al. (2012) reported seasonal trends in diatom assemblages for catchments draining humic soils in Finland; this pattern was not observed by this study.

Uncertainty in indices and consequences for water body status

The data presented in this study indicated that biotic indices do fluctuate spatially and seasonally as predicted by H_3. It was not possible to accurately ascertain whether indices in this study showed predictable relationships to catchment characteristics or anthropogenic stress gradients as indices developed to assess anthropogenic stress gradients have not been calibrated for acid sensitive peatland systems. Among the pressures highlighted for peatland streams as arising from forestry activities, are increased acidification (Allott et al., 1997) and a potential for eutrophication (Rodgers et al., 2010). Diatom assemblages are linked to pH as well as nutrient supply, and the underlying pH gradients, when not accounted for, can interfere with expected responses to nutrient supply (Schneider et al., 2013; O'Driscoll et al., 2013). Higher diatom ACID and TDI scores were associated with increasing alkalinity and greater pH dilution downstream and away from the constraining influence of the peat. However, the TDI-EQR, demonstrated consistent scores across seasons and sites, indicating that the inclusion of the alkalinity and season correction factor accounted for the natural variation occurring in these streams. This result, however, has to be considered with caution as the TDI-EQR which was designed to correct for alkalinity included relatively few low alkalinity sites (Kelly et al.,

2008; O'Driscoll et al., 2012). Furthermore, O'Driscoll et al. (2013) demonstrated that even though the phosphorus concentrations rose after a forest clearfelling event, the diatom assemblages showed no response. These findings suggest that the current metrics may not be capable of detecting anthropogenic stress in acid-sensitive peat-fed catchments. The unique nature of these systems requires the calibration/validation of newly developed diatom (Juggins & Kelly, 2012) and macroinvertebrate (Moe et al., 2010) indices to allow partitioning of anthropogenic stress from responses to pH in naturally acidic catchments.

The seasonal variability in diatom index scores within water bodies was very low, indicating low temporal heterogeneity. These results suggest that the reliability of diatom-based biotic indices in low alkaline peat catchments is largely independent of seasonal variation, and a fairly consistent index value will be obtained, irrespective of sampling time. This is in contrast to Kelly et al. (2009) who, considering a more complete alkalinity gradient over a range of systems, suggested that at least six replicates over 2/3 years are required to provide a reliable status class using the TDI-EQR. The lack of seasonal variation in the diatom assemblages and resulting biotic indices from the current study may be a reflection of hydro-morphology of these types of rivers, where frequent floods may prevent larger diatom species to develop (Biggs, 2000). This finding suggests that this metric may be less capable of detecting nutrient enrichment in catchments with a potential for eutrophication due to flash flooding. It has been recommended that, to accurately measure nutrient enrichment, phytobenthic quantity, and composition should be considered (EU, 2000; Kelly, 2013); however, the validity of measuring phytobenthic quantity in spate catchments prone to frequent flooding is questionable as these parameters were highly variable throughout the duration of this study.

Significant seasonal variation was presented in the EPT index scores, with higher values obtained in winter and spring and lower values obtained in the summer/autumn period in contrast with previous work (Sprules, 1947; Vlek, 2006; Šporka et al., 2006). Sprules (1947) reported that while the number of Plecoptera species decreased with increasing temperature, Ephemeroptera species increased, thus avoiding seasonal differences in EPT index scores. However, there is a relatively low number of sensitive Ephemeroptera observed in the acid sensitive Glennamong, and so when the number of Plecoptera species decreased, they were not replaced by Ephemeroptera species. It is clear

that the macroinvertebrate assemblages of blanket peat catchments change with seasonal phenological cues such as daylight hours and water temperature. Sampling strategies adopted my many European countries are a trade off between biological reliability and economic considerations and there is a general consensus regarding the period most suited for sampling macroinvertebrates, i.e., in the summer/autumn period when the pressures (high temperature, low DO, and high nutrient/organic pressure) are likely to be at their greatest (Šporka et al., 2006). The data presented here indicated that sampling during this period would classify these sites as ‘‘moderately polluted’’. However, anthropogenic nutrient stress gradients were not supported by water chemistry and diatom data in this study or supported by studies which have previously set out to assess eutrophication impacts in these rivers (Rodgers et al., 2010; O'Driscoll et al., 2012, 2013). This leads to believe that this lowering of status class is a reflection of life-cycle histories. Sampling twice a year would incorporate this natural variation, although this would be more expensive.

Ecological classifications, as specified by the WFD should be defined at the level of water bodies; however, bio-assessments are usually carried out at one location in the main river channel and exclude headwaters. Management of downstream sections does not necessarily protect ecosystem processes of whole river systems (Clarke et al., 2008; Mykrä et al., 2012). Variation within a water body arising from interaction between the water body and anthropogenic pressure is acceptable as EQRs have been designed to highlight such issues. However, if the variation within a water body arises from distinctiveness between headwaters and sites further downstream, either the classification of a water body or a correction factor in the EQR should account for this. While the raw biotic index scores calculated for water bodies in this study did vary, the EQRs accounted for this variation in support of H_4 (EQRs derived from the raw biotic indices would account for the variation, and sections within a water body would show consistent ecological statuses).

Summary

This study has provided a detailed insight into the dynamics of macroinvertebrate and diatom assemblages in rivers draining afforested peatland. Despite having impaired assemblages (Eyre et al.,

2005; Feeley et al., 2011), there is still capacity for spatial and seasonal trends in afforested peatland streams. Associations between diatom species previously reported in the literature have been identified by this study, and mesocosm experiments which can control some variability may be necessary to disentangle some of the variables (Kelly, 2013). Seasonal trends have been reported for diatom assemblages for a range of systems (Kelly et al., 2009) and more recently for catchments draining humic soils (Mykrä et al., 2012), however, this finding was not observed by this study. Owing to the unique nature of these systems, current macroinvertebrate (Moe et al., 2010) and diatom (Andrén & Jarlman 2008; Juggins & Kelly 2012) metrics may not be capable of detecting anthropogenic stress supporting the requirement of a new index which would allow for the partitioning of anthropogenic stress from responses to pH in naturally acidic catchments (Schneider et al., 2013). The use of phytobenthic composition and quantity metrics as indicators of nutrient enrichment in catchments with a potential for eutrophication needs to be exercised with caution in cases where catchments are prone to flash flooding. However, the potential for eutrophication may be limited if there is no potential for accumulation of biomass (Kelly, 2013). The macroinvertebrate EQR fluctuated with season giving a lower ‘‘moderately polluted status’’ class in the summer/autumn period. This is likely a reflection of life-cycle histories rather than nutrient pressure (Rodgers et al., 2010; O'Driscoll et al., 2012). Studies such as this which consider the use of developed metrics and sampling strategies in peat-fed rivers are important for contributing a basis for robust decisions in the protection of peatland catchments if the limited budgets available to governments for biomonitoring are to be used wisely.

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Figure 1 Geographical location of the study site in Ireland (left) and of the 12 sampling sites along each of the Glennamong and Srahrevagh.

Figure 2 Hydrographs of the Glennamong (a) and Srahrevagh (b) rivers over the duration of the study.

Figure 3 (a) MDS plot of invertebrate samples taken from the Glennamong at four seasons (autumn, spring, summer and winter). Vectors represent the environmental variables which best explain the assemblage pattern, as calculated using the BVSTEP routine in Primer 6. Rank correlation = 0.556. (b, c) SIMPER analysis results highlighting the average abundances of species responsible for the (b) seasonal and (c) site variation in the Glennamong invertebrates.

Figure 4 (a) MDS plot of invertebrate samples taken from the Srahrevagh at four seasons (autumn, spring, summer and winter). Vectors represent the environmental variables which best explain the assemblage pattern, as calculated using the BVSTEP routine. Rank correlation = 0.433. (b, c) SIMPER analysis results highlighting the average abundances of species responsible for the (b) seasonal and (c) site variation in the Srahrevagh invertebrates.

Figure 5 (a) MDS plot of diatom samples taken from the Glennamong at four sites (L-lower, M-Middle, MU – Mid upper and Up- Upper). Vectors represent the environmental variables which best explain the assemblage pattern. Rank correlation = 0.786. (b)SIMPER analysis results highlighting the average abundances of species most responsible for the site variation in the Glennamong diatoms.

Figure 6 (a) MDS plot of diatom samples taken from the Srahrevagh at four sites (L-lower, M-Middle, MU – Mid upper and Up- Upper). Vectors represent the environmental variables which best explain the assemblage pattern. Rank correlation = 0.588. (b)SIMPER analysis

results highlighting the average abundances of species most responsible for the site variation in the Srahrevagh diatoms.

Figure 7 Average abundances of diatom species most affected by the summer 2009 flood event before the flood and in the two years after (tested with t-test and revealed to be significantly different between years 2009 and 2010)

Figure 8 (a and b) Seasonal variations (Spring, Summer, Autumn, Winter) at each section (UP – Upper, MU – Mid upper, M – Middle and L – Lower) in EPT (top), SI, ACID and TDI(bottom) in the Srahrevagh (a) and the Glennamong (b) respectively. The error bars indicate the variation in each sample (n=3).

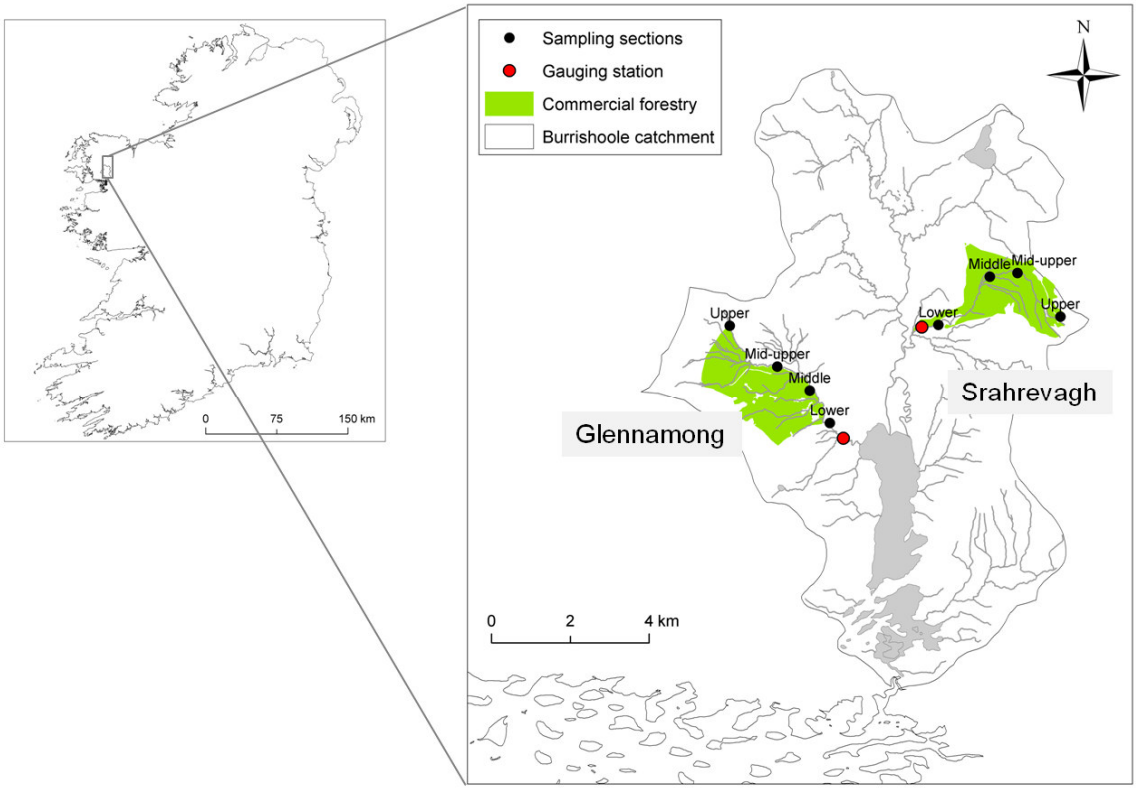
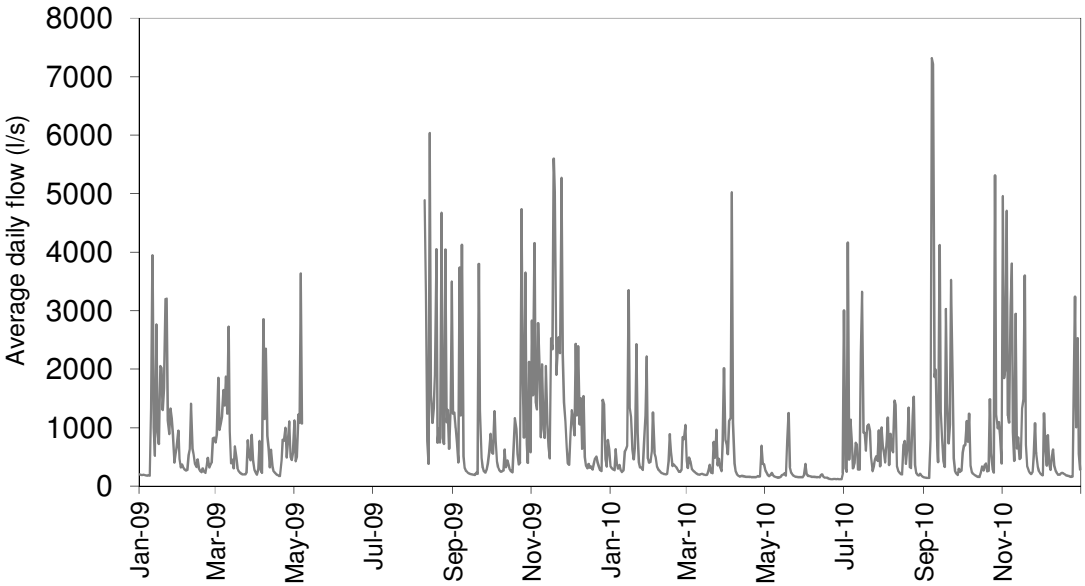
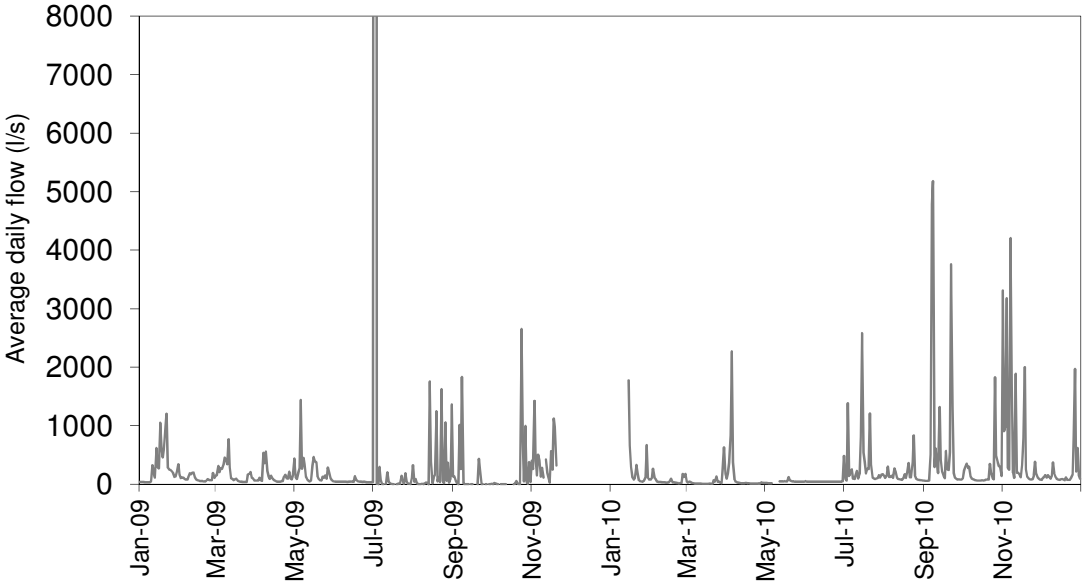

Sampling sections
Gauging station
Commercial forestry
Burrishoole catchment
N
0
75
150 km
Upper
Mid-upper
Middle
Lower
Middle
Mid-upper
Upper
Lower
Glennamong
Srahrevagh
0
2
4 km

Figure 1

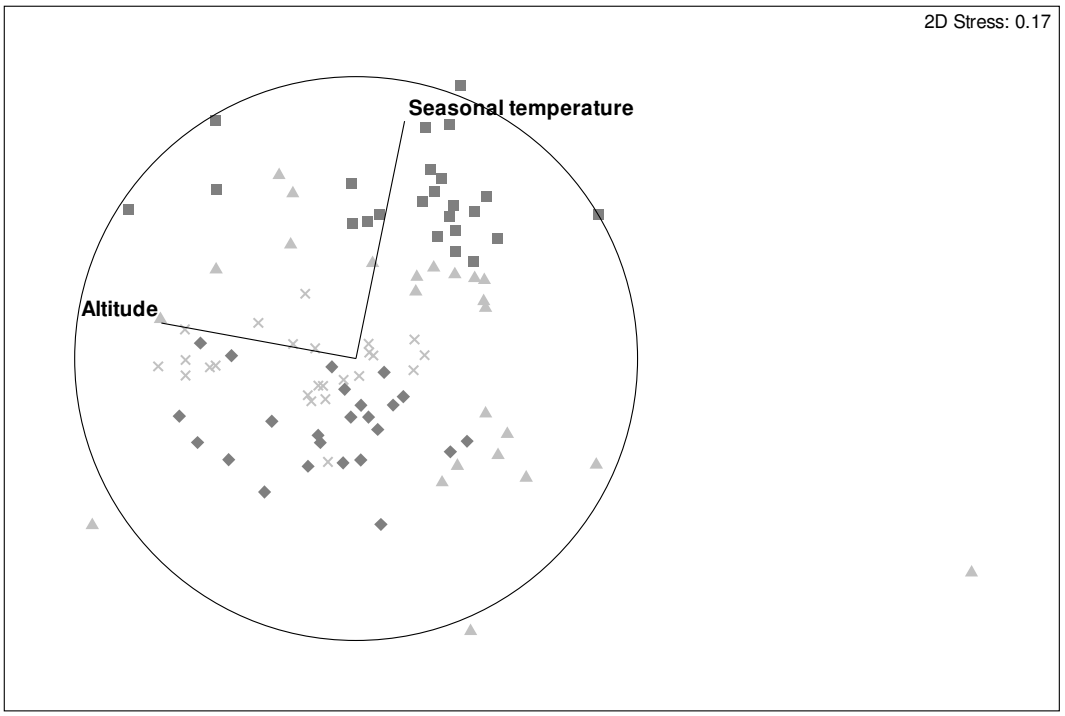

Average daily flow (l/s)
8000
7000
6000
5000
4000
3000
2000
1000
0
Jan-09
Mar-09
May-09
Jul-09
Sep-09
Nov-09
Jan-10
Mar-10
May-10
Jul-10
Sep-10
Nov-10

a)

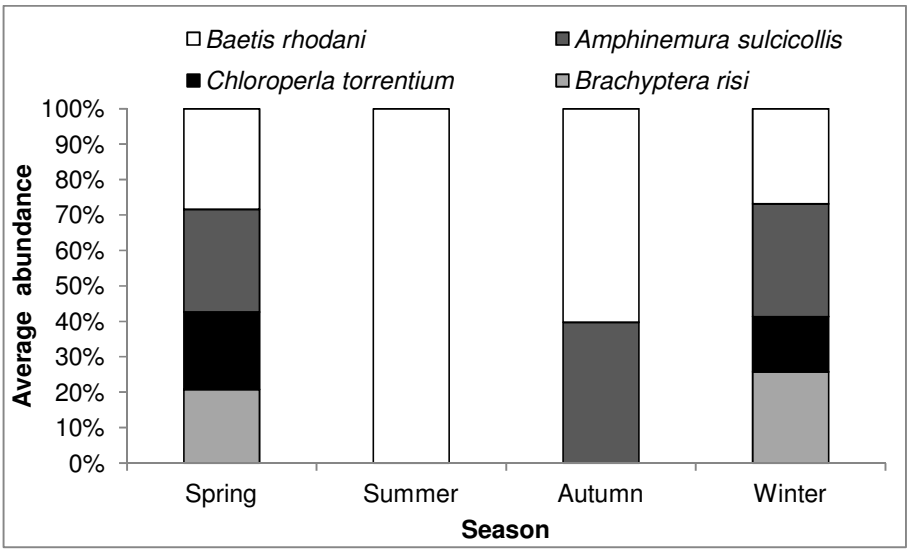

Average daily flow (l/s)
8000
7000
6000
5000
4000
3000
2000
1000
0
Jan-09
Mar-09
May-09
Jul-09
Sep-09
Nov-09
Jan-10
Mar-10
May-10
Jul-10
Sep-10
Nov-10

b)

Figure 2


2D Stress: 0.17
Seasonal temperature
Altitude

a)


Baetis rhodani
Amphinemura sulcicollis
Chloroperla torrentium
Brachyptera risi
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Average abundance
Spring
Summer
Autumn
Winter
Season

b)

c)

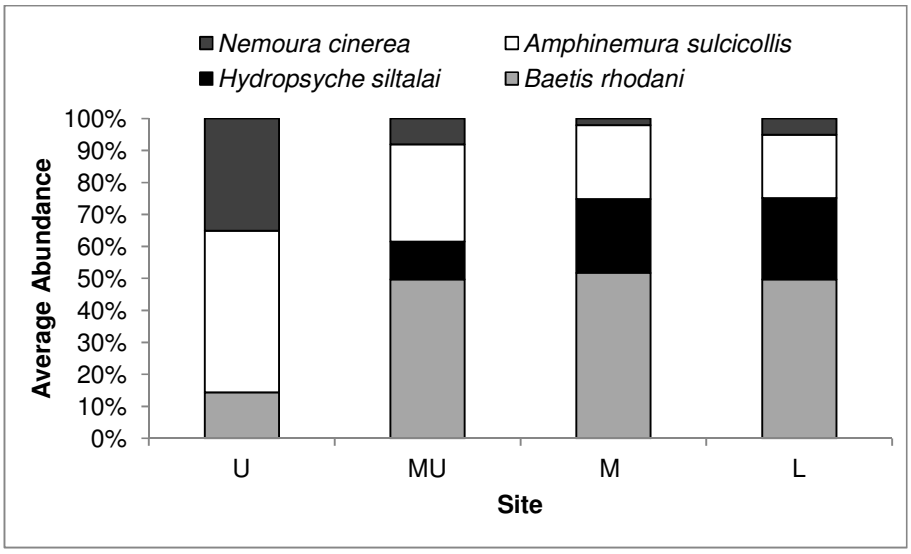
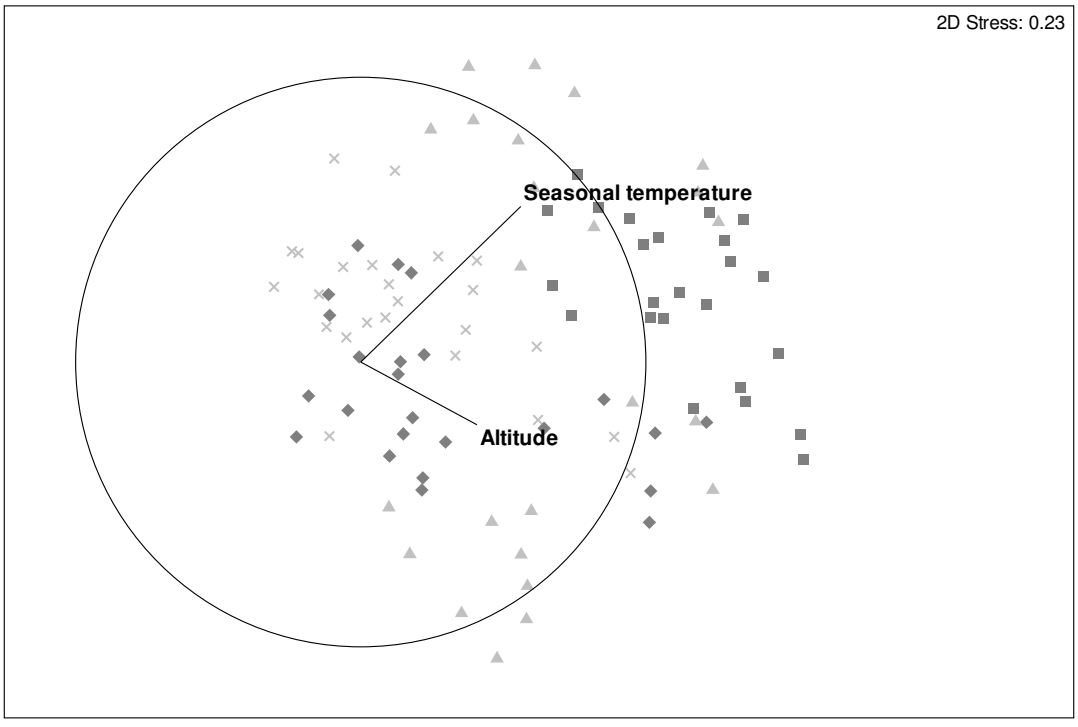
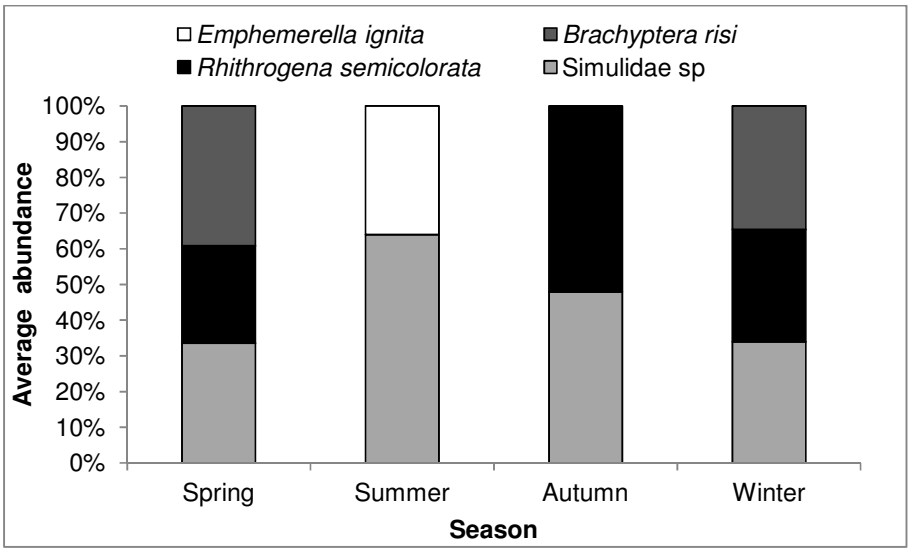


Figure 3 (a, b and c)

a)



b)



c)

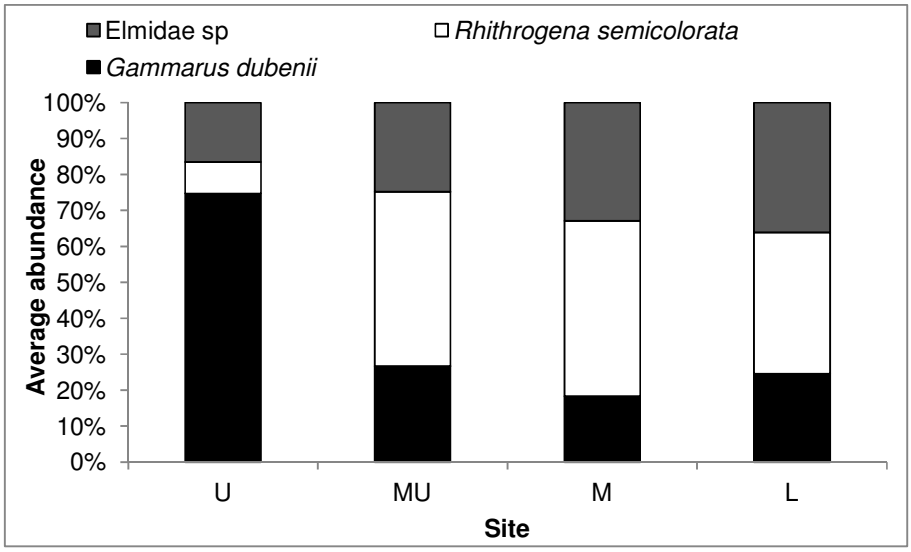
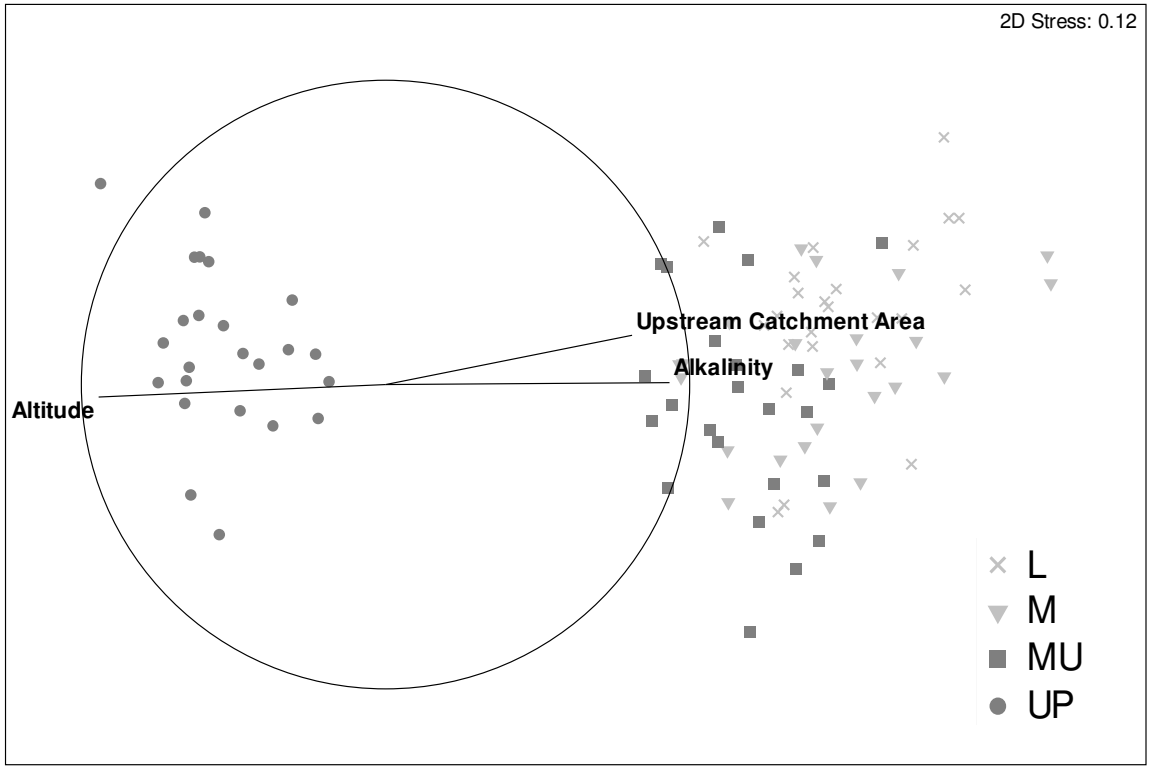
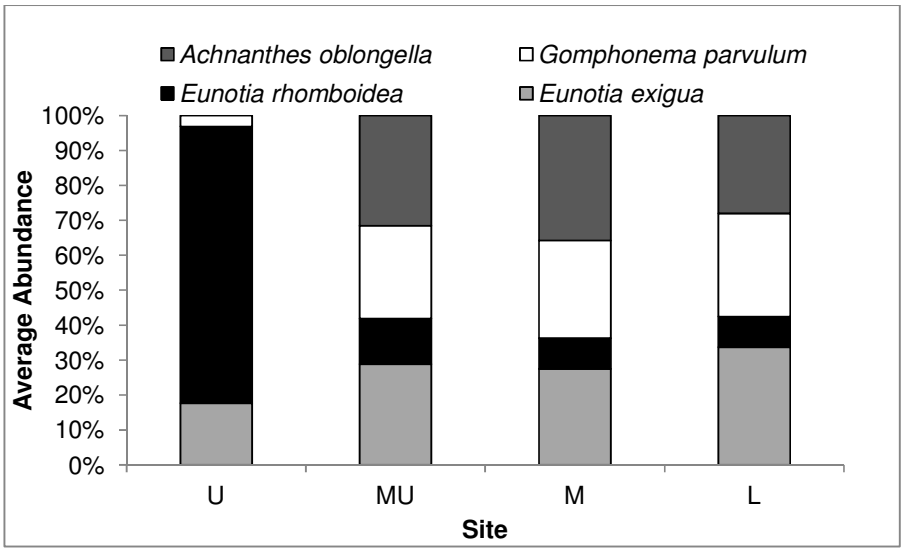


Figure 4 (a, b and c)

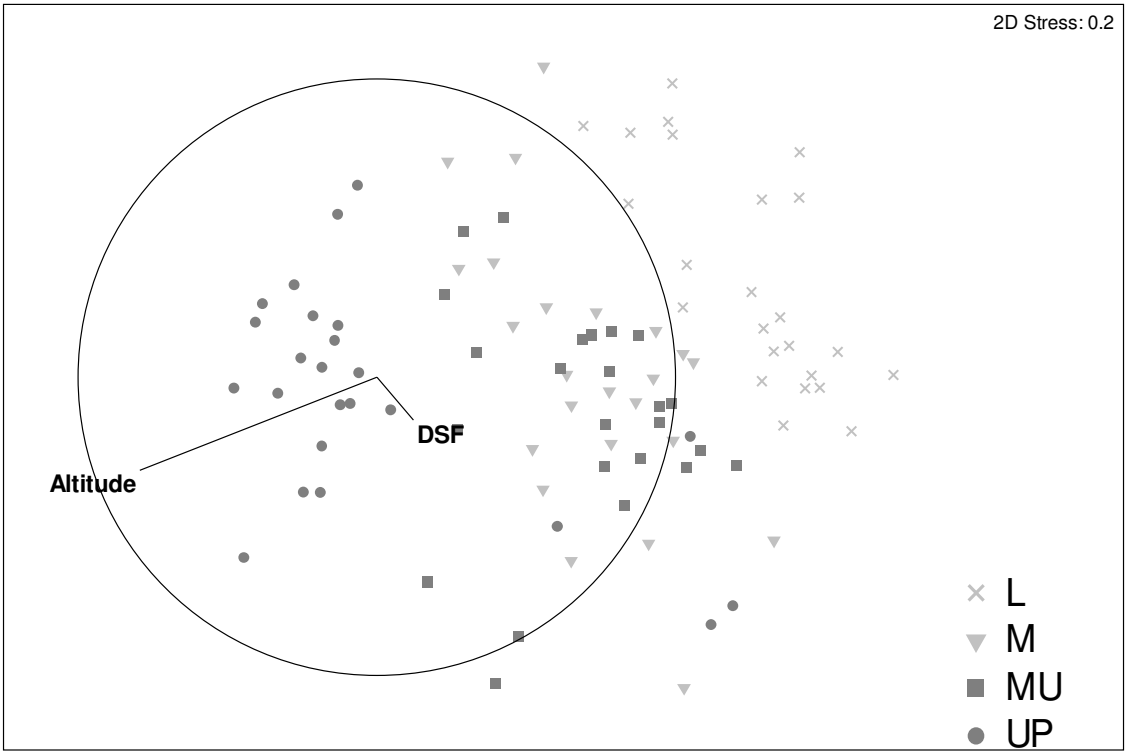


a)

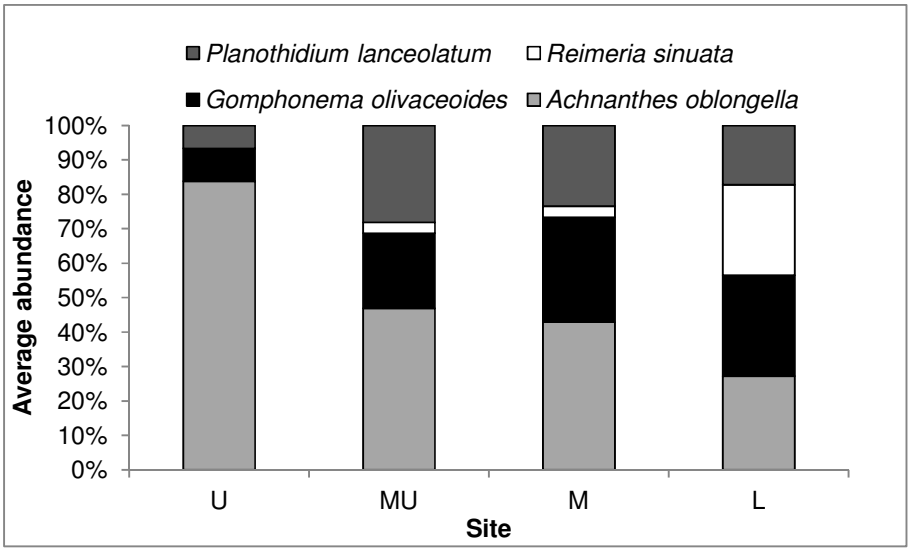


b)

Figure 5 (a and b)



a)



b)

Figure 6 (a and b)

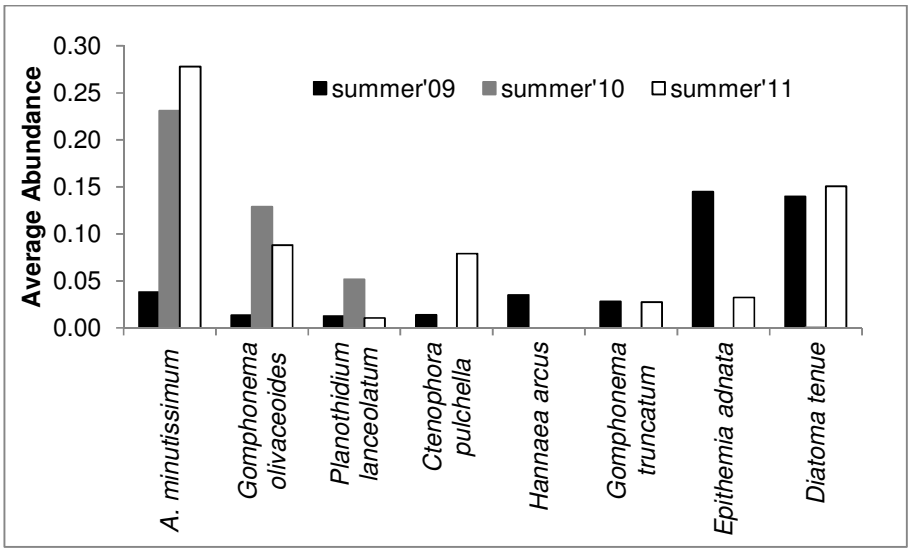
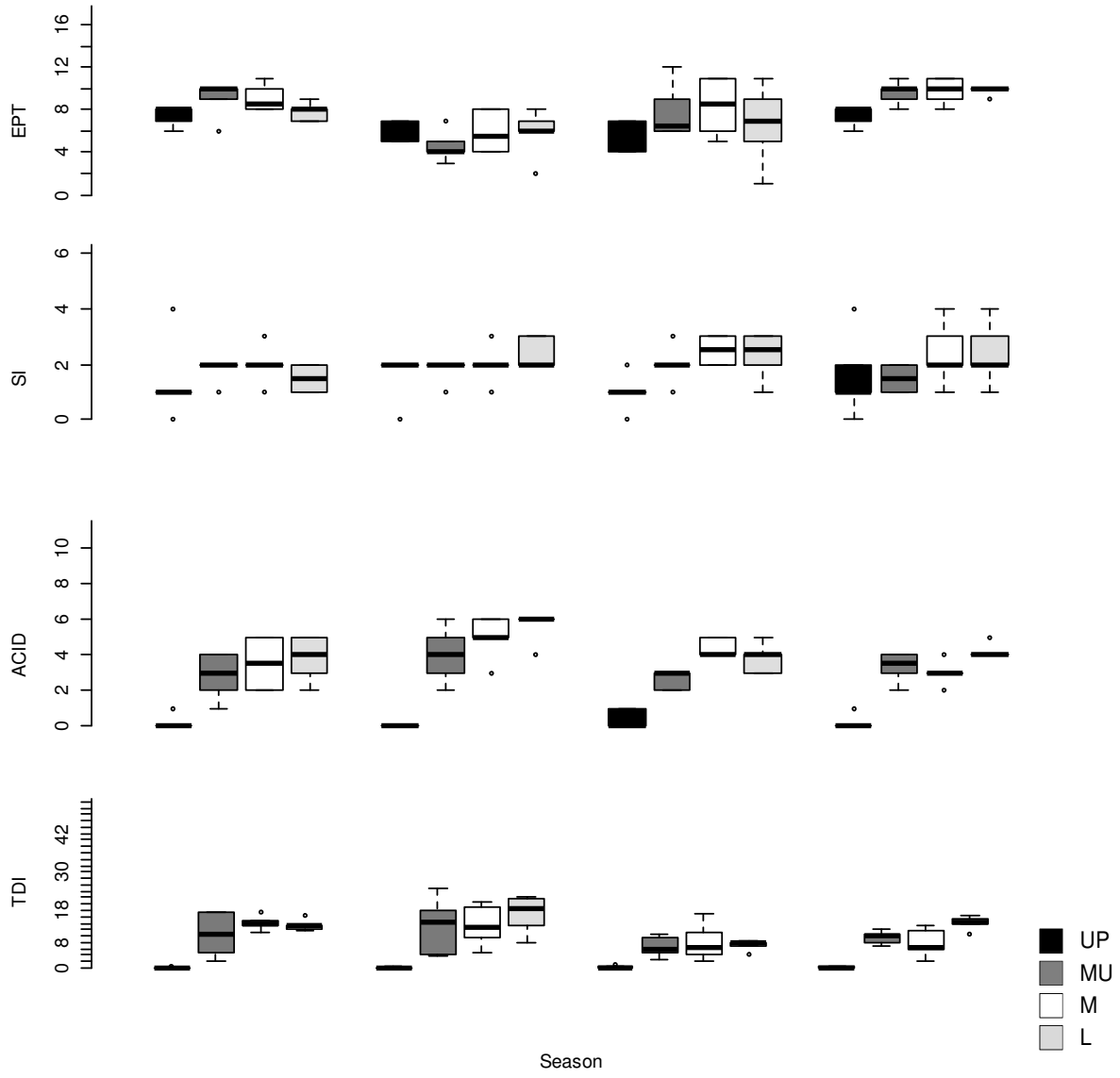
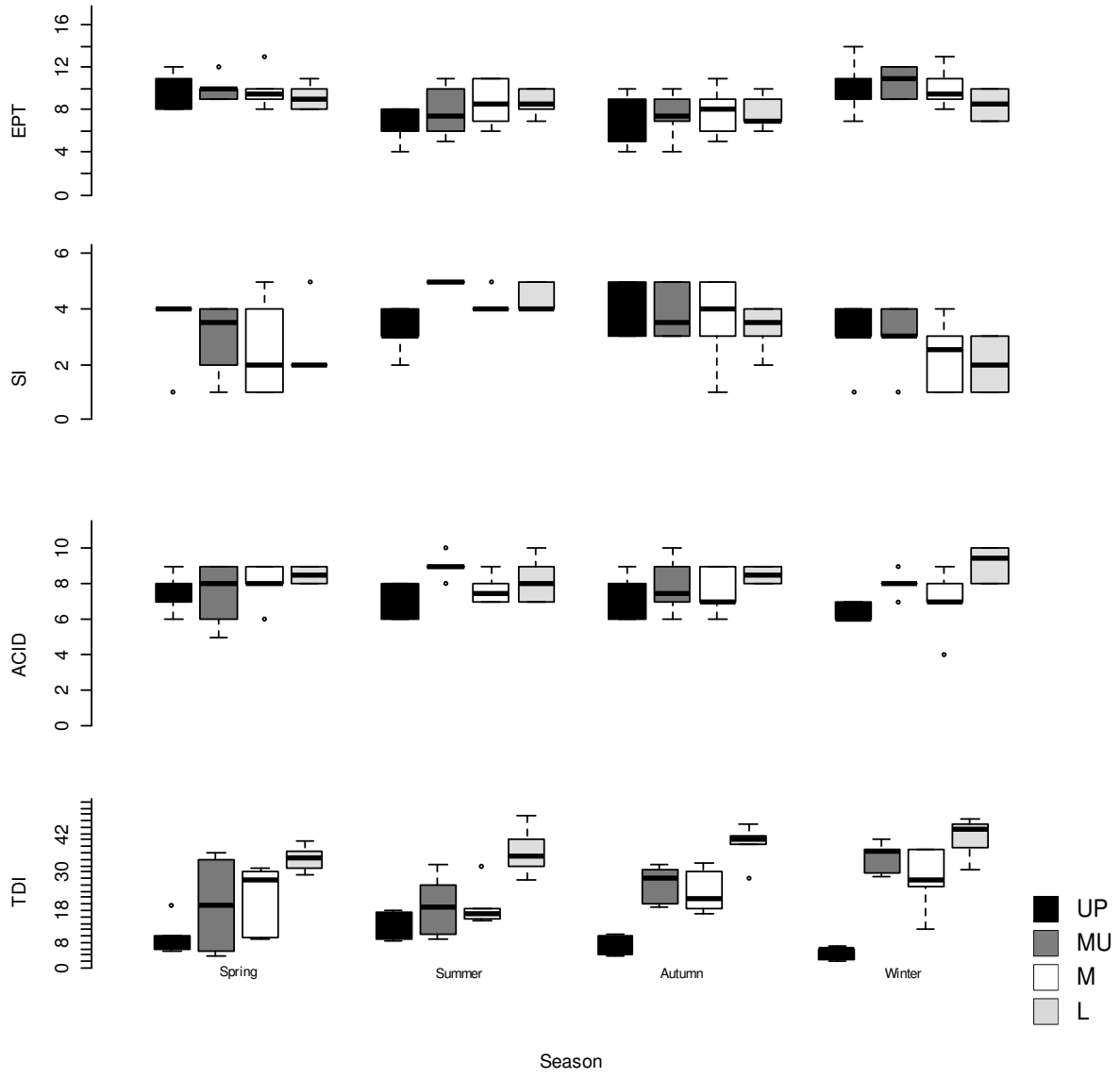

Average Abundance
0.30
0.25
0.20
0.15
0.10
0.05
0.00
summer'09
summer'10
summer'11
A. minutissimum
Gomphonema olivaceoides
Planothidium lanceolatum
Ctenophora pulchella
Hannaea arcus
Gomphonema truncatum
Epithemia adnata
Diatoma tenue

Figure 7


EPT
0
4
8
12
16
SI
0
2
4
6
ACID
0
2
4
6
8
10
TDI
0
8
18
30
42
UP
MU
M
L
Season

a)



b)

Figure 8 (a and b)

Table 1 Kruskal-Wallis results to determine the significance of factors site and season for all environmental variables.

		Glennamong				Srahrevagh			
		H	df	N	*p*	H	df	N	*p*
Season	Temperature	5.12	3	96	0.163	74.81	3	96	<0.001
	Conductivity	7.24	3	96	0.065	20.08	3	96	<0.001
	pH	40.10	3	96	<0.001	24.02	3	96	<0.001
	Colour	15.76	3	96	0.001	36.66	3	96	<0.001
	Alkalinity	48.11	3	96	<0.001	20.91	3	96	<0.001
	NH4	0.71	3	96	0.871	27.81	3	96	<0.001
	SRP	3.05	3	96	0.385	15.31	3	96	0.002
	TON	50.66	3	96	<0.001	61.51	3	96	<0.001
Site	Temperature	71.73	3	96	<0.001	5.25	3	96	0.154
	Conductivity	26.11	3	96	<0.001	23.55	3	96	<0.001
	pH	9.46	3	96	0.024	9.29	3	96	0.026
	Colour	63.02	3	96	<0.001	40.49	3	96	<0.001
	Alkalinity	36.41	3	96	<0.001	58.71	3	96	<0.001
	NH4	30.79	3	96	<0.001	1.34	3	96	0.720
	SRP	9.09	3	96	0.028	20.84	3	96	<0.001
	TON	13.89	3	96	0.003	14.59	3	96	0.002

Table 2 PERMANOVA results for the (a) macroinvertebrate and (b) diatom assemblages

Glennamong						Srahrevagh					
Source	df	SS	MS	F	P(MC)	Source	df	SS	MS	F	P(MC)
YE	1	5615	5615	15.99	< 0.01	YE	1	6394	6394	8.95	< 0.01
SI	3	19591	6530	19.89	< 0.01	SI	3	24072	8024	23.43	< 0.01
SE	3	32101	10700	14.9	< 0.01	SE	3	33177	11059	12.1	< 0.01
YExSI	3	1054	351	1.07	0.37	YExSI	3	2142	714	2.09	0.01
YExSE	3	8624	2875	4.96	< 0.01	YExSE	3	10146	3382	3.86	< 0.01
SIxSE	9	6462	718	2.19	< 0.01	SIxSE	9	8223	914	2.67	< 0.01
YExSIxSE	9	5221	580	1.77	< 0.01	YExSIxSE	9	7886	876	2.56	< 0.01
Residual	64	21017	328			Residual	64	21918	342		
Total	95	99685				Total	95	113959			

Glennamong						Srahrevagh					
Source	df	SS	MS	F	P(MC)	Source	df	SS	MS	F	P(MC)
YE	1	2466	2466	8.4	< 0.01	YE	1	3621	3621	1.75	0.15
SI	3	64069	21356	93.47	< 0.01	SI	3	30861	10287	25.38	< 0.01
SE	3	10706	3569	4.54	< 0.01	SE	3	10038	3346	2.53	< 0.01
YExSI	3	881	294	1.29	0.23	YExSI	3	6202	2067	5.1	< 0.01
YExSE	3	6955	2318	4.07	< 0.01	YExSE	3	16167	5389	6.62	< 0.01
SIxSE	9	7076	786	3.44	< 0.01	SIxSE	9	11891	1321	3.26	< 0.01
YExSIxSE	9	5131	570	2.5	< 0.01	YExSIxSE	9	7328	814	2.01	< 0.01
Residual	64	14623	228			Residual	64	25943	405		
Total	95	111906				Total	95	112051			

Df = degrees of freedom, SS = square sum, MS=mean sum, *F* = Fisher's univariate *F*-statistic, *P* (MC) = *p* values using Monte Carlo permutations. n=3.

Table 3 ANOVA results for within waterbody variation in biotic indices.

		WE 322441 (Glennamong)			WE 322781 (Srahrevagh)		
	Source of variation	d.f.	F-ratio	*p*	d.f.	F-ratio	*p*
Diatoms							
Acid	Season	3	7.92	<0.01	-	-	n.s.
	Site	2	7.21	<0.01	3	10.53	<0.01
	Site x Season	9	2.42	0.02	-	-	n.s.
TDI	Season	3	8.62	<0.01	3	2.96	0.04
	Site	-	-	n.s.	3	60.41	<0.01
	Site x Season	-	-	n.s.	9	2.21	0.03
Invertebrates							
EPT	Season	3	18.41	<0.01	3	10.58	<0.01
	Site	-	-	n.s.	-	-	n.s.
	Site x Season			n.s.	-	-	n.s.
SI	Season	-	-	n.s.	3	8.689	<0.01
	Site	-	-	n.s.	-	-	n.s.
	Site x Season	-	-	n.s.	-	-	n.s.

Appendix A Macroinvertebrate taxa, number of occurrences and % abundance in samples in the Glennamong and Srahrevagh. Only the taxa that were observed in at least 5 % of the 96 samples from 2009 – 2010 are included.

Macroinvertebrate Taxa	Occurrence in Srahrevagh # Samples	Occurrence in Glennamong # Samples	% Total Glennamong Abundance	% Total Srahrevagh Abundance
Amphinemura sulcicollis Stephens	44	68	7.02	0.97
Baetis rhodani Pictet	96	77	27.74	36.03
Brachypteri risi Morton	48	44	2.72	11.29
Chironomid species	56	79	6.44	1.28
Chloroperla torrentium Pictet	23	45	2.07	0.28
Dicranota species	56	58	0.98	0.63
Ecdyonururs insignis Eaton	47	/	/	1.18
Elmidae species	60	46	0.59	1.86
Emphemerella ignita Poda	18	/	/	1.21
Gammarus dubenii Lilljeborg	71	/	/	3.44
Halesus digitatus Schrank	40	32	0.32	0.75
Helodes species	52	7	0.04	0.93
Heptagenidae species	9	/	/	0.12
Hydropsyche siltalai Dohler	47	50	1.18	1.12
Isoperla grammatica Poda	54	46	1.09	0.73
Isotomurus palustris Müller	7	/	/	0.05
Leuctra hippopus Kempny	81	94	0.55	5.34
Limnius volckmari Panzer	30	70	0.41	0.78
Lumbricidae species	25	5	0.05	0.18
Nemoura cinerea Retzius	4	37	0.78	0.04
Oulimnius tuberculatus Müller	8	9	0.11	0.20
Perla bipunctata Pictet	6	/	/	0.04
Philopotamus montana Donovan	65	/	/	2.64
Polycentropus kingi McLachlan	25	77	1.69	0.38
Protonemura meyeri Pictet	51	90	10.57	0.84
Rhyacophila dorsalis Curtis	64	5	0.14	0.93
Rhithrogena semicolorata Curtis	63	/	/	10.59
Silo pallipes Fabricus	49	25	0.41	0.88
Simulidae species	90	86	16.19	15.27

1

Appendix B Diatom taxa with ACID and TDI classifications, number of occurrences and % abundance in samples in the Glennamong and Srahrevagh. Only the taxa that were observed in at least 5 % of the 96 samples from 2009 – 2010 are included.

Diatom taxa	ACID group	TDI group	Occurrence in Srahrevagh # Samples	Occurrence in Glennamong # Samples	% Total Glennamong Abundance	% Total Srahrevagh Abundance
Achnanthes oblongella Østrup	3	1	95	71	18.20	34.82
*Achnanthidium minutissimum type 1 * sensu* Potapova et Hamilton	3	2	95	50	3.68	16.41
*Achnanthidium minutissimum type 2 * sensu* Potapova et Hamilton	3	2	81	13	0.11	3.76
*Achnanthidium minutissimum type 3 * sensu* Potapova et Hamilton	3	2	61	7	0.26	1.43
Brachysira neoexilis Lange-Bertalot	1	1	19	47	0.57	0.12
Cocconeis placentula Ehrenberg	4	3	21	/	/	0.78
Ctenophora pulchella (Ralfs ex Kützing) Williams & Round	4	2	17	/	/	0.24
Diatoma mesodon (Ehrenberg) Kützing	3	3	31	/	/	0.28
Diatoma tenue Agardh	4	3	7	/	/	0.46
Encyonema gracile Ehrenberg	2	2	12	49	1.24	0.07
Encyonema silesiacum (Bleisch in Rabenhorst) Mann in Round, Crawford & Mann	3	3	18	/	/	0.09
Epithemia adnata (Kützing) Rabenhorst	5	1	6	/	/	0.48
Eunotia bilunaris var. mucophila Lange-Bertalot and Norpel	2	1	17	26	0.24	0.11
Eunotia exigua complex	1	1	62	85	13.09	1.27
Eunotia implicata Norpel, Lange-Bertalot & Alles	2	1	22	59	2.54	0.41
Eunotia incisa W. Smith ex W. Gregory	2	1	11	80	4.63	0.18
Eunotia microcephala Krasske ex Hustedt	2	1	/	29	1.02	/
Eunotia minor (Kützing) Grunow in Van Heurck	2	1	5	15	0.15	0.03
Eunotia paludosa Grunow	1	1	4	8	0.06	0.02
Eunotia rhomboidea Hustedt	2	1	10	66	12.27	0.19
Eunotia subarcuatoides Alles, Norpel, Lange-Bertalot	1	1	14	59	0.76	0.12
Fragilaria capucina var. gracilis (Østrup) Hustedt	3	2	70	33	3.59	4.75
Fragilaria capucina var. rumpens (Kützing) Lange-Bertalot	3	2	27	/	/	0.45
Frustulia rhomboides (Ehrenberg) De Toni	2	1	30	75	3.52	0.15
Gomphonema clavatum Ehrenberg	3	3	18	/	/	0.17
Gomphonema gracile Ehrenberg	3	3	22	19	0.46	0.56
Gomphonema minutum (Agardh) Agardh	3	4	9	20	0.48	0.09

Gomphonema olivaceoides Hustedt	3	2	70	8	0.04	9.20
Gomphonema parvulum complex	3	3	87	74	10.47	9.23
Gomphonema pumilum (Grunow) Reichardt & Lange-Bertalot	5	3	64	11	0.32	2.11
Gomphonema truncatum Ehrenberg	4	3	10	/	/	0.18
Hannaea arcus (Ehrenberg) Patrick. in Patrick and Reimer	4	1	9	/	/	0.28
Meridion circulare (Greville) Agardh	4	2	57	11	0.09	1.04
Navicula gregaria Donkin	4	5	39	/	/	0.55
Navicula lanceolata (Agardh) Kützing	4	5	44	/	/	0.85
Navicula species	3	5	7	/	/	0.05
Nitzschia species	4	5	33	/	/	0.33
Pinnularia appendiculata (Agardh) Cleve	2	1	50	85	5.90	0.60
Pinnularia species	2	1	4	21	0.43	0.02
Pinnularia subcapitata Gregory	2	1	33	72	5.58	0.29
Planothidium lanceolatum (Brébisson ex Kützing) Lange-Bertalot	4	5	70	/	/	3.40
Reimeria sinuata (Gregory) Kociolek & Stoermer	3	4	33	/	/	3.22
Stauroneis anceps Ehrenberg	3	5	8	/	/	0.03
Surirella species	3	3	7	5	0.02	0.04
Synedra ulna (Nitzsch) Ehrenberg	3	2	13	/	/	0.36
Tabellaria flocculosa (Roth) Kützing	2	1	33	94	10.28	0.79

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13 Appendix C Physical and chemical characteristics of the sites studied in spring, summer, autumn and winter

Site	Upper				Mid-Upper				Middle				Lower			
Season	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter
n=	9	9	6	6	9	9	6	6	9	9	6	6	9	9	6	6
Srahrevagh																
Altitude (m)	342	/	/	/	186	/	/	/	129	/	/	/	33	/	/	/
Upstream catchment area (ha)	13	/	/	/	168	/	/	/	266	/	/	/	690	/	/	/
Temperature	8.8	15.1	9.7	4.6	9.9	15.4	10.5	5.0	11.5	17.6	10.6	5.5	12.0	17.9	12.4	5.5
	0.41	*0.86*	*0.21*	*0.27*	*0.42*	*0.51*	*0.34*	*0.26*	*0.48*	*0.85*	*1.38*	*0.13*	*0.65*	*0.61*	*0.70*	*0.12*
Conductivity (µS cm-1)	83	81	68	62	111	107	79	85	115	114	82	85	164	149	122	112
	2.95	*3.60*	*8.83*	*5.75*	*4.60*	*9.72*	*9.34*	*12.24*	*6.61*	*11.82*	*10.03*	*11.07*	*6.09*	*18.48*	*17.00*	*12.45*
Alkalinity (mg L^{-1} CaCO3)	13.3	13.5	16.8	8.2	23.9	37.0	19.0	14.0	22.5	37.9	17.9	14.9	50.6	68.8	45.8	32.8
	0.18	*0.41*	*0.21*	*0.07*	*0.64*	*0.28*	*0.15*	*0.50*	*2.46*	*2.61*	*2.67*	*1.76*	*0.45*	*0.15*	*0.16*	*0.48*
PO4-P (µg L-1)	5.27	6.75	4.07	5.54	7.05	7.47	6.84	6.60	7.68	7.12	6.94	7.88	8.44	9.52	6.91	7.58
	0.67	*1.68*	*0.22*	*0.56*	*0.90*	*1.24*	*1.39*	*1.58*	*0.83*	*1.28*	*0.64*	*0.46*	*0.78*	*2.11*	*0.73*	*0.41*
NH4-N (µg L^{-1})	45.08	40.18	17.59	29.21	45.56	32.38	18.53	51.02	48.87	28.95	25.88	32.19	43.73	26.18	12.89	31.57
	11.11	*8.98*	*6.51*	*6.12*	*4.47*	*8.42*	*7.54*	*17.61*	*5.83*	*8.69*	*10.93*	*1.37*	*3.56*	*9.89*	*6.10*	*5.55*
pH	6.70	7.00	7.14	6.03	7.21	7.20	7.14	6.44	6.98	6.96	6.93	6.21	7.60	7.70	7.33	6.93
	0.66	*0.52*	*0.20*	*0.85*	*0.31*	*0.47*	*0.37*	*0.26*	*0.34*	*0.33*	*0.31*	*0.85*	*0.51*	*0.68*	*0.73*	*0.56*
Colour (PtCo)	16	38	29	38	29	49	70	68	41	41	74	75	42	56	87	98
	1.32	*1.74*	*3.12*	*6.54*	*2.52*	*0.83*	*0.42*	*1.84*	*0.88*	*1.69*	*1.28*	*5.53*	*0.93*	*0.29*	*0.42*	*0.97*
Glennamong																
Altitude (m)	237	/	/	/	90	/	/	/	52	/	/	/	17	/	/	/

Upstream catchment area (ha)	191	/	/	/	605	/	/	/	1145	/	/	/	1549	/	/	/
Temperature	9.6	13.4	10.7	5.4	11.0	14.9	11.4	5.3	12.6	15.4	13.2	4.9	13.1	15.6	15.1	4.5
	0.32	*0.22*	*0.51*	*0.35*	*0.36*	*0.40*	*0.74*	*0.77*	*0.70*	*0.46*	*1.12*	*0.30*	*0.80*	*0.58*	*1.28*	*0.02*
Conductivity ($\mu S\ cm^{-1}$)	64	54	62	66	72	61	59	60	80	68	76	64	80	70	67	64
	4.48	*3.71*	*2.91*	*1.12*	*3.94*	*5.25*	*1.57*	*2.17*	*4.11*	*7.13*	*13.51*	*3.24*	*4.19*	*6.98*	*1.72*	*0.40*
Alkalinity ($mg\ L^{-1}$ CaCO3)	0.1	0.1	0.1	0.1	6.7	6.7	6.7	6.7	10.7	10.7	10.7	10.7	9.4	9.4	9.4	9.4
	0.01	*0.01*	*0.02*	*0.02*	*0.21*	*0.21*	*0.27*	*0.27*	*0.12*	*0.12*	*0.15*	*0.15*	*0.08*	*0.08*	*0.11*	*0.11*
PO4-P ($\mu g\ L^{-1}$)	6.55	10.04	4.51	6.29	9.18	8.13	5.72	6.65	6.29	8.05	5.26	6.53	6.83	6.99	4.54	5.66
	0.97	*2.11*	*0.80*	*0.49*	*1.38*	*1.43*	*0.60*	*0.90*	*0.89*	*1.54*	*0.52*	*0.55*	*0.96*	*1.39*	*0.63*	*0.26*
NH4-N ($\mu g\ L^{-1}$)	47.46	43.31	17.38	30.58	38.35	36.01	18.64	30.58	46.80	33.73	23.75	27.58	59.44	32.56	21.60	29.99
	6.92	*9.59*	*4.79*	*6.00*	*6.29*	*7.41*	*7.15*	*6.69*	*7.51*	*7.29*	*12.27*	*3.20*	*12.12*	*7.79*	*12.81*	*5.77*
pH	5.78	4.93	4.86	5.14	6.78	6.12	6.02	5.96	7.18	6.49	6.24	6.01	6.76	6.35	6.06	5.99
	0.98	*0.56*	*0.14*	*0.52*	*0.37*	*0.76*	*0.73*	*0.35*	*0.21*	*0.75*	*0.88*	*0.32*	*0.75*	*0.77*	*1.22*	*0.14*
Colour (PtCo)	73	104	136	107	52	82	104	104	41	81	82	108	43	68	86	102
	2.19	*2.91*	*2.92*	*3.94*	*1.96*	*1.86*	*9.41*	*5.15*	*0.50*	*1.36*	*0.63*	*1.52*	*2.08*	*0.60*	*0.21*	*1.32*

Italics indicate the standard deviation

Appendix D Table 2 Summary of average biotic index values in the Srahrevagh and Glennamong rivers.

Site	Upper				Mid-Upper				Middle				Lower			
Season	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter
Sample No. Srahrevagh	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
Q	0.8	0.7	0.7	0.8	0.8	0.7	0.8	0.8	0.8	0.7	0.7	0.8	0.8	0.7	0.8	0.8
	/	*/*	*0.08*	*/*	*0.03*	*0.05*	*0.05*	*/*	*0.03*	*/*	*0.05*	*0.04*	*0.04*	*0.03*	*0.05*	*/*
EPT	9	7	7	10	10	8	8	11	10	7	8	10	9	7	8	9
	1.41	*1.50*	*2.37*	*2.32*	*1.51*	*2.26*	*2.07*	*1.37*	*1.39*	*2.51*	*2.14*	*1.79*	*1.58*	*2.13*	*1.51*	*1.38*
SI	3	3	4	3	3	5	4	3	3	4	4	2	2	4	3	2
	1.30	*0.88*	*0.89*	*1.10*	*1.17*	*0.71*	*0.98*	*1.10*	*2.28*	*0.93*	*1.51*	*1.21*	*1.00*	*0.87*	*0.82*	*0.89*
ACID	7	7	7	6	8	9	8	8	8	8	8	7	9	8	8	9
	0.92	*0.71*	*1.43*	*0.30*	*1.26*	*0.59*	*1.37*	*0.74*	*1.69*	*0.56*	*1.33*	*1.45*	*0.46*	*1.47*	*0.64*	*0.85*
TDI	9	12	7	5	22	20	26	35	24	22	24	28	36	35	39	41
	4.37	*3.97*	*3.02*	*2.07*	*13.57*	*8.03*	*5.52*	*4.63*	*10.22*	*6.58*	*6.80*	*9.25*	*4.03*	*5.82*	*5.68*	*5.93*
TDI-EQR	1	1	1	1	1	1	1	0.8	1	1	1	1	1	1	1	1
	/	*/*	*/*	*/*	*0.04*	*0.02*	*0.05*	*0.06*	*0.05*	*0.01*	*0.07*	*0.07*	*0.04*	*0.08*	*0.07*	*0.08*
Chl a ($g\ m^{-2}$)	6.31	6.79	2.43	2.09	1.24	2.13	1.75	2.08	1.45	3.25	2.63	2.74	3.22	6.11	1.23	2.97
	7.18	*4.64*	*1.81*	*0.91*	*1.02*	*1.61*	*2.53*	*2.54*	*0.67*	*3.43*	*1.19*	*2.65*	*4.08*	*6.25*	*0.46*	*3.20*
AFDM ($g\ m^{-2}$)	2105.67	4942.17	6257.68	2408.47	910.57	1684.58	1652.45	1634.61	1331.76	3135.03	2331.96	3691.51	1154.60	5096.57	1116.03	1270.41

	1731.04	*2902.94*	*5306.55*	*920.50*	*412.00*	*246.06*	*652.94*	*690.10*	*1515.06*	*1201.79*	*528.94*	*4757.13*	*598.50*	*2818.81*	*244.78*	*832.29*
TP ($g\ m^{-2}$)	7.73	6.85	23.29	19.51	5.51	12.31	16.28	9.65	2.56	14.68	37.77	6.45	8.21	24.85	13.16	14.28
	7.09	*0.94*	*7.99*	*10.31*	*4.15*	*10.41*	*5.38*	*4.21*	*2.16*	*9.43*	*13.90*	*3.98*	*6.00*	*17.37*	*7.86*	*8.92*
Glennamong																
Q	0.8	0.8	0.8	0.8	0.8	0.7	0.8	0.8	0.8	0.7	0.7	0.8	0.8	0.7	0.7	0.8
	/	*0.05*	*/*	*/*	*/*	*/*	*0.05*	*/*	*/*	*0.04*	*0.05*	*/*	*/*	*0.05*	*0.08*	*/*
EPT	8	6	6	8	9	5	8	10	9	6	8	10	7	5	7	10
	0.73	*0.78*	*1.38*	*0.84*	*1.73*	*1.20*	*2.42*	*1.03*	*1.12*	*1.51*	*2.50*	*1.17*	*1.13*	*1.80*	*3.50*	*0.41*
SI	1	2	1	2	2	2	2	2	2	2	3	2	2	3	2	2
	1.12	*0.71*	*0.63*	*1.38*	*0.33*	*0.44*	*0.63*	*0.55*	*0.50*	*1.01*	*0.55*	*1.03*	*0.50*	*0.73*	*0.82*	*1.03*
ACID	0	0	0	0	3	3	3	3	4	5	4	3	4	5	4	4
	0.25	*0.17*	*0.36*	*0.32*	*1.11*	*1.24*	*0.61*	*0.89*	*1.35*	*1.14*	*0.55*	*0.67*	*1.29*	*1.22*	*0.48*	*0.30*
TDI	0	0	0	0	14	11	7	10	16	11	8	8	16	14	7	14
	0.26	*0.12*	*0.35*	*0.26*	*8.36*	*7.44*	*2.94*	*1.90*	*5.67*	*5.90*	*5.28*	*3.97*	*7.03*	*6.83*	*1.63*	*2.03*
TDI-EQR	0.5	0.7	0.3	0.3	1	1	1	0.7	1	1	1	1	1	1	1	0.5
	0.03	*0.05*	*0.19*	*/*	*0.07*	*0.03*	*0.03*	*0.07*	*0.04*	*/*	*0.02*	*0.10*	*0.06*	*0.01*	*/*	*0.19*
Chl a ($g\ m^{-2}$)	5.35	8.26	7.44	5.11	12.42	3.55	1.85	1.95	14.09	3.74	7.68	8.18	10.67	4.15	5.55	4.88
	2.70	*7.56*	*4.64*	*1.46*	*7.63*	*2.30*	*1.14*	*1.62*	*9.47*	*1.94*	*6.71*	*5.48*	*9.55*	*2.11*	*5.26*	*2.58*
AFDM ($g\ m^{-2}$)	2618.61	3880.46	4449.49	2755.43	3292.57	3448.11	2172.03	1237.98	4321.44	3578.42	3954.34	2284.99	5269.23	4037.27	3531.53	2220.61
	1037.91	*1813.78*	*1507.66*	*401.10*	*1365.22*	*2711.74*	*581.71*	*345.58*	*2725.07*	*2683.50*	*1245.74*	*1002.27*	*3036.16*	*2783.77*	*954.26*	*725.05*
TP ($g\ m^{-2}$)	5.78	7.05	18.72	17.14	9.21	13.20	10.80	11.85	7.16	12.07	13.63	2.91	6.48	12.80	12.62	5.08
	3.18	*2.80*	*2.95*	*12.07*	*2.99*	*10.21*	*4.44*	*8.92*	*6.06*	*5.01*	*7.63*	*14.10*	*2.35*	*6.94*	*12.24*	*3.05*

Italics indicate the standard deviation