

1 Habitat-specific invertebrate responses to hydrological variability, anthropogenic flow alterations and hydraulic  
2 conditions

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## Abstract

1. Quantifying ecological responses to river flow regimes is a key scientific approach underpinning many environmental flow (e-flow) strategies. Incorporating habitat-scale influences (e.g. substrate composition and organic matter cover) within e-flow frameworks has the potential to provide a broader understanding of the causal mechanisms shaping instream communities, which may be used to guide river management strategies.
2. In this study, we examined invertebrate communities inhabiting three distinct habitat groups (HGs - defined by coarse substrates, fine sediments, and the fine-leaved macrophyte *Ranunculus* sp.) across four rivers (each comprising two study sites) within a single catchment. We tested the structural and functional responses of communities inhabiting different HGs to three sets of flow-related characteristics: (i) antecedent hydrological (discharge –  $\text{m}^3\text{s}^{-1}$ ) variability; (ii) antecedent anthropogenic flow alterations (percentage of discharge added to or removed from the river by human activity) and (iii) proximal hydraulic conditions (characterized by the Froude number). The former two were derived from groundwater model daily time series in the year prior to the collection of each invertebrate sample, while the latter was collected at the point of sampling.
3. While significant effects of hydrological and anthropogenic flow alteration indices were detected, Froude number exerted the greatest statistical influence on invertebrate communities. This highlights that habitat-scale hydraulic conditions to which biota are exposed at the time of sampling are a key influence on the structure and function of invertebrate communities.
4. Mixed-effect models testing invertebrate community responses to flow-related characteristics, most notably Froude number, improved when a HG interaction term was incorporated. This highlights that different mineralogical and organic habitat patches mediate ecological responses to hydraulic conditions. This can be attributed to HGs supporting distinct taxonomic and functional compositions and/or providing unique ecological functions (e.g. flow refuges) which alter how instream communities respond to hydraulic conditions.
5. While the individual importance of both flow and small-scale habitat effects on instream biota has been widely reported, this study provides rare evidence on how their interactive effects have a significant influence on riverine ecosystems. These findings suggest that river management strategies and e-flow frameworks should not only aim to create a mosaic of riverine habitats that support ecosystem functioning, but also consider the management of local hydraulic conditions within habitat patches to support specific taxonomic and functional compositions.

## 1) Introduction

Flow regime variability is widely recognised as a primary factor shaping riverine ecosystems (Monk *et al.*, 2006; Ledger and Milner, 2015; Thompson *et al.*, 2018; Poff, 2018). However, land use changes (Chadwick *et al.*, 2006; López-Moreno *et al.*, 2014) and water resource management practices have profoundly altered river flow regimes (Lehner *et al.*, 2011; de Graaf *et al.*, 2014; Gleeson and Richter, 2018), significantly threatening the integrity of lotic ecosystems globally (Bunn and Arthington, 2002; Poff *et al.*, 2010, Vörösmarty *et al.*, 2010). For example, groundwater abstraction substantially reduces river discharges worldwide (de Graaf, *et al.*, 2014) and profoundly alters lotic ecosystems (Bradley *et al.*, 2014; 2017; Kennen *et al.*, 2014). Conversely, some management activities elevate river discharges (e.g. effluent water returns and low-flow alleviation schemes) which also prompt significant ecological responses (Wright and Berrie, 1987; Luthy *et al.*, 2015). As such, there remains a pressing need to understand how water resources can be managed to meet human needs while conserving lotic ecosystems and the services they provide (Arthington *et al.*, 2010; Poff *et al.*, 2017; Poff, 2018).

Environmental flows (e-flows) represent the management of river discharges to conserve specific societal and ecological attributes (Arthington *et al.*, 2010). Establishing statistical relationships between flow regime properties and targeted ecological responses (i.e. flow-ecology relationships) represents a key scientific process underpinning many e-flow methodologies (Tharme, 2003; Poff and Zimmerman, 2010; Davies *et al.*, 2014; Poff, 2018). Scientists now widely advocate the construction of flow-ecology relationships to guide the implementation of region-wide e-flow strategies, in part due to limited resources restricting the collection of detailed ecological and hydrological information on a river by river basis (Arthington *et al.*, 2006; Poff *et al.*, 2010; Chen and Olden, 2018). As such, the functional properties of biotic communities are being increasingly utilized within flow-ecology relationships (e.g. Mims and Olden, 2013; Ruhi *et al.*, 2018), with such responses being more likely to transcend multiple river basins as they are not confined by the biogeographical constraints of individual species and community structural properties (White *et al.*, 2017a; Poff, 2018).

Despite the advantages of flow-ecology relationships in guiding regional e-flow strategies, such statistical relationships do not necessarily reflect the underlying mechanisms structuring instream

communities (Lancaster and Downes, 2010; Acreman *et al.*, 2014). Quantifying community responses to different flow-related characteristics (e.g. hydrological variability, flow alterations and hydraulic conditions) at the habitat-scale has the potential to provide more ecologically meaningful evidence to guide e-flow strategies (Acreman *et al.*, 2014; Rosenfeld, 2017; Arthington *et al.*, 2018). E-flow methodologies accounting for habitat-scale characteristics (e.g. ‘habitat simulation’ techniques – see Tharme, 2003) often focus on channel areas defined by depth-velocity relationships because of the widely recognised influence of hydraulic conditions on fish species with a high socioeconomic value (e.g. Bovee *et al.*, 1998; Lamouroux and Jowett, 2005; Harby *et al.*, 2007). At the regional scale, stream velocities often respond comparably to changes in discharge (Rosenfeld, 2017), which allows ecohydraulic principles (e.g. ecological preferences towards shear velocity conditions) to be integrated within studies utilising hydrological (river discharge) time series (e.g. Monk *et al.*, 2006; 2008; Armanini *et al.*, 2014). However, directly examining ecological responses to hydraulic conditions has been reported to facilitate a greater understanding of the underlying causal mechanisms structuring communities as they provide a proximal characterisation of the stream flow forces experienced by biota (Turner and Stewardson, 2014; Lamouroux *et al.*, 2017; Monk *et al.*, 2018).

The mineralogical (e.g. gravel and silt) and organic (e.g. macrophyte and macroalgae) habitat patches occurring in lotic environments are shaped by hydrological, hydraulic and geomorphic controls (Kemp *et al.*, 1999). Anthropogenic flow alterations have been shown to modify biotic communities indirectly *via* changes to habitat heterogeneity (e.g. Armitage and Pardo, 1995; Storey and Lynas, 2007). However, how communities inhabiting different mineralogical and organic habitat patches respond to different flow-related characteristics has not been widely explored (rare examples being Palmer *et al.*, 1996; Lind *et al.*, 2006; Clarke *et al.*, 2010) and has been seldom incorporated within e-flow methodologies globally.

In this study, we examine invertebrate community responses to three sets of flow-related characteristics: antecedent hydrological (discharge –  $\text{m}^3\text{s}^{-1}$ ) variability, antecedent anthropogenic flow alterations (daily percentage of discharge added to or removed from the river by human activities) and hydraulic conditions. The former two were derived from daily time series outputted from a groundwater model

over the year prior to the collection of each invertebrate sample, while the latter was measured at the point of invertebrate sampling. We tested whether community responses to these flow-related characteristics varied between distinct lotic habitat groups (HGs – comprising fine and coarse substrate classes and *Ranunculus* sp. - a fine-leaved macrophyte). This study aimed to quantify the structural and functional responses of invertebrate communities to: (i) differences in HGs; (ii) the individual influence of each flow-related characteristic across different HGs and (iii) the most statistically influential ('optimal') flow-related indices across different HGs.

## **2) Materials and methodology**

### *2.1) Study area*

Four rivers, each comprising two sampling sites, were examined across the Hampshire Avon catchment (Hampshire, United Kingdom; Fig. 1) between May 2015 and January 2016; a period characterised by intermediate river discharges within the context of long-term hydrological time series (Barker *et al.*, 2016; White, 2018). The Hampshire Avon is classified as a Special Area of Conservation (SAC) under the EU Habitats Directives (92/43/EEC), with areas of the catchment also being designated as 'Sites of Special Scientific Interest' (SSSI; Natural England, 1996). The catchment is primarily underlain by a chalk lithology (Heppell *et al.*, 2017), a fine-grained limestone which exhibits a relatively low specific yield, although it can develop high transmissivities as groundwaters move through small fissures (Soley *et al.*, 2012). As such, chalk is considered a 'highly productive aquifer' (see BGS, 2018; see Fig. 1) and overlaying rivers typically convey seasonally consistent flow regimes as groundwater levels rise and fall in accordance with antecedent climatic conditions (Sear *et al.*, 1999). However, the Hampshire Avon is also underlain by bands of greensand (a 'moderately productive aquifer') and clay (possessing 'essentially no groundwater') in the west of the catchment (see Fig. 1 and BGS, 2018 for nomenclature), which facilitate quicker hydrological responses to rainfall (Heppell *et al.*, 2017). The land use across the four sub-catchments studied is predominantly arable agriculture (although the Wylfe exhibits a higher proportion of grassland coverage) with minimal urban coverage (see Table 1). Rivers across the Hampshire Avon exhibit comparable physico-chemical properties due to the strong calcareous geological influence and similar land uses between sub-catchments. The rivers examined are

characterized by alkaline waters and high electrical conductivity, dissolved oxygen and nutrient levels (Table 2).

Within the Hampshire Avon, groundwater abstraction is the primary water resource management mechanism reducing river discharges, with the regional water company (Wessex Water plc.) operating 21 groundwater supply wells across the catchment (White *et al.*, 2018). However, outflows from effluent water returns and low flow alleviation strategies (which utilise groundwater to augment discharges in select river channels that fall below threshold discharge values) results in some river reaches conveying a greater volume of flow than would naturally occur. All study sites exhibit perennial flow regimes.

## 2.2) Defining habitat groups

Three ‘Habitat Groups’ (HGs) were established based on their prevalence over a 50m reach for each of the study sites. Two HGs comprised different sedimentological characteristics which were present across all study sites - (i) coarse substrates and (ii) fine sediments. Coarse substrates included bare mineralogical coverings dominated by gravel and/or cobble sized substrates (between 2-64mm – Kemp *et al.*, 1999), while fine sediment habitats comprised sand and silts sized particles (<2mm), often deposited between macrophyte stands growing in the river margins (e.g. *Apium nodiflorum*, *Callitriche* sp., *Sparganium erectum*). The third HG comprised (iii) *Ranunculus* sp., a fine-leaved, submerged macrophyte which is widespread within many calcareous rivers regionally (Westwood *et al.*, 2006). It is typically located in central areas of channel cross-sections conveying higher flow velocities (Westwood *et al.*, 2006). *Ranunculus* sp. has been shown to support diverse invertebrate communities and is a key refuge for faunal assemblages during extreme hydrological conditions (Bickerton *et al.*, 1993; Wright and Symes, 1999). *Ranunculus* sp. was sampled within five of the eight study sites.

## 2.3) Biological data

Field sampling was undertaken during spring (May), summer (July), autumn (October) 2015 and winter (January) 2016, although high river levels prohibited sampling at one site along the R. Nadder during winter 2016. Invertebrate samples were collected using a Surber sampler (0.03m<sup>2</sup>, 250-µm mesh size),

disturbing the sediment and/or plant material (at the base of the *Ranunculus* sp. bed where leaves are most highly concentrated) for 15-seconds for each sample. Three replicate samples were collected from each HG present within each study site across all sampling occasions (n = 237; 48-69 samples taken from each river - which varied depending on the presence of *Ranunculus* sp. between study sites and seasons). Invertebrate samples were collected from separate HG patches within each study site (spanning a 50-metre reach). A total of 93 samples were collected from both coarse substrates and fine sediments, while 51 samples were taken from *Ranunculus* sp. All samples were preserved using 4% formaldehyde solution in the field prior to being processed and stored within 70% industrial methylated spirit in the laboratory. Specimens were identified to lowest possible taxonomic level (typically species or genus), but in some cases taxa were resolved to family level (primarily Diptera larvae); while Hydracarina, Oligochaeta (class), Ostracoda (subclass) and Collembola (order) were identified as such.

#### 2.4) Velocity data

A 30-second averaged flow velocity reading was taken immediately adjacent to each invertebrate sample at 60% of the channel depth using a Valeport Electromagnetic Current Meter. From this, the Froude number was calculated (Table 3) to enable a direct comparison of hydraulic measurements across different habitat conditions (Jowett, 1993), as well as between reaches and seasons, given that the influence of flow velocity is scaled by the channel depth (Wadeson and Rowntree, 1998)

#### 2.5) Wessex Basin groundwater model

The 'Wessex Basin' regional groundwater model (see Soley *et al.*, 2012) was used to characterize the hydrological variability predicted to occur at each study site by modelling a 'historic' discharge time series. Daily historic discharge time series were obtained from the model between 1/1/2014 -31/1/2016 so that the hydrological variability could be derived from the 12-month period preceding all invertebrate samples. Outputs from the Wessex Basin model were also used to quantify antecedent anthropogenic flow alterations across the same time period. This was derived from the daily percentage difference between naturalized (i.e. modelled discharges subject to no hydrological alterations) and the historic discharge time series. This 'anthropogenic flow alteration' time series accounts for any groundwater

abstractions operated by Wessex Water plc., in addition to all hydrological inputs (e.g. effluent water returns or low flow alleviation strategies).

The hydrogeological mechanisms underpinning the Wessex Basin model have been described elsewhere (Heathcote *et al.*, 2004; Soley *et al.*, 2012; White *et al.*, 2018) and are summarized here. The model divides the Wessex Water plc. region underlain by chalk and upper greensand into 250x250m grid cells, with stream cells (for which discharge time series are outputted) being positioned along the valley floors. The Wessex Basin model has been adapted from the MODFLOW model (see McDonald and Harraugh, 1988), with the interaction between stream cells and groundwater levels being calculated at  $\approx 10$ -day intervals (3 modelled outputs per month). This has been combined with daily outputs from a 4R (Rainfall, Recharge and Runoff Routing) hydrological model to provide an estimate of total daily discharge conveyed by each stream cell. Errors in mean long-term (1970-2013) historic discharges (outputted by the Wessex Basin model) were within  $\pm 10\%$  of observed discharges (ENTEC, 2016), which were obtained from flow gauges (sourced from the National River Flow Archive – NRFA, 2018). As such, the Wessex Basin model was considered indicative of a ‘very good’ hydrological model (see Hain *et al.*, 2018; for additional hydrological model fit statistics of sampling sites positioned close to flow gauges, see Supplementary Material, Appendix A, Table A1). In addition, the Wessex Basin model has been externally reviewed and is considered to accurately model daily river discharges by the environmental regulator within England (the Environment Agency). Although it should be noted that an incorrectly modelled 10-day drying event at the R. Wylfe 1 during September 2015 was excluded when deriving hydrological indices (see below) as a nearby flow gauge (positioned  $\approx 3$ km upstream from the R. Wylfe 1 – see Fig. 1) indicated permanent flowing conditions throughout the study period (NRFA, 2018 - site ‘43806 - Wylfe at Brixton Deverill’).

### **3) Data analysis**

#### *3.1) Invertebrate community response metrics*

Invertebrate taxonomic (community abundances) and functional trait multivariate compositions were examined. Functional traits were derived from the European database compiled by Tachet *et al* (2010).



The functional traits database adopts a fuzzy-coding procedure, whereby faunal affinities to individual traits range from zero (indicating no affinity) to three or five (indicating high affinity – the upper limit depending on the amount of available information reported in existing literature – Tachet *et al.*, 2010). Trait information within the database is typically available at species- or genus-level and taxa resolved to a coarser resolution than that specified within the database were excluded from the trait analyses. Trait values for all qualifying taxa were standardized across all ‘grouping features’ (a functional trait category - e.g. ‘maximum body size’) so that ‘traits’ (modalities residing within grouping features - e.g. ‘ $\leq 0.25\text{cm}$ ’, ‘ $\geq 8\text{cm}$ ’; for nomenclature, see Schmera *et al.*, 2015) summed to 1 to ensure equal taxonomic weighting. These standardized values were then used to derive univariate functional diversity indices (see below). To calculate the multivariate functional trait compositions, standardized values were multiplied by  $\ln(x+1)$  transformed community abundances (see Schmera *et al.*, 2014) to create a trait-abundance array. Finally, each trait was averaged across all sampled taxa and standardized across all grouping features to account for spatially and temporally driven changes in taxonomic abundances (Gayraud *et al.*, 2003; Demars *et al.*, 2012). Eleven grouping features comprising 63 traits were examined containing information on the biological properties of invertebrate taxa (see Supplementary Material, Appendix B, Table B1).

Seven univariate community response metrics were derived and calculated within R studio (operated within R version 3.3.1; R Development Core Team, 2014). Five structural responses of invertebrate communities were examined: i) total community abundance - ‘Abundance’, ii) taxonomic richness - ‘TaxRic’, iii) taxonomic diversity (obtained from the inverse Simpson’s diversity index; Oksanen, 2016) – ‘TaxDiv’ iv) ‘Berger-Parker’ index (Seaby and Henderson, 2007) and v) the percentage of Ephemeroptera, Plecoptera and Trichoptera taxa – ‘%EPT’. The functional richness ‘FRic’ and functional evenness ‘FEve’ metrics were calculated using the dbFD function in the ‘FD’ package (Laliberté *et al.*, 2015) and were derived from a Bray-Curtis dissimilarity matrix created from standardized trait values (see above). FRic characterizes the volume of functional space occupied by invertebrate communities and FEve describes the regularity of abundances within this space (Villéger *et al.*, 2008).

### 3.2) Antecedent hydrological and anthropogenic flow alteration indices

All subsequent statistical analyses were performed in R Studio. Given that some hydrological indices have been shown to be influenced by river catchment sizes (Monk *et al.*, 2006), historic discharge time series from each study site were transformed to z-scores. As anthropogenic flow alterations are dimensionless (the percentage difference between naturalized and historic discharges), these were not transformed. Subsequently, 47 indices were derived to characterise both the hydrological ('Q' – derived from historic discharge time series) and anthropogenic flow alteration ('AF') time series (94 indices in total) at each sampling site prior to each sampling event. These indices were calculated as they have been highlighted as ecologically influential within groundwater dominated rivers in the UK (see Worrall *et al.*, 2014) and characterise different components of the flow regime (i.e. 'magnitude', 'frequency', 'duration', 'timing' and 'rate of change' - see Poff *et al.*, 1997). The indices derived included the 33 hydrological indices outlined in the 'Indicators of Hydrological Alteration' methodology (Richter *et al.*, 1996) and 14 additional variables which have been demonstrated to significantly influence invertebrate communities within UK groundwater dominated streams (Wood *et al.*, 2000; Wood and Armitage, 2004; Monk *et al.*, 2006; Worrall *et al.*, 2014; see Supplementary Material, Appendix C, Table C1). Hydrological indices dominated by a single value (>50%) or with a lack of unique values (n<10) were excluded from subsequent analyses (13 in total, leaving 81 Q and AF indices – see Supplementary Material, Appendix C, Table C1).

Separate 'Principal Component Analyses' (PCAs) were performed on Q and AF indices using a correlation matrix (Olden and Poff, 2003). The statistical significance of each PCA axis was determined via a broken-stick methodology using the '*PCAsignificance*' function within the BiodiversityR package (Kindt, 2018). Subsequently, the dominant 25 Q and AF indices (50 in total) were derived following the data redundancy procedure outlined by Olden and Poff (2003) and Monk *et al* (2007); with the number of indices selected from each significant PCA axis being proportional to the amount of statistical variation that the axis itself explained. This procedure accounts for the major sources of statistical variation and minimizes redundancy between hydrological indices. To account for collinearity between the selected indices, 'Variation Inflation Factors' (VIFs) were calculated for the Q

and AF indices derived from the PCA procedure, as well as 2 hydraulic metrics (mean velocity and Froude number), with variables being iteratively removed until all VIFs were below 3 (Zuur *et al.*, 2010).

### 3.3) Analytical framework

The following statistical analyses are reported in three subsections corresponding to each study aim. An analytical framework for this is presented schematically in Fig. 2 (although it should be noted that an alternative analytical framework was explored to test the influence of HGs and each set of flow-related characteristics on invertebrate communities – see Supplementary Material, Appendix D, Table D1).

#### 3.3.1) Structural and functional community differences between habitat groups

Multivariate differences in the taxonomic and functional trait compositions of invertebrate communities between HGs were examined by pooling the three replicate samples from each HG within each study site (taxonomic abundances were summed; functional traits were averaged). This was tested *via* a ‘Permutational Analysis of Variance’ (PERMANOVA) using the ‘*adonis*’ function in the Vegan package (Oksanen *et al.*, 2017). Pairwise PERMANOVAs were used to test how communities differed between each paired combination of HGs. ‘Principal Coordinate Analysis’ (PCoA) plots were constructed using a Bray-Curtis dissimilarity index to visualize community differences between HGs. PCoA was performed using the ‘*cmdscale*’ function and displayed using the ‘*ordispider*’ function (both in Vegan).

To examine whether each univariate community response metric differed between HGs, ‘Linear Mixed-effect Models’ (LMMs) were constructed using the ‘*lmer*’ function in the lme4 package (Bates *et al.*, 2017). For this, HG was examined as a fixed-effect and the following procedures were adopted (and applied to all LMMs used throughout the study herein): (i) river and season were used as random effects to account for a potential lack of spatial and temporal independence between samples; (ii) random intercept models were fitted using a maximum-likelihood approximation; (iii) Quantile-Quantile plots were inspected to ensure that model residuals were normally distributed, while fitted values were plotted against Pearson residuals to examine the homogeneity of variances and identify outliers (Bolker *et al.*,

2009; a maximum of six data points were removed from each LMM); (iv) community abundance was  $\log(x)$  transformed to satisfy model assumptions when used as a dependent variable within LMMs; (v) the significance of all LMMs were obtained *via* likelihood-ratio tests and (vi) the statistical variation explained by the fixed-effects within each LMM was examined through marginal pseudo  $r^2$  values ( $r^2_m$ ; see Nakagawa and Schielzeth, 2013) obtained from the '*r2beta*' function in the *r2glmm* package (Jaeger, 2017). Differences in the community response metric values between HGs were graphically presented using the *ggplot2* package (Wickman and Chang, 2016).

### 3.3.2) Community responses to different sets of flow-related characteristics

LMMs were used to quantify the influence of each flow-related characteristic (i.e. the separate influence of Q, AF indices and the Froude number - see Fig. 2) on each of the seven community response metrics. For this, Q and AF indices were scaled (i.e. z-scores calculated) to facilitate model convergence (Bolker *et al.*, 2009). In total, six sets of statistical models were prepared, each consisting of seven LMMs testing the response of each community response metric (dependent variable). These six sets of LMMs comprised three statistical 'pairs' corresponding to each flow-related characteristic. The first set of LMMs modelled the additive influences of Q indices ('flow-ecology' relationships) as fixed effects (independent variables), with the second set of LMMs incorporating an interaction term between Q indices and HG ('HG.flow-ecology') - these two sets represented the first statistical 'pair'. This process was repeated by replacing the Q indices with AF indices (pair 2 – 'flow alteration-ecology' and 'HG.flow alteration-ecology') and the Froude number (pair 3). As such, the inclusion of a HG interaction term represented the only difference between each set of LMMs comprising each statistical pair. Comparisons between LMMs testing the same community response metric within each statistical pair were performed to test whether community responses to each flow-related characteristic differed between HGs. Comparisons were conducted through multiple lines of statistical evidence: (i) likelihood ratio tests were performed to test if LMMs differed significantly; (ii) the amount of statistical variation explained by LMMs were derived from  $r^2_m$  values and (iii) the statistical likelihood of the model was determined by comparing 'Akaike Information Criterion' (AIC) values. For this,  $\Delta AIC \geq 2$  indicated a difference in the statistical likelihood of the two LMMs, which provides a greater understanding of

whether the inclusion of a HG interaction term improved the model fit (specifically given its penalizing function associated with a greater number of explanatory variables).

### 3.3.3) Community responses to the most statistically influential flow-related indices

To examine community responses to the most statistically influential flow-related indices (Q, AF and Froude number), seven LMMs were constructed that tested each community response metric (dependent variable) against the additive influences of all flow-related characteristics selected following PCA and VIF analyses (these were used as fixed-effects, whereby z-scores were calculated to facilitate model convergence). Subsequently, a backwards stepwise procedure was performed *via* the ‘step’ function in the lmerTest package (Kuznetsova *et al.*, 2017) to identify the significant fixed-effects comprising each LMM. For this, the significant  $\alpha$  level (0.05) was adjusted *via* the Bonferroni correction to prevent overfitting models. ‘Optimal’ LMMs were constructed that comprised the additive influences of statistically significant indices (identified from the stepwise procedure) as fixed-effects. Subsequently, ‘HG.optimal’ LMMs were constructed that examined the interaction between HG and the flow-related indices comprising each optimal LMM. The statistical significance (likelihood ratio tests), explanatory power ( $r^2_m$ ) and statistical likelihood (AIC) of all optimal and HG.optimal LMMs was quantified and compared. This was also calculated for each individual fixed-effect, with the statistical variation explained by each variable being quantified by semi-partial  $r^2_m$  values using the ‘r2beta’ function and the significance of each individual fixed-effect being obtained from the ‘anova’ function in lmerTest. Graphics displaying the responses of the most sensitive community response metrics to flow-related indices within the HG.optimal LMMs were prepared using the ‘effects’ package (Fox *et al.*, 2017).

## 4) Results

### 4.1) Hydrological variability and anthropogenic flow alterations

All rivers examined displayed seasonally consistent changes in hydrological variability, with peak discharges occurring between late winter and early spring, before declining across the summer and autumn months (Fig. 3; although this was least evident for Nadder 1 – see Fig. 3c). On average, naturalised discharges were reduced by 3.88% across the eight sampling sites over the study period.

The rivers Ebble (Figs. 3a and 3b) and Bourne (Figs. 3g and 3h) displayed the most buffered hydrographs. Anthropogenic flow alterations within the Ebble deviated marginally from 0 (-2.89% to 3.89%), but the Bourne exhibited greater reductions in discharge (-13.14% to -0.43%). The Nadder displayed a more variable flow regime, with sharp rises and falls in discharge occurring (Figs. 3c and 3d). Flow alterations fluctuated marginally around 0 (-0.59% to 0.83%) at Nadder 1, while Nadder 2 exhibited small net reductions in discharge (-4.35% to 0%). The Wylye displayed some short-term (daily to weekly) spikes in discharges (Figs. 3e and 3f), although not to the same degree as the R. Nadder. Anthropogenic flow alterations in the Wylye were greater (-48.33% to 9.16%) compared to the other rivers studied.

The PCA of hydrological (Q – river discharge) indices was used to select the 25 Q indices used in subsequent analyses, which were derived from PCA axes 1-6; all of which were significant (as indicated by the broken-stick procedure) and explained 97.40% of the total statistical variation. The 25 AF indices were derived from PCA axes 1-7 (all of which were significant) and accounted for 98.15% of the statistical variation. After VIFs were calculated to check for collinearity, 11 indices (5 Q and AF indices, in addition to the Froude number) were utilized in subsequent analyses (Table 3).

#### 4.2) Invertebrate community responses

The following results are divided into three sub-sections, which correspond directly to the three study aims (see Section 3.3 and Fig. 2).

##### 4.2.1) Structural and functional community differences between habitat groups

PERMANOVAs indicated that invertebrate taxonomic ( $F = 11.14$ ,  $p\text{-value} \leq 0.001$ ) and functional trait compositions ( $F = 8.82$ ,  $p\text{-value} \leq 0.001$ ) differed significantly between HGs, which accounted for 23% ( $r^2 = 0.23$ ) and 16% ( $r^2 = 0.16$ ) of the total statistical variation, respectively. Pairwise PERMANOVAs indicated that taxonomic and functional trait compositions differed significantly between all HG pairs ( $p\text{-values} = <0.001 - 0.007$ ). Greater amounts of statistical variation were explained when comparing taxonomic compositions supported by ‘*Ranunculus* sp.’ versus ‘coarse substrate’ ( $r^2 = 0.19$ ;  $F = 11.02$ ) and ‘fine sediment’ ( $r^2 = 0.24$ ;  $F = 14.57$ ) HGs, compared to coarse substrates versus fine sediments ( $r^2 = 0.12$ ;  $F = 8.44$ ). Pairwise PERMANOVAs examining differences in functional trait compositions

between HGs explained the lowest amount of statistical variation when testing coarse substrates versus *Ranunculus* sp. ( $r^2 = 0.07$ ;  $F = 3.23$ ), compared to fine sediments versus coarse substrates ( $r^2 = 0.12$ ;  $F = 8.33$ ) and *Ranunculus* sp. ( $r^2 = 0.15$ ;  $F = 8.43$ ). A PCoA plot indicated that each HG supported distinct invertebrate taxonomic compositions (Fig. 4a). There was a greater overlap in the functional trait compositions supported by each HG, although communities inhabiting ‘fine sediments’ were slightly more functionally distinct (Fig. 4b).

LMMs highlighted that Abundance ( $r^2 = 0.39$ ,  $X^2 = 122.72$ ), TaxRic ( $r^2 = 0.36$ ,  $X^2 = 116.05$ ), TaxDiv ( $r^2 = 0.15$ ,  $X^2 = 39.53$ ), Berger-Parker ( $r^2 = 0.12$ ,  $X^2 = 28.72$ ), %EPT ( $r^2 = 0.14$ ,  $X^2 = 38.23$ ), FRic ( $r^2 = 0.38$ ,  $X^2 = 101.34$ ) and FEve ( $r^2 = 0.20$ ,  $X^2 = 50.12$ ) all differed significantly (all  $p$ -values  $\leq 0.001$ ) between HGs. *Ranunculus* sp. supported greater Abundance (Fig. 5a), TaxRic (Fig. 5b), %EPT (Fig. 5e) and FRic (Fig. 5f) values. TaxDiv was highest within coarse substrates (Fig. 5c), while fine sediments supported communities characterized by a higher structural dominance (Berger-Parker index - Fig. 5d), but a greater functional evenness (FEve – Fig. 5g).

#### 4.2.2) Community responses to hydrological variability and anthropogenic flow alterations

Community response metrics typically displayed limited responses to the additive influences of hydrological (Q) and anthropogenic flow alteration (AF) indices, respectively termed ‘flow-ecology’ and ‘flow alteration-ecology’ relationships (see Fig. 2). LMMs detected 2 significant flow-ecology relationships and only 1 flow alteration-ecology relationship (see Table 4). Significant flow-ecology ( $r^2m = 0.19$ ,  $X^2 = 12.87$ ,  $p$ -value = 0.025) and flow alteration-ecology relationships ( $r^2m = 0.10$ ,  $X^2 = 14.71$ ,  $p$ -value = 0.012) were observed for FEve. Incorporating a HG interaction with Q indices (i.e. ‘HG.flow-ecology relationships’) significantly improved model fits for 4 community response metrics (Abundance, TaxDiv, FRic and FEve – but  $\Delta AICc$  for TaxDiv  $> -2$ , see Table 4) and accounted for a higher amount of statistical variation compared to all respective flow-ecology relationships (up to 23% -  $\Delta r^2m = 0.23$  for FRic; see Table 4). HG.flow alteration-ecology relationships significantly improved model fits for 3 response metrics (Abundance, TaxRic and %EPT – but  $\Delta AICc$  for %EPT  $> -2$ , see Table 4) and explained a greater amount of statistical variation compared to all respective flow alteration-ecology relationships (up to 34% -  $\Delta r^2m = 0.34$  for Abundance). The Froude number had a significant

influence on all invertebrate community response metrics and these results are presented in the subsequent sub-section to avoid repetition (these findings were congruent with the outputs of the alternative analytical framework considered and outlined in Appendix D, Table D2).

#### 4.2.3) Community responses to the most statistically influential flow-related indices

The backwards stepwise selection procedure performed on LMMs testing the additive influence of all flow-related indices (Q, AF and Froude number) demonstrated that all invertebrate response metrics were most significantly modelled using 1-4 variables as fixed-effects. The ‘optimal’ model testing TaxRic incorporated various flow-related indices (Froude number, AFJulianMin, QMax30 and QJulianMin) and accounted for 50% of the total statistical variation ( $r^2m = 0.50$ ), which increased by 4% within the ‘HG.optimal’ model (Table 5). Froude number was included within all optimal models and its individual effect within the optimal models accounted for a greater amount of statistical variation (6-38% -  $r^2m = 0.06-0.38$ ) compared to all other significant flow-related indices (Table 5). Abundance and TaxRic both exhibited a positive relationship with Froude number across ‘coarse’ and ‘*Ranunculus* sp.’ HGs, but this was less evident within ‘fine’ sediment habitats (Figs. 6a and 6b). TaxDiv responded positively with Froude number within coarse substrates and fine sediments but displayed a strong negative relationship within *Ranunculus* sp. (Fig. 6c). FRic also exhibited a positive relationship with Froude number within sedimentological HGs but did not display a clear directional change within *Ranunculus* sp. (Fig. 6d). In total, 4 Q indices were incorporated within 3 optimal models, although these individually explained up to 9% of the statistical variation ( $r^2m = 0.09$  – Table 5). AF indices were included within 2 optimal LMMs when TaxRic and FEeve were modelled against AFJulianMin (the Julian day number when the minimum flow alteration occurred) and AFMay (the average flow alteration value in May), respectively; these statistical relationships accounted for 9-20% ( $r^2m = 0.09-0.20$ ) of the statistical variation (Table 5). HG.optimal models exhibited a higher statistical power and differed significantly from each respective optimal model in all instances (Table 5). The Froude number exhibited a significantly greater statistical influence when its interaction with HG was considered in all instances, but this was not observed for Q and AF indices incorporated within optimal models (Table 5).



## 5) Discussion

### 5.1) Invertebrate community differences between habitat groups

This study aimed to quantify how invertebrate communities inhabiting distinct lotic habitats responded to three sets of flow-related characteristics: antecedent hydrological variability; antecedent anthropogenic flow alterations (daily percentage of discharge removed or added to the river) and proximal hydraulic conditions (characterized by the Froude number). HGs supported distinct taxonomic compositions, supporting the findings of many studies reporting structural differences in invertebrate communities between mineralogical and organic habitat patches (e.g. Robson and Chester, 1999; Li *et al.*, 2012). Functional trait compositions also differed between HGs, but there was a greater degree of overlap than for taxonomic compositions, particularly between *Ranunculus* sp. and coarse substrates. This contradicts the limited evidence reporting that the functional properties of invertebrate communities are more distinct between mineralogical and organic habitat patches than for taxonomic compositions (Demars *et al.*, 2012; White *et al.*, 2017b).

*Ranunculus* sp. supported the highest community abundances (Abundance), taxonomic and functional richness (TaxRic and FRic, respectively) and proportional number of taxa within Ephemeroptera, Plecoptera and Trichoptera within each sample (%EPT) compared to other HGs. This reflects the suite of ecological functions that *Ranunculus* sp. provides, including the provision of cover from predators, a habitat to lay eggs and attach egg sacks to, or a platform from which fauna can consume food resources (Ladle *et al.*, 1972; Gunn, 1985). Invertebrate communities inhabiting fine sediments displayed a high structural dominance (Berger-Parker), but a high degree of functional evenness (FEve). Greater FEve values occur when there is a high degree of taxonomic evenness or when functional distances among species are more regularly distributed (Villéger *et al.*, 2008). As such, the latter must be true for invertebrate communities sampled from fine sediments given that greater FEve values occurred (relative to other HGs) despite exhibiting high Berger-Parker values (indicating a lower taxonomic evenness). The more even distribution of taxa across functional trait space (indicated by higher FEve values) within fine sediments suggests that the loss of taxa (TaxRic) occurred randomly, rather than clusters of taxa exhibiting comparable functional niches being extirpated (Barnum *et al.*, 2017). Larsen and Ormerod

(2014) highlighted that fine sediment deposition led to random co-occurrences of species as biotic interactions weakened. Such ecological and community demographical processes could explain the higher FEve values occurring within fine sediments in the present study. Given that fine sediments are regularly disturbed and entrained in lotic environments (e.g. Gibbins *et al.*, 2007), higher FEve values within fine sediments indirectly contradicts previous findings highlighting that FEve decreases with higher disturbance frequencies (e.g. Schriever *et al.*, 2015; Barnum *et al.*, 2017).

## 5.2) Invertebrate community responses to hydrological variability and anthropogenic flow alterations

Two invertebrate community response metrics (TaxRic and FEve) were significantly influenced by antecedent hydrological conditions (derived from historic discharge time series – flow-ecology relationships). Such significant flow-ecology relationships support the plethora of evidence reported globally demonstrating the importance of river flow regimes in shaping the structure (e.g. Kennen *et al.*, 2010; Warfe *et al.*, 2014; Steel *et al.*, 2018) and function of instream communities (e.g. Mims and Olden, 2013; Schriever *et al.*, 2015; White *et al.*, 2017b), although the latter has been comparatively understudied worldwide (Arthington *et al.*, 2018; Poff, 2018). However, statistical models in this study did not detect a significant influence of hydrological characteristics for some community response metrics and flow-ecology relationships explained relatively low ( $\leq 10\%$ ) amounts of statistical variation, which potentially reflects the following five factors. First, samples were collected across a single catchment (eight sites) over one year and specifically during a time when intermediate discharges occurred (i.e. no extreme flow events were recorded – Barker *et al.*, 2016; White, 2018). As such, communities were exposed to a relatively limited range of hydrological conditions compared to studies undertaken across greater spatial and temporal scales (e.g. Monk *et al.*, 2006; Chen and Olden, 2018). Second, river flow regimes are widely recognised as a strong environmental ‘filter’ (*sensu* Poff, 1997) operating across large (catchment to regional) spatial scales (see Lytle and Poff, 2004; Biggs *et al.*, 2005). As such, riverine invertebrate species pools are confined to taxa adapted to region-wide hydrological variations, which are then subjected to smaller scale environmental filters (e.g. habitat conditions - Poff, 1997). This helps explain the findings of this study given that statistical models did not consistently detect significant flow-ecology relationships and instream communities were more

responsive to habitat-scale controls (HGs and hydraulic conditions, see below). This suggests that the filtering effect of river flow regimes at the regional scale could not be statistically detected within this study conducted across a single catchment. Third, habitat replicates within the same reach used in this study shared the same discharge-related (i.e. hydrological variability and anthropogenic flow alteration) values, which may have resulted in weaker statistical associations and highlights the difficulty in integrating flow-related characteristics across different spatial scales (see Biggs *et al.*, 2005). Fourth, river flow regimes may act in concert with other environmental variables (e.g. water quality and morphological alterations) to exert a synergistic effect on instream ecological processes (see Booker *et al.*, 2015). As such, flow-ecology relationships testing the individual effect of hydrological characteristics may overlook significant interactive effects with alternative environmental variables, as demonstrated with HGs in this study. Fifth, the nature and strength of flow-ecology relationships are artefacts of the underpinning ecological (Cuffney and Kennen, 2018) and hydrological (Wilby *et al.*, 2017) information and the data used within this study may have had a key influence on the results. For example, within the family Chironomidae (Order: Diptera), species-specific flow-ecology relationships are likely to have occurred (e.g. Cañedo-Argüelles *et al.*, 2016), which would not have been detected in this study due to their consideration at the family level. However, it should be noted that invertebrate taxa were identified consistently and to the lowest practical resolution within this study, which has been demonstrated to provide the basis for developing consistent and robust flow-ecology relationships (see Monk *et al.*, 2012).

Functional Evenness (FEve) responded significantly to anthropogenic flow alterations, highlighting its potential use as a tool for underpinning significant flow-ecology (see above) and flow alteration-ecology relationships. This provides additional evidence supporting recent calls for the functional properties of biota to be incorporated into environmental flow (e-flow) science (e.g. Arthington *et al.*, 2018; Poff, 2018). Non-significant flow alteration-ecology relationships observed in this study may be an artefact of the five factors discussed above. However, it is also likely that flow alterations across the rivers studied were not of sufficient magnitude to yield consistent, statistically detectable ecological responses. Long-term improvements in water management operations have occurred across the study

region to limit extreme flow alterations (Bowles and Henderson, 2012). Discharges were reduced on average by just 3.88% across all sampling sites over the study period, which is much lower than extreme flow alterations being reported elsewhere globally (e.g. c. 100% reduction in discharge due to groundwater abstraction reported by Bradley *et al.*, 2014; 2017). Moreover, although the daily reductions in historic discharges of up to 48.33% occurred at a single site in this study, in a UK study Bradley *et al* (2017) only detected negative ecological effects of groundwater abstraction when river discharges were reduced by at least 50%. Such findings may explain the absence of significant flow alteration-ecology relationships observed in this study. Notwithstanding, this study represents the first of its kind to test ecological responses to a suite of indices characterising anthropogenic flow alterations (centred on the five facets of the flow regime – see Poff *et al.*, 1997) that incorporates both subsurface (groundwater abstraction) and surface (e.g. effluent water returns) hydrological changes. There is a paucity of information on how groundwater abstraction influences riverine ecosystems globally (Poff and Zimmerman, 2010; Gleeson and Richter, 2018). Given that groundwater abstraction practices are increasingly and severely depleting subsurface water resources (Gleeson *et al.*, 2012) and substantially reducing river discharges globally (de Graaf *et al.*, 2014), studies such as this are vital for guiding e-flow science and sustainable groundwater management operations.

Community responses to hydrological indices were stronger when incorporating their interaction with HGs (i.e. HG.flow-ecology relationships), which significantly improved the statistical fit of Abundance, FRic and FEve models. This highlights that hydrological controls on the total abundance and functional diversity of communities differs between HGs, which may have significant implications for the wider food web (Power *et al.*, 2008; Ledger *et al.*, 2013; Greenwood and Booker, 2015). Similarly, various community responses (most notably Abundance and TaxRic) to anthropogenic flow alterations were stronger when a HG interaction term was incorporated (HG.flow alteration-ecology relationships). Other studies have also reported habitat-specific invertebrate responses to flow alterations, including marginal habitats (which become regularly disconnected from the channel - Storey and Lynas, 2007) and riffles (due to the loss of rheophilic taxa - Brooks *et al.*, 2011). In contrast, Bradley *et al* (2017) reported that instream community responses to groundwater abstraction did not differ between substrate

size classes. Variable ecological responses to flow alterations have been reported at global (e.g. Poff and Zimmerman, 2010), national (e.g. Mims and Olden, 2013), regional (e.g. Chen and Olden, 2018) and even system-specific scales (Thompson *et al.*, 2018). The results of the present study provide evidence that ecological responses to anthropogenic flow alterations vary at the habitat-scale and specifically between distinct mineralogical and organic habitat patches, which have seldom been incorporated within e-flow research thus far (but see Lind *et al.*, 2006; Finn *et al.*, 2009; Bradley *et al.*, 2017).

### *5.3) Statistically optimal flow-related characteristics driving ecological responses.*

The Froude number exerted a significant influence on all structural and functional community response metrics examined within this study. Froude number has been demonstrated to have a significant influence on the structural (Rempel *et al.*, 2000) and functional (Lamouroux *et al.*, 2004) properties of river invertebrate communities as it characterises the hydraulic conditions experienced by biotic communities (Turner and Stewardson, 2014). Previous research has highlighted that the morphological properties of invertebrate species govern community responses to Froude number, such as organisms with streamlined body forms responding positively to higher flow velocities (Rempel *et al.*, 2000; Lamouroux *et al.*, 2004). In addition, the behavioural responses of invertebrates to hydraulic conditions shapes community responses to Froude number, such as species migrating to different surface (Lancaster *et al.*, 2006) or subsurface refuges (Holomuzki and Biggs, 2000) during adverse hydraulic conditions.

Examining changes in optimal hydraulic conditions (based on the preference of target organisms) over a range of river discharges has been a core part of ‘habitat simulation’ e-flow methodologies (Lamouroux and Jowett, 2005). Various authors have demonstrated the application of such techniques within e-flow frameworks (e.g. Strevens, 1999; Nikghalb *et al.*, 2017). For example, Lamouroux and Olivier (2015) used a hydraulic habitat model to reliably predict changes in fish populations in response to a restored flow regime. Findings from the present study reinforce the benefits of incorporating hydraulic observations within e-flow studies. Hydraulic observations provide an improved characterization of the forces to which biota are exposed to at the time of sampling compared to the use

of discharge-related statistics alone (e.g. Malcolm et al., 2012; Monk et al., 2018). Given the crucial importance of hydraulic forces in shaping the structural and functional properties of communities, further observational and experimental studies are required to elucidate the causal mechanisms underpinning ecological responses to hydraulic characteristics to guide the practical application of future e-flow science (Arthington et al., 2018).

Froude number was utilised in this study due to its comparability between habitats, rivers and seasons (Jowett 1993; Wadeson and Rowntree, 1998). Unsurprisingly, Froude number was highly correlated with flow velocities and is therefore intrinsically linked to the entrainment threshold of riverbed sediments, a widely recognised disturbance affecting instream communities (e.g. Gibbins *et al.*, 2007). Froude number has also been demonstrated to reliably characterise the average shear stresses occurring between submerged plant strands within lotic environments (Folkard, 2011). However, differences in ecological responses to hydraulic conditions between different mineralogical and organic habitat patches (HGs in this study) has not been widely explored, in part due to the difficulties obtaining reliable hydraulic observations between macrophyte strands (see Marjoribanks *et al.*, 2014).

This study demonstrated that invertebrate community responses to Froude number differed between HGs, highlighting how mineralogical and organic habitat patches mediate the structural and functional responses of biota to hydraulic conditions. This potentially reflects HGs supporting distinct communities which respond differently to Froude, such as various rheophilic taxa (e.g. *Rhyacophila dorsalis*, *Limnius volckmari* and *Elmis aenea*; see Appendix E, Table E1 and Extence *et al.*, 1999) inhabiting coarse substrates and *Ranunculus* sp. patches and benefit from higher flow velocities. Alternatively, the significant interactive effects of Froude number and HGs on the structure and function of invertebrate communities could be attributed to mineralogical and organic habitat patches providing unique ecological functions which alter how instream communities respond to hydraulic conditions. For example, *Ranunculus* sp. is typically located in channel areas exhibiting high flow velocities, which deliver high quantities of detritus between the porous plant stands. Many filter-feeding invertebrates (e.g. *Brachycentrus subnubilus*, *Hydropsyche* sp. and various Simuliidae species - see Appendix E,

Table E1) occupy *Ranunculus* sp. patches in order to consume food resources by attaching themselves to plant stands suspended in water column (Ladle *et al.*, 1972; Wharton *et al.*, 2006).

#### 5.4) Incorporating small-scale habitat features into environmental flow frameworks

The need to conserve and/or create ecologically favorable habitat conditions in order to enhance the effectiveness of river management strategies has received considerable research attention (see Palmer *et al.*, 2010). This has been most widely considered within the context of morphological river restoration efforts applied at the reach-scale (e.g. Kemp *et al.*, 1999; White *et al.*, 2017b). However, incorporating habitat-scale features within regional environmental flow (e-flow) strategies may be hindered by limited resources restricting the ability of scientists and practitioners to collect such fine-scale data across larger geographical scales (see Chen and Olden, 2018). Notwithstanding, a limited body of research has highlighted how flow regimes could be managed to indirectly benefit instream communities by modifying the composition of small-scale lotic habitats (e.g. Armitage and Pardo, 1995; Storey and Lynas, 2007). This study further emphasizes how hydrological and hydraulic controls on habitat compositions could be incorporated into e-flow research. Moreover, the findings from this study provides rare evidence that the ecological benefits of e-flow frameworks and river restoration practices could be further improved by considering the hydraulic conditions occurring within distinct small-scale habitat patches. Further research is required to understand how flow characteristics shape riverine communities at the habitat-scale in order to provide a causal basis for guiding the development of regional e-flow strategies.

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## Tables

**Table 1** – Land use coverage (%) for each of the studied river catchments (Source: NRFA, 2018).

	<b>Ebble</b>	<b>Nadder</b>	<b>Wylfe</b>	<b>Bourne</b>
National River Flow Archive (NRFA) site	43011 – Ebble at Bodeham	43006 – Nadder at Wilton	43008 – Wylfe at South Newton	43004 – Bourne at Laverstock
Woodland	6.00	15.98	9.38	10.07
Arable agriculture	55.83	48.94	31.01	40.45
Grassland	31.30	29.94	50.70	39.07
Heathland	0.51	0.80	0.17	0.00
Urban	2.86	3.18	2.70	4.54

**Table 2** – Average water quality measurements from different study sites between January 2014 – January 2016 (Source: Environment Agency, 2017). N.B. All water quality (WQ) sites located within 2.5km of the study sampling sites. There is no regulatory WQ monitoring site close to Ebble 2 and some WQ measurements are not routinely recorded at Bourne1.

	<b>Ebble 1</b>	<b>Nadder 1</b>	<b>Nadder 2</b>	<b>Wylfe 1</b>	<b>Wylfe2</b>	<b>Bourne1</b>	<b>Bourne2</b>
Environment Agency WQ site	SW-50250291	SW-C0235000	SW-50220284	SW-50250634	SW-50240461	SW-50240226	SW-50240116
pH	7.88	8.09	8.07	8.33	8.22	7.84	8.05
Conductivity (µs/cm)	551	496.25	507.85	455.49	580.17	535.97	551.25
Dissolved oxygen (% saturation)	95.57	95.28	94.08	103.73	105.34	NA	98.75
Nitrates (mg/l)	7.04	4.40	5.53	6.31	5.89	7.29	7.63
Orthophosphate (reactive) (mg/l)	0.05	0.16	0.12	0.09	0.16	NA	0.05

994 **Table 3** – Hydrological (Q), anthropogenic flow alteration (AF) and hydraulic (Froude) indices  
 995 included within the final analyses.

Index	Flow-related characteristic	Flow regime components	Description
QMay	Hydrological ( $\text{m}^3\text{s}^{-1}$ )	Magnitude Timing	Mean average discharge in May
QJulianMin	Hydrological ( $\text{m}^3\text{s}^{-1}$ )	Magnitude Timing	Julian day of the minimum discharge occurrence.
QMax30	Hydrological ( $\text{m}^3\text{s}^{-1}$ )	Magnitude	Maximum discharge in the 30-days prior to sampling.
QMin30	Hydrological ( $\text{m}^3\text{s}^{-1}$ )	Magnitude	Minimum discharge in the 30-days prior to sampling.
QMin90	Hydrological ( $\text{m}^3\text{s}^{-1}$ )	Magnitude	Minimum discharge in the 90-days prior to sampling.
AFMay	Anthropogenic flow alteration (%)	Magnitude Timing	The average flow alteration in May.
AFJul	Anthropogenic flow alteration (%)	Magnitude Timing	The average flow alteration in July.
AFJulianMin	Anthropogenic flow alteration (%)	Magnitude Timing	Julian day of the minimum percentage modified discharge.
AFLPD	Anthropogenic flow alteration (%)	Magnitude Duration	The average duration that flow alterations $<75^{\text{th}}$ percentile.
AbMax7	Anthropogenic flow alteration (%)	Magnitude	Maximum flow alteration in the 7-days prior to sampling.
Froude	Hydraulic	NA	The ratio between inertial and gravitational forces within the water column. $Fr = v / \sqrt{gD}$ . $v$ = average velocity ( $\text{ms}^{-1}$ ); $g$ = gravitational acceleration ( $\text{ms}^{-2}$ ) and $D$ = sample depth (m).



**Table 4.** Invertebrate community responses to the influences of hydrological variability and anthropogenic flow alterations ('flow-ecology' and 'flow alteration-ecology' relationships, respectively) and their interaction with HG ('HG.flow-ecology' and 'HG.flow alteration-ecology' relationships, respectively). Shaded boxes highlight significant differences whereby each statistical 'pair' differs significantly and possesses higher  $r^2m$  and  $\Delta AICc$  values  $\leq 2$  when a HG interaction is incorporated. Stars denote the degree of significance: =  $p \leq 0.05$ ; \*\* =  $p \leq 0.01$ ; \*\*\* =  $p \leq 0.001$ ; NS = non-significant. See section 3.3.2 and Fig. 2 for statistical model descriptions and nomenclature.

Response	Statistic	Hydrological variability			Anthropogenic flow alterations		
		Flow-ecology	HG.Flow-ecology	Difference	Flow alteration-ecology	HG.Flow alteration-ecology	Difference
Abundance	AIC	322.70	312.44	-	325.39	269.13	-
	$r^2m$	0.06	0.15	-	0.03	0.37	-
	$X^2$	4.36	34.63	30.26	1.67	77.93	76.26
	<i>p-value</i>	0.499(NS)	0.003**	<0.001***	0.893(NS)	<0.001***	<0.001***
TaxRic	AIC	1457.50	1465.00	-	1462.30	1458.90	-
	$r^2m$	0.10	0.15	-	0.08	0.37	-
	$X^2$	13.56	26.07	12.51	8.75	32.18	23.43
	<i>p-value</i>	0.019*	0.037*	0.252(NS)	0.119(NS)	0.006**	0.009**
TaxDiv	AIC	880.47	881.01	-	904.35	913.56	-
	$r^2m$	0.04	0.12	-	0.01	0.05	-
	$X^2$	9.10	28.56	19.46	1.36	12.15	10.79
	<i>p-value</i>	0.105(NS)	0.018*	0.035*	0.929(NS)	0.668(NS)	0.374(NS)
Berger-Parker index	AIC	-165.05	-155.67	-	-143.17	-129.85	-
	$r^2m$	0.03	0.08	-	0.01	0.04	-
	$X^2$	7.48	18.10	10.63	1.99	8.67	6.68
	<i>p-value</i>	0.188(NS)	0.257(NS)	0.387(NS)	0.851(NS)	0.894(NS)	0.755(NS)
%EPT	AIC	1863.50	1871.00	-	1857.60	1857.10	-
	$r^2m$	0.08	0.13	-	0.06	0.22	-
	$X^2$	6.22	18.80	12.57	7.48	28.04	20.56
	<i>p-value</i>	0.285(NS)	0.223(NS)	0.249(NS)	0.187(NS)	0.021*	0.024*
FRic	AIC	-668.30	-703.85	-	-678.33	-667.80	-
	$r^2m$	0.03	0.26	-	0.03	0.08	-
	$X^2$	6.68	62.23	55.56	6.98	16.45	9.47
	<i>p-value</i>	0.246(NS)	<0.001***	<0.001***	0.222(NS)	0.353(NS)	0.488(NS)
FEve	AIC	-393.45	-425.47	-	-391.86	-386.52	-
	$r^2m$	0.10	0.25	-	0.19	0.27	-
	$X^2$	12.87	54.88	42.02	14.71	29.37	14.66
	<i>p-value</i>	0.025*	<0.001***	<0.001***	0.012*	0.014*	0.145(NS)

**Table 5.** Invertebrate community responses to statistically ‘optimal’ (derived from a backwards stepwise selection procedure) flow-related indices (Q, AF and Froude), as well as their interaction to HG. F = F-value obtained from *anova* for each individual covariate, X<sup>2</sup> derived from likelihood ratio tests for each full model (highlighted in bold). Shaded boxes highlight significant differences between optimal and HG.optimal (likelihood ratio test) and when the latter possesses a higher r<sup>2</sup>m and ΔAICc values ≤-2. Stars denote the degree of significance: = p ≤ 0.05; \*\* = p ≤ 0.01; \*\*\* = p ≤ 0.001; NS = non-significant. See section 3.3.2 and Fig. 2 for statistical model descriptions and nomenclature.

Response	Optimal and HG.optimal model summaries					Difference	
	Covariates	r <sup>2</sup> m	AIC	F / X <sup>2</sup>	p-value	X <sup>2</sup>	p-value
Abundance	Froude	<b>0.30</b>	<b>238.57</b>	<i>100.80 / 80.66</i>	<b>&lt;0.001***</b>		
	HG × Froude	<b>0.34</b>	<b>216.73</b>	<i>45.52 / 106.26</i>	<b>&lt;0.001***</b>	<b>25.60</b>	<b>&lt;0.001***</b>
TaxRic	Froude	0.38	1344.50	<i>148.07</i>	<0.001***		
	HG × Froude	0.44	1324.80	<i>61.47</i>	<0.001***	23.66	<0.001***
	AFJulianMin	0.20	1423.70	<i>17.58</i>	<0.001***		
	HG × AFJulianMin	0.19	1426.10	<i>8.35</i>	<0.001***	1.55	0.460(NS)
	QMax30	0.08	1428.00	<i>20.52</i>	<0.001***		
	HG × QMax30	0.07	1425.30	<i>6.16</i>	<0.001***	6.67	0.036*
	QJulianMin	0.04	1423.50	<i>8.28</i>	0.004**		
	HG × QJulianMin	0.06	1424.70	<i>5.09</i>	0.002**	2.81	0.246(NS)
	<b>Froude + AFJulianMin+QMax30+QJulianMin</b>	<b>0.50</b>	<b>1310.80</b>	<b>123.45</b>	<b>&lt;0.001***</b>		
	<b>HG × (Froude + AFJulianMin+QMax30+QJulianMin)</b>	<b>0.54</b>	<b>1294.10</b>	<b>156.16</b>	<b>&lt;0.001***</b>	<b>32.70</b>	<b>&lt;0.001***</b>
TaxDiv	Froude	<b>0.11</b>	<b>850.23</b>	<i>26.86 / 25.38</i>	<b>&lt;0.001***</b>		
	HG × Froude	<b>0.17</b>	<b>835.93</b>	<i>16.03 / 43.68</i>	<b>&lt;0.001***</b>	<b>18.29</b>	<b>&lt;0.001***</b>
Berger-Parker	Froude	<b>0.06</b>	<b>-172.03</b>	<i>14.39 / 13.83</i>	<b>&lt;0.001***</b>		
	HG × Froude	<b>0.12</b>	<b>-184.33</b>	<i>10.73 / 30.12</i>	<b>&lt;0.001***</b>	<b>16.30</b>	<b>&lt;0.001***</b>
%EPT	Froude	<b>0.11</b>	<b>1893.00</b>	<i>28.28 / 24.23</i>	<b>&lt;0.001***</b>		
	HG × Froude	<b>0.15</b>	<b>1881.20</b>	<i>15.22 / 40.07</i>	<b>&lt;0.001***</b>	<b>15.83</b>	<b>&lt;0.001***</b>
FRic	Froude	0.24	-718.49	<i>66.94</i>	<0.001***		
	HG × Froude	0.39	-769.32	<i>45.64</i>	<0.001***	<b>54.83</b>	<b>&lt;0.001***</b>
	QMax30	0.05	-671.13	<i>10.41</i>	0.001**		
	HG × QMax30	0.02	-667.86	<i>1.32</i>	0.270(NS)	0.74	0.692(NS)
	<b>Froude + QMax30</b>	<b>0.24</b>	<b>-726.65</b>	<b>57.64</b>	<b>&lt;0.001***</b>		
	<b>HG × (Froude + QMax30)</b>	<b>0.39</b>	<b>-767.23</b>	<b>106.22</b>	<b>&lt;0.001***</b>	<b>48.58</b>	<b>&lt;0.001***</b>

<b>FEve</b>	Froude	0.10	<b>-413.37</b>	<i>25.65</i>	<0.001***		
	HG × Froude	0.15	<b>-429.76</b>	<i>14.73</i>	<0.001***	20.39	<0.001***
	QMax30	0.09	<b>-405.16</b>	<i>18.25</i>	<0.001***		
	HG × QMax30	0.07	<b>-402.75</b>	<i>5.01</i>	0.003**	1.59	0.453(NS)
	AFMay	0.09	<b>-405.46</b>	<i>10.81</i>	0.001**		
	HG × AFMay	0.10	<b>-402.40</b>	<i>4.06</i>	0.008**	0.94	0.625(NS)
	<b>Froude + QMax30 + AFMay</b>	<b>0.20</b>	<b>-433.18</b>	<b>39.81</b>	<b>&lt;0.001***</b>		
	<b>HG × ( Froude + QMax30 + AFMay)</b>	<b>0.25</b>	<b>-440.69</b>	<b>59.32</b>	<b>&lt;0.001***</b>	<b>19.51</b>	<b>0.003**</b>

## Figure captions

**Fig. 1** – The location of the study sites within the Hampshire Avon. Square within the inset = study region, dashed line = Hampshire Avon catchment boundary and circles = sampling sites. Dark grey = ‘highly productive aquifer’, light grey = ‘moderately productive aquifer’ and white = ‘low productivity aquifer’ or ‘rocks with essentially no groundwater’ (for classification, see BGS, 2018).

**Fig. 2** – A flow chart outlining the analytical framework adopted within this study. Dashed lines = 1<sup>st</sup> aim/results subsection, grey lines = 2<sup>nd</sup> aim/results subsection and solid black lines = 3<sup>rd</sup> aim/results subsection. The nomenclature for different sets of statistical models is outlined in apostrophes.

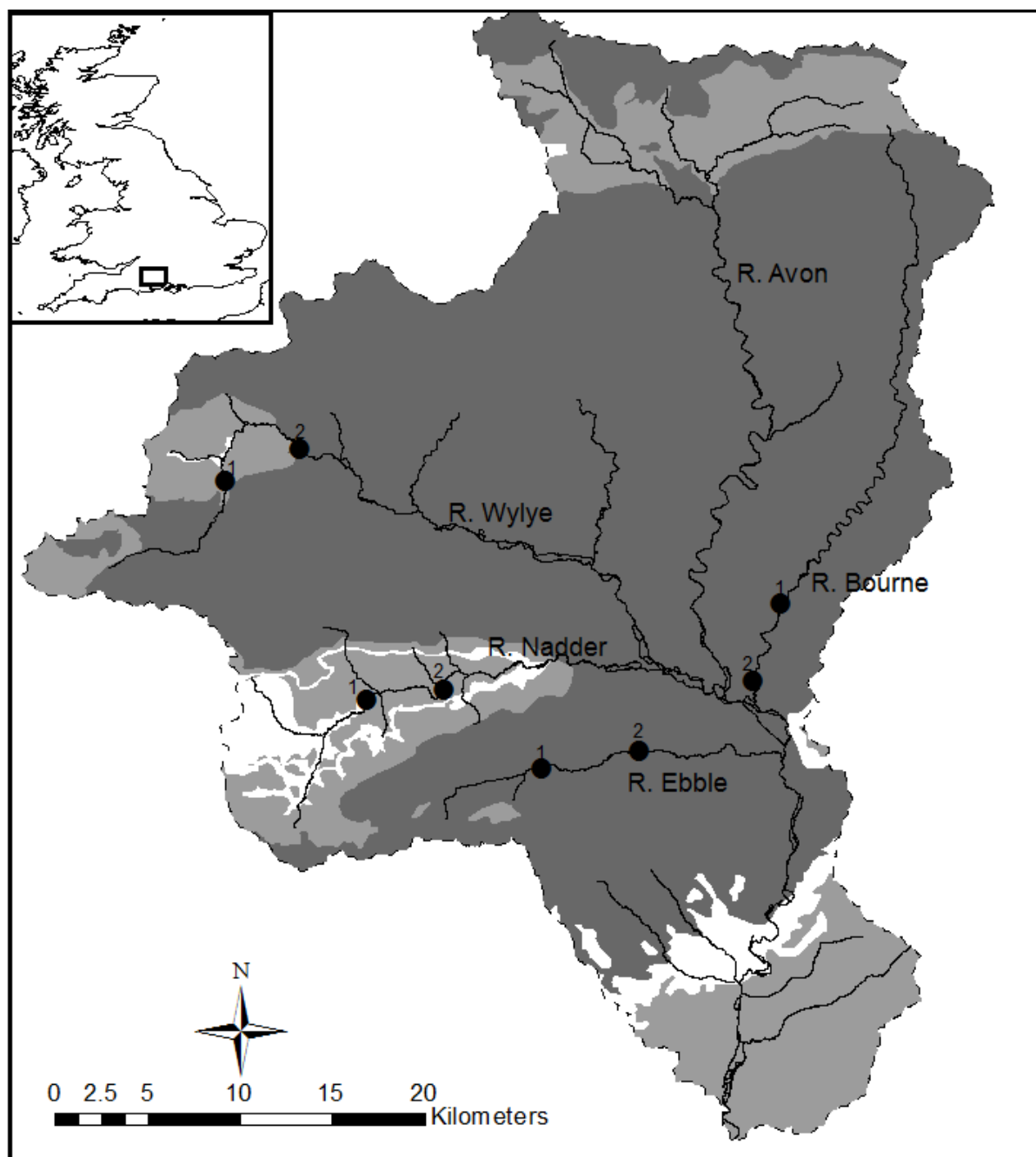
**Fig. 3** – A daily time series of historical discharges (black) and anthropogenic flow alterations (grey) occurring at each study site: (a) Ebble 1; (b) Ebble 2; (c) Nadder 1; (d) Nadder 2; (e) Wylye 1; (f) Wylye 2; (g) Bourne 1 and (h) Bourne 2.

**Fig. 4** – PCoA plot of invertebrate communities between habitat groups for (a) taxonomic and (b) functional trait compositions. Dark blue = Fine sediments; light blue = coarse substrates and green = ‘*Ranunculus* sp.’ (these lines are dashed to aid visual interpretation).

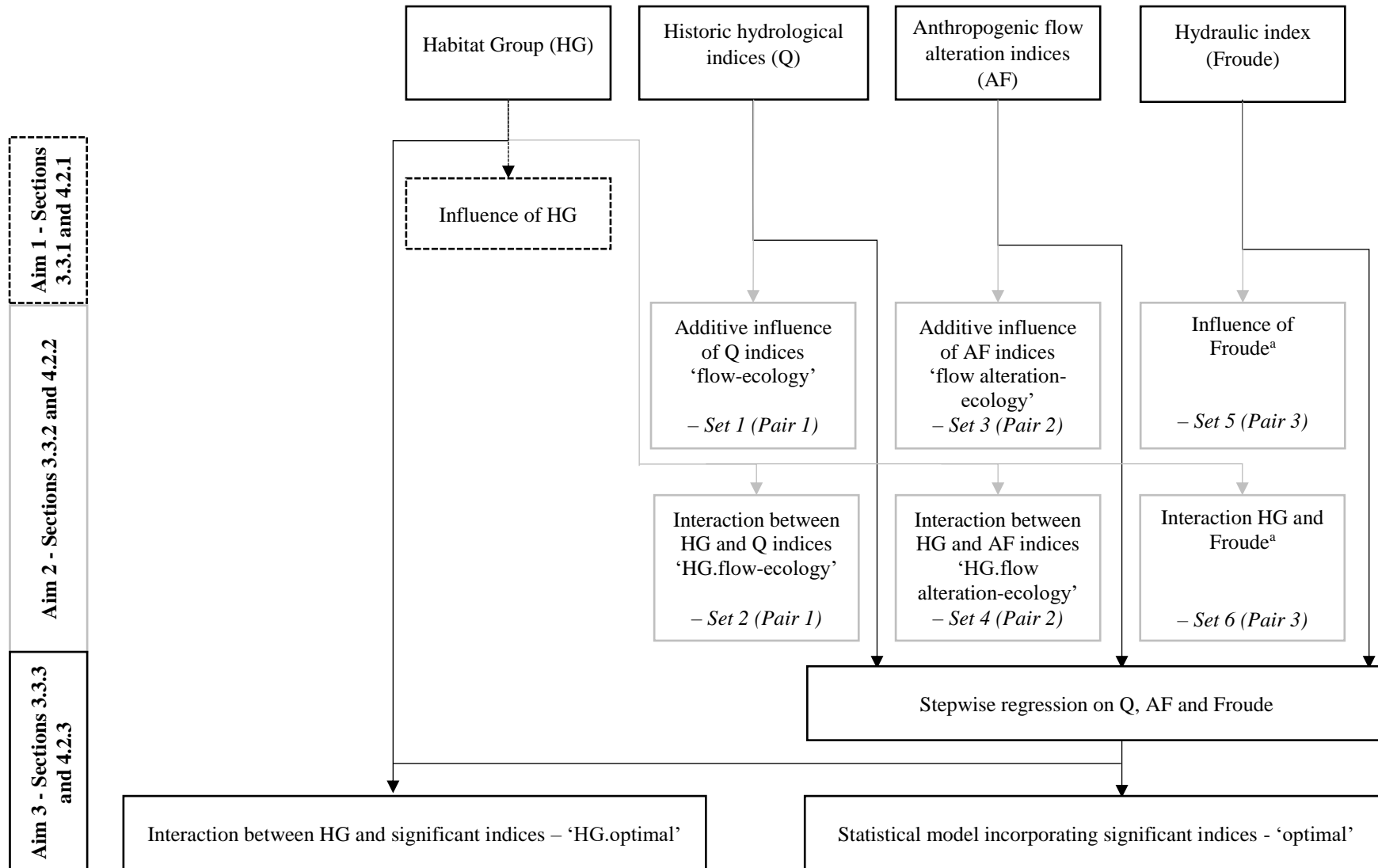
**Fig. 5** - Average ( $\pm 1$  standard error) values for invertebrate response metrics between different Habitat Groups (HGs). (a) Abundance; (b) TaxRic; (c) TaxDiv; (d) Berger-Parker index; (e) %EPT; (f) FRic and (g) FEve. Dark blue = Fine sediments; light blue = coarse substrates and green = ‘*Ranunculus* sp.’.

**Fig. 6** – Statistical relationships between invertebrate community responses to Froude across different HGs, with 95% confidence intervals obtained from LMMs. (a) Abundance; (b) TaxRic; (c) TaxDiv and (d) FRic. Dark blue = Fine sediments; light blue = coarse substrates and green = ‘*Ranunculus* sp.’ (these lines are dashed to aid visual interpretation).

Fig. 1



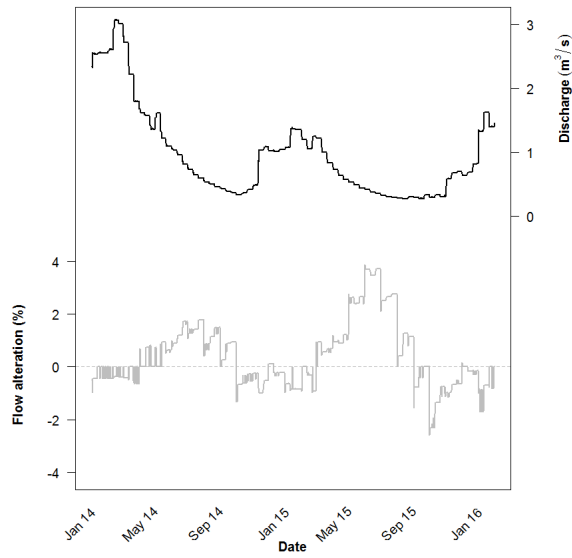
**Fig. 2**



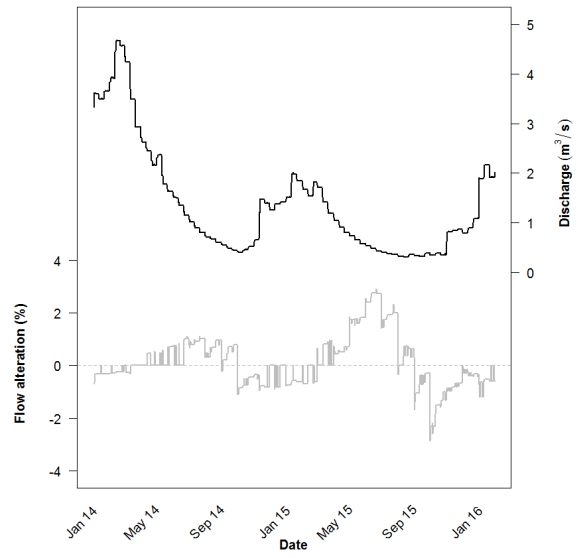
a) To avoid repetition, these results are presented in the third sub-section of the results

**Fig. 3**

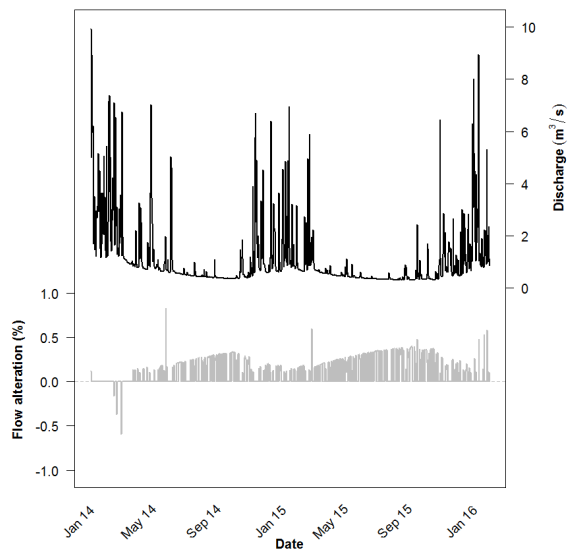
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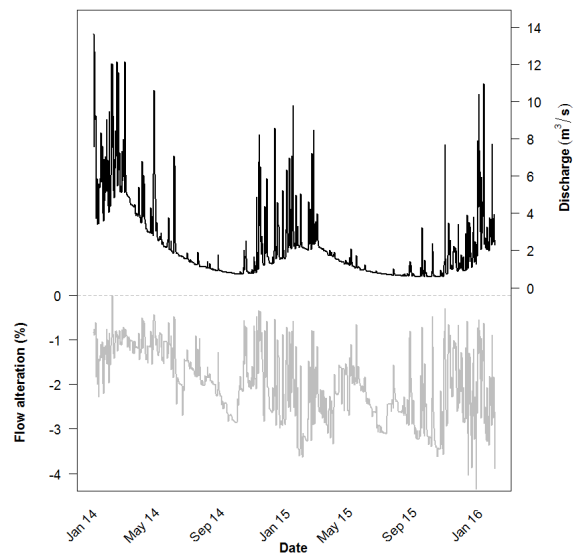
b)



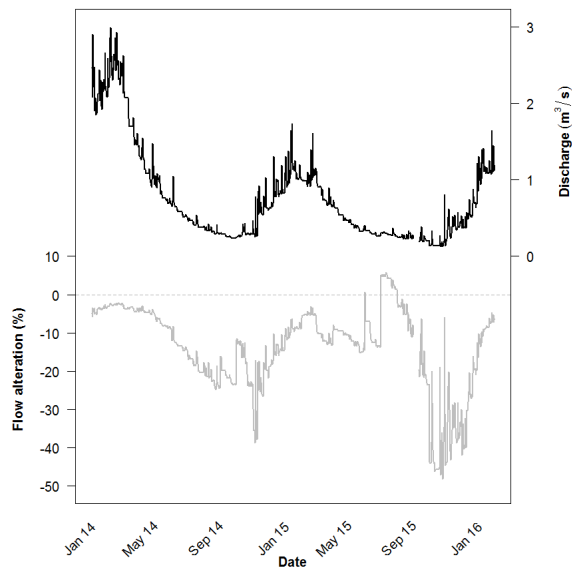
c)



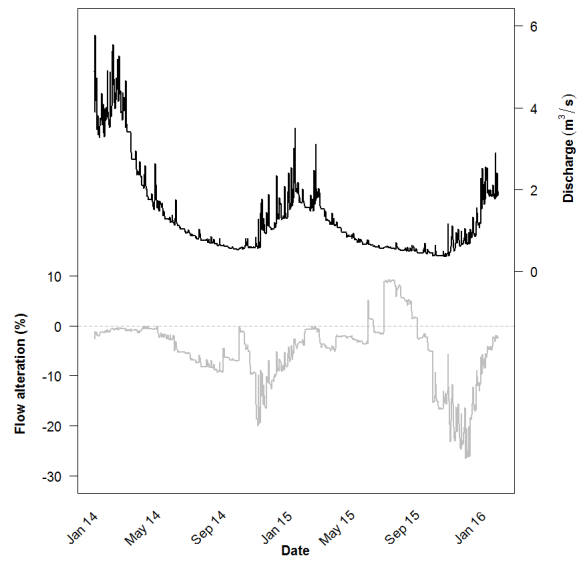
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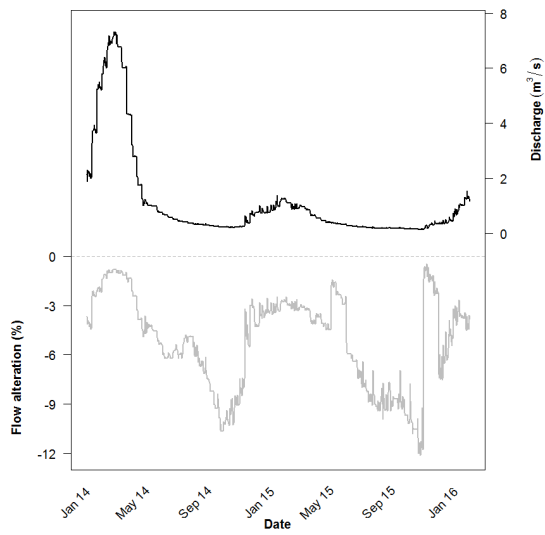
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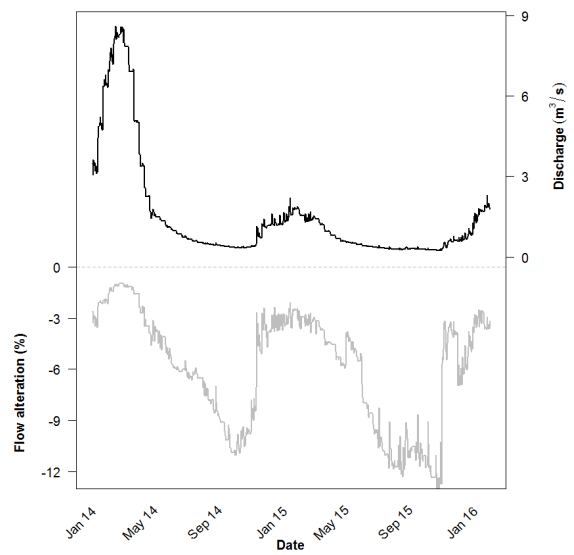
f)



g)



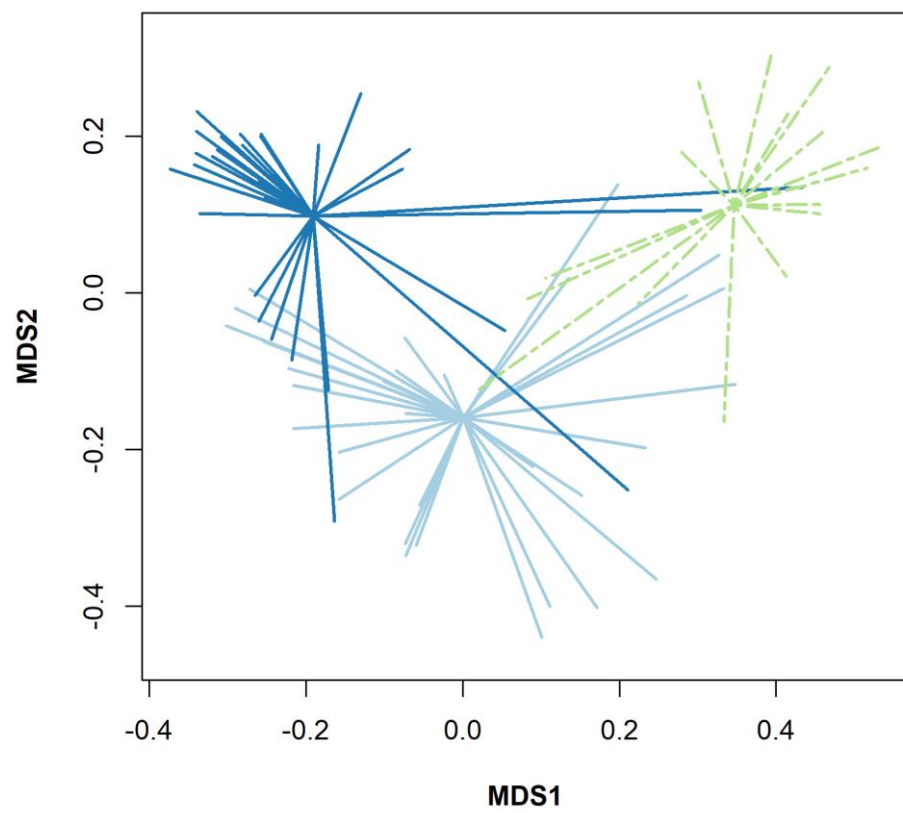
h)





**Fig 4**

a)



b)

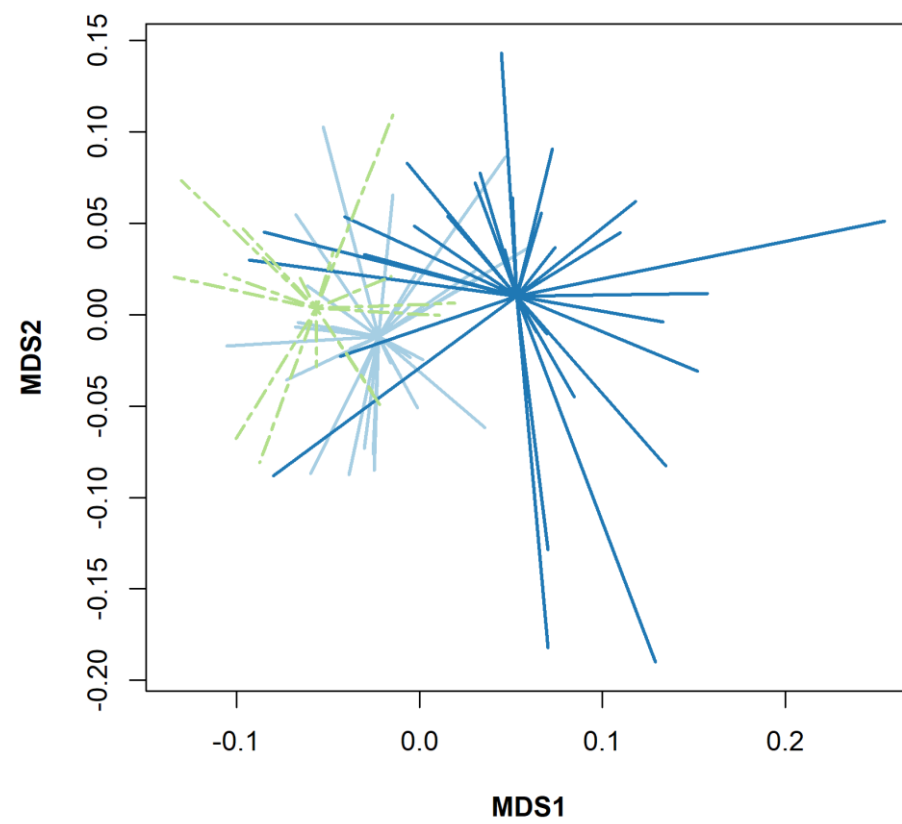
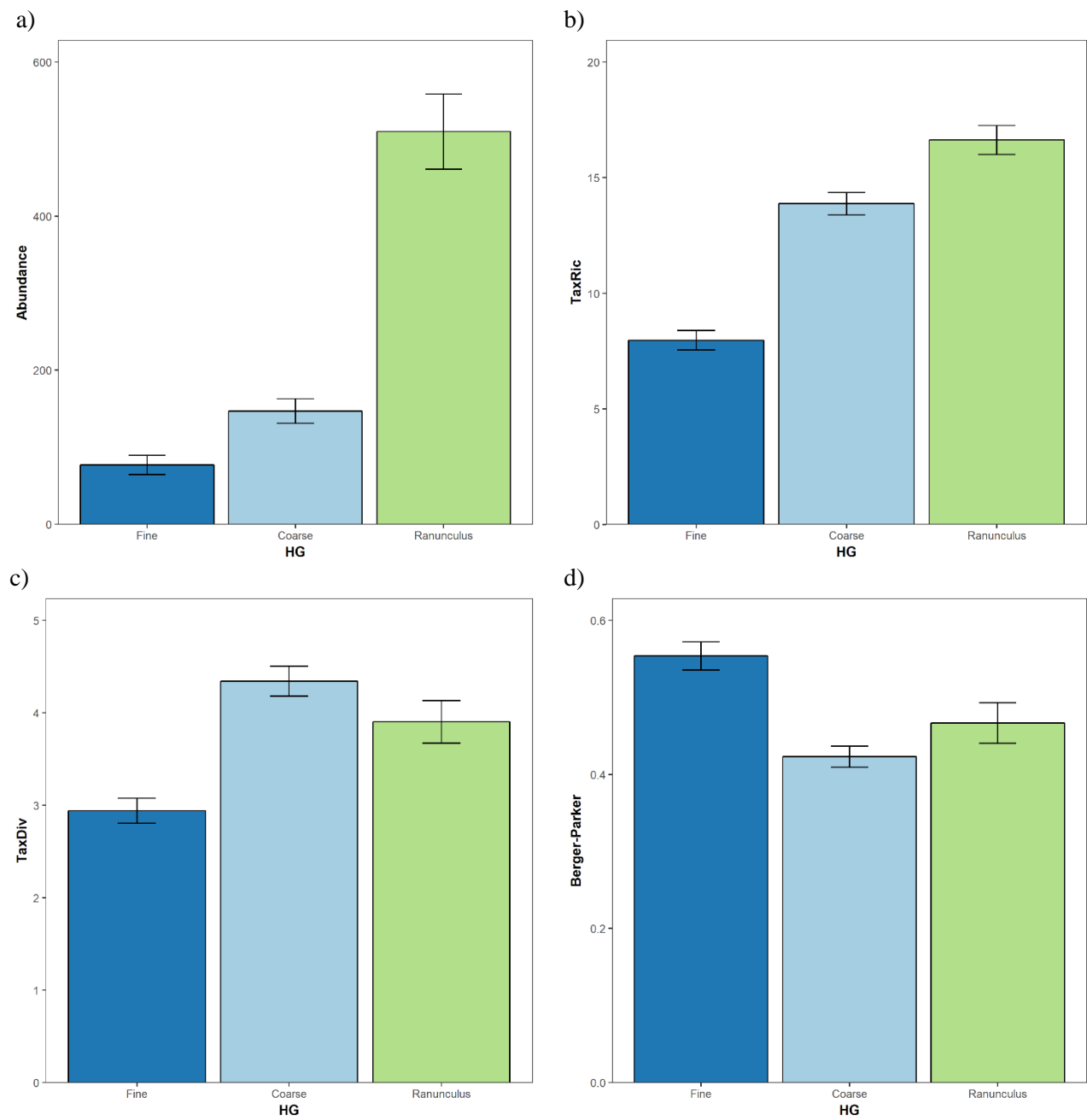
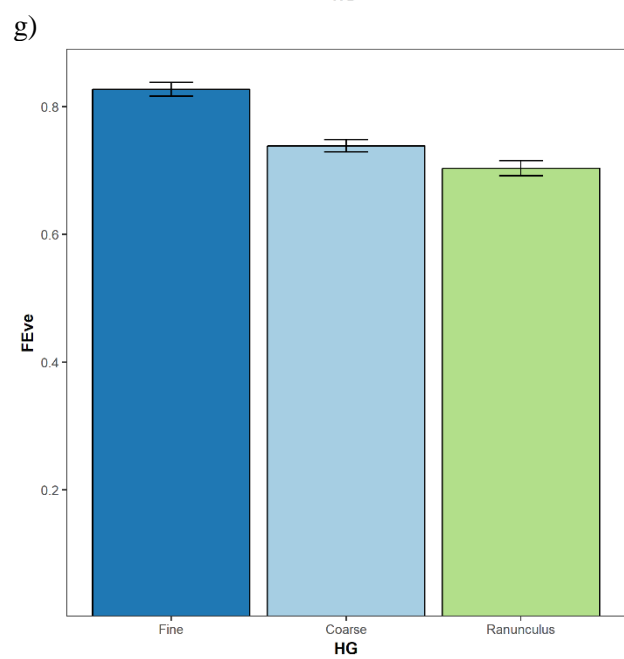
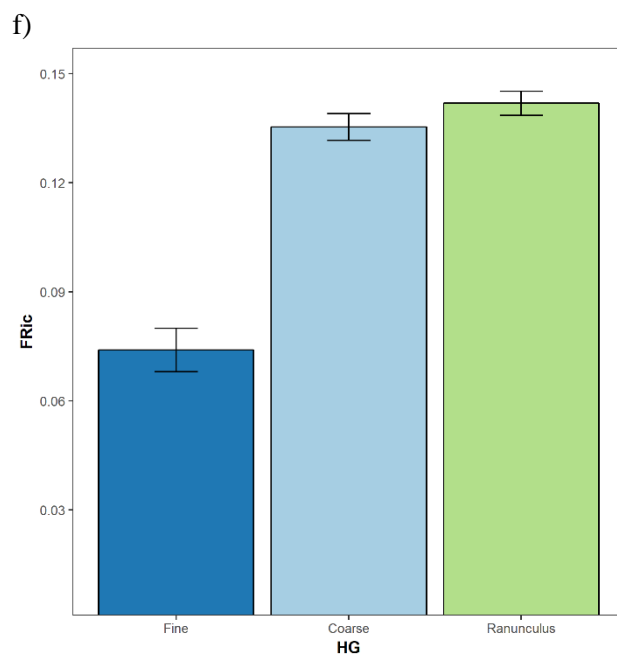
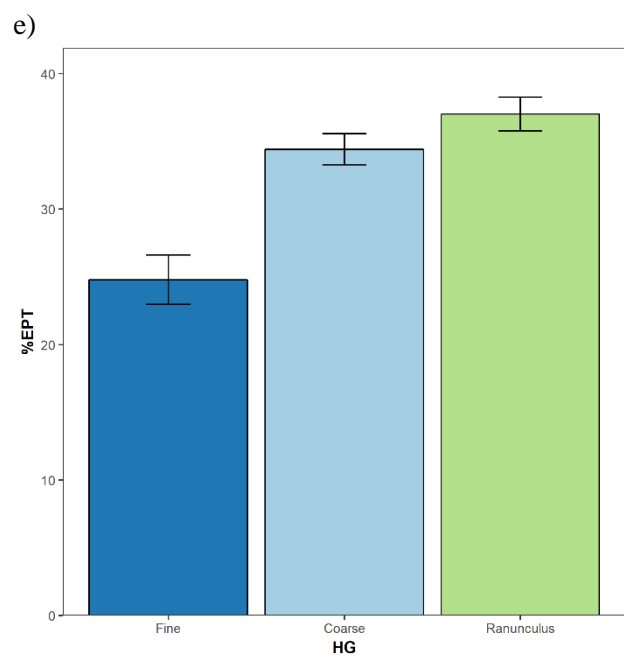


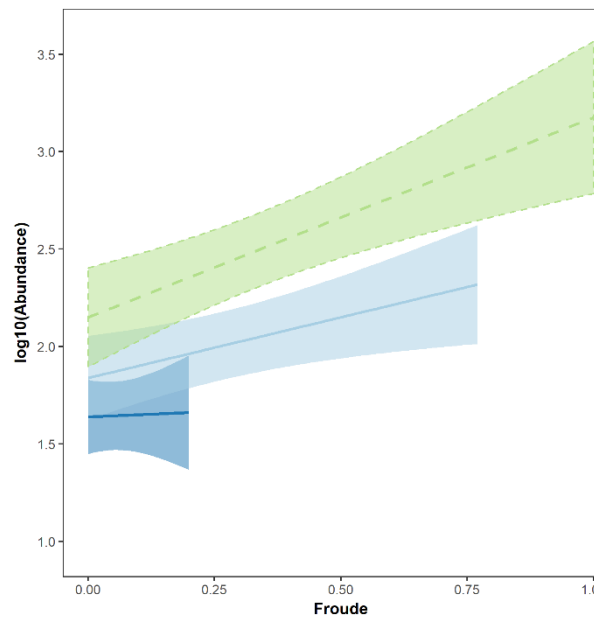
Fig. 5



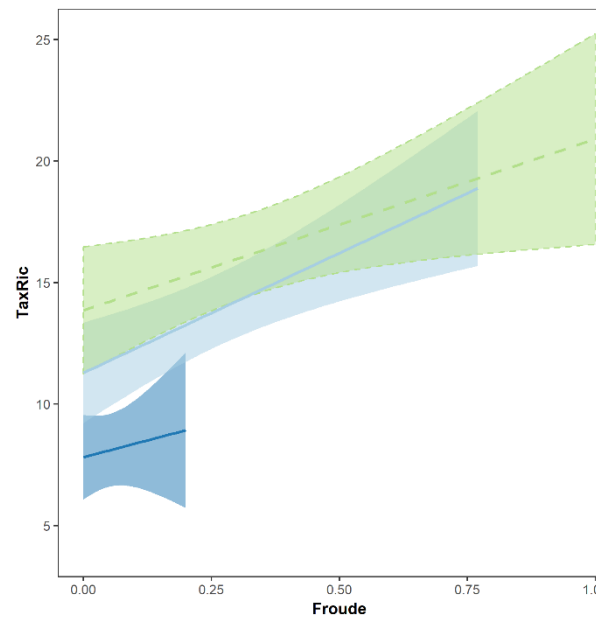


**Fig. 6**

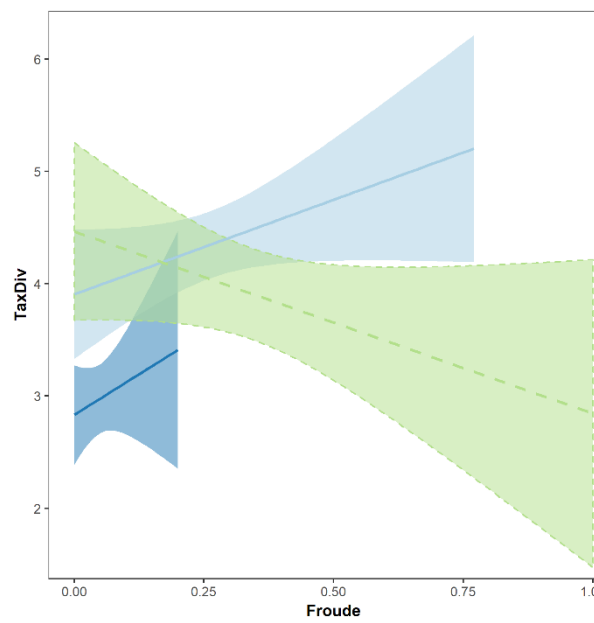
a)



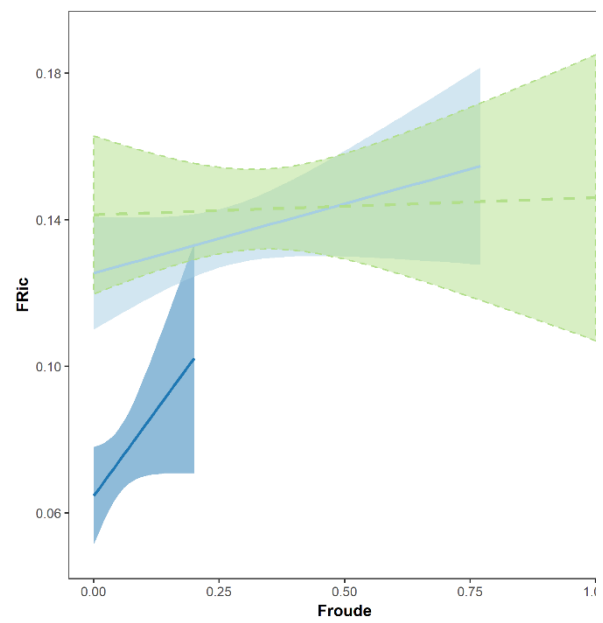
b)



c)



d)



## Supplementary Information

### Appendix A

This appendix outlines various hydrological model fit statistics highlighting the accuracy of the Wessex Basin groundwater model outputs in modelling daily river discharges. Summaries are presented for the three sampling sites positioned within a close proximity (<1km) of existing flow gauges recording river discharges for environmental regulators from the National River Flow Archive (NRFA, 2018). The Nash-Sutcliffe (Nash and Sutcliff, 1970), Root Mean Square Error (RMSE) and  $r^2$  (of a linear regression) model fit summaries are presented.

NRFA site	Nash-Sutcliffe	RMSE	$r^2$
43806 – Wylfe at Brixton Deverill	0.58	0.47	0.83
43012 – Wylfe at Norton Bavant	0.86	0.39	0.88
43004 – Bourne at Laverstock	0.85	0.78	0.98

### References

Nash, J. E. and Sutcliffe, J. V. (1970). River flow forecasting through conceptual models part I - A discussion of principles. *Journal of Hydrology*, 10. 282–290.

National River Flow Archive (2018) National River Flow Archive, Centre for Ecology and Hydrology. Available online at <<http://nrfa.ceh.ac.uk>>. [Accessed 04/04/2018].

## Appendix B

This appendix outlines the functional traits examined within this study.

Grouping feature	Trait	Grouping feature	Trait
Maximum potential size	$\leq 0.25\text{cm}$	Locomotion and substrate relation	Flier
	$>0.25- 0.5\text{cm}$		Surface swimmer
	$>0.5- 1\text{cm}$		Full water swimmer
	$>1- 2\text{cm}$		Crawler
	$> 2- 4 \text{ cm}$		Burrower
	$>4- 8\text{cm}$		Interstitial
	$>8\text{cm}$		Temporarily attached
Life-cycle duration	$\leq 1 \text{ year}$	Respiration method	Permanently attached
	$>1 \text{ year}$		Gill
Voltinism	$<1$		Plastron
	1		Spiracle
	$>1$		Hydrostatic vesicle
Aquatic stages	Egg		Tegument
	Larva	Food consumed	Microorganisms
	Nymph		Detritus $<1\text{mm}$
	Adult		Dead plant $\geq 1\text{mm}$
Reproduction strategy	Ovoviviparity		Living microphytes
	Isolated, free eggs		Living macrophytes
	Isolated, cemented eggs		Dead animal $\geq 1\text{mm}$
	Clutches, cemented		Living microinvertebrates
	Clutches, free		Living invertebrates
	Clutches, in vegetation		Vertebrates
	Clutches, terrestrial	Feeding group	Absorber
	Asexual		Deposit feeder
Dispersal strategy	Aquatic passive		Shredder
	Aquatic active		Scraper
	Aerial passive		Filter-feeder
	Aerial active		Piercer
Resistance form	Eggs/statoblasts		Predator
	Cocoons		Parasite
	Housings against desiccation		
	Diapause / dormancy		
	None		

## Appendix C

The following appendix outlines hydrological (Q – derived from historic discharge time series) and anthropogenic flow alteration (AF) indices. All indices were derived from flow time series extending 1-year prior to the date of each sampling event, except for mean average monthly values which were calculated from the 12-months prior to the beginning of the month of the sampling event. Asterisk denotes indices excluded from the PCA analyses. Flow regime component refers to those outlined within Richter *et al* (1996) and Poff *et al* (1997); M = Magnitude, F = Frequency; D = Duration, T = Timing and R = Rate of change.

**Table C1** – Descriptions of Q and AF indices examined within this study.

Flow regime component	Q index	Description (derived from historic discharge time series - m <sup>3</sup> /sec)	AF index	Description (derived from daily percentage differences between naturalised and historic discharge time series - %)
M,T	QJan*	Average January discharge	AFJan*	Average flow alteration in January
M,T	QFeb*	Average February discharge	AFFeb*	Average flow alteration in February
M,T	QMar*	Average March discharge	AFMar*	Average flow alteration in March
M,T	QApr*	Average April discharge	AFApr*	Average flow alteration in April
M,T	QMay	Average May discharge	AFMay	Average flow alteration in May
M,T	QJun	Average June discharge	AFJun	Average flow alteration in June
M,T	QJul	Average July discharge	AFJul	Average flow alteration in July
M,T	QAug	Average August discharge	AFAug	Average flow alteration in August
M,T	QSep	Average September discharge	AFSep	Average flow alteration in September
M,T	QOct	Average October discharge	AFOct	Average flow alteration in October
M,T	QNov	Average November discharge	AFNov	Average flow alteration in November
M,T	QDec	Average December discharge	AFDec	Average flow alteration in December
M,D	Q1Min	Minimum 1-day average discharge	AF1Min	Minimum 1-day average altered flows
M,D	Q3Min	Minimum 3-day average discharge	AF3Min	Minimum 3-day average altered flows
M,D	Q7Min	Minimum 7-day average discharge	AF7Min	Minimum 7-day average altered flows
M,D	Q30Min	Minimum 30-day average discharge	AF30Min	Minimum 30-day average altered flows
M,D	Q90Min	Minimum 90-day average discharge	AF90Min	Minimum 90-day average altered flows
M,D	Q1Max	Maximum 1-day average discharge	AF1Max	Maximum 1-day average altered flows
M,D	Q3Max	Maximum 3-day average discharge	AF3Max	Maximum 3-day average altered flows
M,D	Q7Max	Maximum 7-day average discharge	AF7Max	Maximum 7-day average altered flows

M,D	Q30Max	Maximum 30-day average discharge	AF30Max	Maximum 30-day average altered flows
M,D	Q90Max	Maximum 90-day average discharge	AF90Max	Maximum 90-day average altered flows
M,T	QJulianMin	Julian date of minimum discharge	AFJulianMin	Julian date of minimum altered flows
M,T	QJulianMax*	Julian date of maximum discharge	AFJulianMax	Julian date of maximum altered flows
M,F,D	QLPC*	Number of daily flow events <Q75	AFLPC*	Number of daily altered flow events <AF75
M,F,D	QLPD	Average number of days flow events <Q75	AFLPD	Average number of days flow events <AF75
M,F,D	QHPC*	Number of daily flow events >Q75	AFHPC*	Number of daily altered flow events >AF25
M,F,D	QHPD	Average number of days flow events >Q25	AFHPD	Average number of days flow events >AF25
F,R	QRises	Number of consecutive days flows increased	AFRises	Number of consecutive days altered flows increased
F,R	RR	Average rate of flow increase on consecutive days	AFRR	Average rate of altered flow increase on consecutive days
F,R	QFalls	Number of consecutive days flows decreased	AFFalls	Number of consecutive days altered flows decreased
F,R	FR	Average rate of flow decrease on consecutive days	AFFR	Average rate of altered flow decrease on consecutive days
M	QMean7	Average flow in the 7-days prior to sampling	AFMean7	Average altered flow in the 7-days prior to sampling
M	QMax7	Maximum flow in the 7-days prior to sampling	AFMax7	Maximum altered flow in the 7-days prior to sampling
M	QMin7	Minimum flow in the 7-days prior to sampling	AFMin7	Minimum altered flow in the 7-days prior to sampling
M	QMean30	Average flow in the 30-days prior to sampling	AFMean30	Average altered flow in the 30-days prior to sampling
M	QMax30	Maximum flow in the 30-days prior to sampling	AFMax30	Maximum altered flow in the 30-days prior to sampling
M	QMin30	Minimum flow in the 30-days prior to sampling	AFMin30	Minimum altered flow in the 30-days prior to sampling
M	QMean90	Average flow in the 90-days prior to sampling	AFMean90	Average altered flow in the 90-days prior to sampling
M	QMax90	Maximum flow in the 90-days prior to sampling	AFMax90	Maximum altered flow in the 90-days prior to sampling
M	QMin90	Minimum flow in the 90-days prior to sampling	AFMin90	Minimum altered flow in the 90-days prior to sampling
M	QMean180	Average flow in the 180-days prior to sampling	AFMean180	Average altered flow in the 180-days prior to sampling
M	QMax180	Maximum flow in the 180-days prior to sampling	AFMax180	Maximum altered flow in the 180-days prior to sampling
M	QMin180	Minimum flow in the 180-days prior to sampling	AFMin180	Minimum altered flow in the 180-days prior to sampling
M	Q10	Discharge exceeded 10% of the time	AF10	Altered flow value exceeded 10% of the time
M	Q95	Discharge exceed 95% of the time	AF95	Altered flow value exceed 95% of the time
M	Baseflow	QMin7 / Mean discharge	AFBaseflow	AFMin7 / Mean altered flow

## References

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## **Appendix D**

### *Introduction*

The main body of text describes an analytical framework outlining the statistical procedures undertaken to test invertebrate responses to different flow-related characteristics, Habitat Group (HG) and their interactive influence. The following appendix describes an alternative framework to test these statistical trends. We refer the reader to the main manuscript for the specific techniques and functions used to undertake the different analyses, and here provide details on the statistical structure and formatting used to carry out the analyses in this appendix.

### *Data analysis*

The axis score values of each sample were obtained from the Principal Component Analyses (PCAs) performed on both Q and AF indices to characterise the antecedent hydrological variability and anthropogenic flow alterations exposed to invertebrate communities, respectively. This was conducted to reduce the number of variables being tested within statistical models (compared to the additive influences of Q and AF indices) and reduce the potential biases associated with model overfitting. For this, axes 1-3 and 1-2 were utilised from PCAs examining Q (Q.PCA) and AF (AF.PCA) indices, respectively. Q.PCA axes 1-3 accounted for 79.2% of the total statistical variation, while AF.PCA axes 1-2 explained 75.2% of the statistical variation. PCA axis scores were utilised as fixed-effects within ‘Linear Mixed-effect Models’ (LMMs). Subsequently, 8 LMMs were established for each of the 7 community response metrics, the structure and rationale of these are outlined in Table D1.

**Table D1** – The format and rationale of the statistical models (LMMs) used to test invertebrate responses to different flow-related characteristics, Habitat Group (HG) and their interactive statistical influence. Q = hydrological variability; AF = anthropogenic flow alterations.

Model number	Respective null model tested against	Variables used as fixed-effects	Rationale
(i)	n/a	Site x Season	Used as a null model to account for differences between reaches (spatial) and seasons (temporal) – i.e. variables not directly tested within this study.
(ii)	(i)	Q.PCA1 + Q.PCA2 + Q.PCA3	Testing invertebrate responses to Q. When compared against (i), it tests whether the influence Q differs from spatial and temporal variations in ecological responses.
(iii)	(ii)	HG x (Q.PCA1 + Q.PCA2 + Q.PCA3)	Testing invertebrate responses to the interaction between Q within each HG. When compared to (ii), it tests whether the ecological influences of Q differ between HGs.
(iv)	(i)	AF.PCA1 + AF.PCA2	Testing invertebrate responses to AF. When compared against (i), it tests whether the influence AF differs from spatial and temporal variations in ecological responses.
(v)	(iv)	HG x (AF.PCA1 + AF.PCA2)	Testing invertebrate responses to the interaction between AF within each HG. When compared to (iv), it tests whether the ecological influences of AF differ between HGs.
(vi)	(i)	Site x Season, HG	Testing invertebrate responses to the influence of HG nested within each specific survey. When compared against (i), it tests whether the influence of HG (within each survey) differs from spatial and temporal variations in ecological responses.
(vii)	(i)	Site x Season, Froude	Testing invertebrate responses to the influence of Froude nested within each specific survey. When compared against (i), it tests whether the influence Froude (within each survey) differs from spatial and temporal variations in ecological responses.
(viii)	(vii)	Site x Season, (HG x Froude)	Testing invertebrate responses to the interactive influence between HG and Froude nested within each specific survey. When compared against (vii), it tests whether the ecological influence of Froude differs between HGs.

## Results and interpretation

The results of the LMMs performed within this appendix are displayed within Table D2.

**Table D2** – Statistical outputs from LMMs. Colours indicate the environment variables being tested: blue = hydrological variability (PCA axis scores derived from Q indices); red = anthropogenic flow alterations (PCA axis scores derived from AF indices); green = HG and yellow = Froude. See Table D1 for further details on the structure of the statistical models.

Response	Model number	r <sup>2</sup> m	AIC	X <sup>2</sup>	p-value	Response	Model number	r <sup>2</sup> m	AIC	X <sup>2</sup>	p-value
Abundance	(i)	0.37	317.03	-	-	%EPT	(i)	0.35	1889.4	-	-
	(ii)	0.21	311.99	48.96	0.020*		(ii)	0.03	1915.3	79.88	<0.001***
	(iii)	0.53	198.56	129.44	<0.001***		(iii)	0.21	1878.9	52.33	<0.001***
	(iv)	0.01	320.36	59.33	<0.001***		(iv)	0.01	1918.1	84.75	<0.001***
	(v)	0.39	208.24	124.12	<0.001***		(v)	0.18	1885	45.1	<0.001***
	(vi)	0.63	194.4	126.63	<0.001***		(vi)	0.47	1845	48.43	<0.001***
	(vii)	0.57	230.54	88.49	<0.001***		(vii)	0.46	1846.6	44.8	<0.001***
	(viii)	0.62	206.64	27.9	<0.001***		(viii)	0.49	1837.7	12.89	0.002**
TaxRic	(i)	0.33	1448.60	-	-	FRic	(i)	0.17	643.58	-	-
	(ii)	0.05	1463.50	68.87	<0.001***		(ii)	0.04	620.21	30.63	0.287(NS)
	(iii)	0.38	1341.40	138.11	<0.001***		(iii)	0.40	533.83	102.38	<0.001***
	(iv)	0.02	1463.10	70.52	<0.001***		(iv)	0.02	622.93	35.35	0.160(NS)
	(v)	0.39	1350.20	124.89	<0.001***		(v)	0.40	530.15	104.78	<0.001***
	(vi)	0.63	1312.00	140.57	<0.001***		(vi)	0.50	535.07	112.5	<0.001***
	(vii)	0.58	1343.10	107.52	<0.001***		(vii)	0.36	589.86	55.72	<0.001***
	(viii)	0.63	1317.20	29.91	<0.001***		(viii)	0.39	582.3	11.56	0.003**
TaxDiv	(i)	0.16	915.13	-	-	FEve	(i)	0.38	-416.09	-	-
	(ii)	0.00	901.23	40.1	0.050(NS)		(ii)	0.17	-408.32	61.77	<0.001***
	(iii)	0.30	834.95	82.27	<0.001***		(iii)	0.38	-454.8	62.48	<0.001***
	(iv)	0.00	899.44	40.31	0.062(NS)		(iv)	0.13	-409.02	63.07	<0.001***
	(v)	0.18	866.52	44.93	<0.001***		(v)	0.31	-455.25	58.23	<0.001***
	(vi)	0.31	871.55	47.58	<0.001***		(vi)	0.54	-478.61	66.52	<0.001***
	(vii)	0.22	899.08	18.05	<0.001***		(vii)	0.47	-449.44	35.35	<0.001***
	(viii)	0.30	877.22	25.86	<0.001***		(viii)	0.51	-462.8	17.35	<0.001***
Berger Parker	(i)	0.21	-146.82	-	-						
	(ii)	0.01	-147.60	53.22	0.002**						
	(iii)	0.25	-197.20	65.60	<0.001***						
	(iv)	0.01	-148.62	54.20	0.002**						
	(v)	0.13	-167.00	30.38	<0.001***						
	(vi)	0.33	-180.12	37.30	<0.001***						
	(vii)	0.26	-158.24	13.42	<0.001***						
	(viii)	0.32	-174.13	19.89	<0.001***						

The results displayed in Table D2 strongly support the findings described within the main body of text. Firstly, models (ii) and (iv) consistently exhibit a much weaker statistical power (i.e. lower r<sup>2</sup>m and higher AIC) compared to the null model (i) (testing for ecological differences between reaches and seasons). This highlights that this study could not detect a strong statistical signature for individual influences of hydrological variability (Q) and anthropogenic flow alterations (AF). However, incorporating a HG interaction terms significantly improved model fits testing the influence of Q and AF, indicating habitat-specific hydrological and flow alteration influences on invertebrate communities. Table

D2 also highlights that HG and Froude consistently yielded a significant influence on all invertebrate community response metrics and explained the highest amounts of statistical variation; with the former exhibiting the strongest statistical trends. Finally, community responses to Froude always improved significantly when accounting for its interaction with HG, highlighting how hydraulic influences on invertebrate communities differed between habitat patches. The implications of these findings are discussed within the main body of text.

## Appendix E

The following appendix presents results from indicator species analysis performed on invertebrate communities inhabiting distinct habitat groups (HGs – see the main text for a full description). For this, a group-equalized ‘Indicator Value’ (IndVal) analysis was conducted *via* the ‘*multipatt*’ function in the ‘*indicspecies*’ package (De Caceres and Jansen, 2015) and performed across 999 permutations to determine its significance.

**Table E1** – Indicator species of different HGs. IV = Indicator value.

Taxa	IV	<i>p</i> -value	Taxa	IV	<i>p</i> -value
<u><b>Fine sediment</b></u>			<u><b>Ranunculus sp. (continued)</b></u>		
<i>Pisidium</i> sp.	0.52	0.014*	Elmis aenea (larvae)	0.77	0.001***
Ostracoda	0.44	0.001***	Elmis aenea (adult)	0.74	0.001***
<i>Mystacides</i> sp.	0.27	0.01**	<i>Gammarus pulex</i>	0.70	0.001***
Dytiscidae larvae	0.26	0.022*	<i>Rhyacophila dorsalis</i>	0.61	0.001***
<u><b>Coarse substrate</b></u>			<i>Hydropsyche siltalai</i>	0.59	0.001***
<i>Limnius volckmari</i> (larvae)	0.77	0.001***	Hydra	0.59	0.001***
<i>Dicranota</i> sp.	0.66	0.001***	<i>Hydropsyche pellicidula</i>	0.50	0.001***
<i>Agapetus fuscipes</i>	0.66	0.001***	<i>Hydropsyche angustipennis</i>	0.48	0.001***
<i>Caenis</i> sp.	0.51	0.014*	<i>Brachycentrus subnubilis</i>	0.48	0.001***
<i>Silo</i> sp.	0.46	0.001***	<i>Lepidostoma hirtum</i>	0.42	0.002**
<i>Leuctra</i> sp.	0.45	0.009**	<i>Oulimnius</i> sp. (adult)	0.42	0.001***
<i>Ancylus fluviatilis</i>	0.42	0.001***	<i>Oulimnius</i> sp. (larvae)	0.42	0.03*
<i>Limnius volckmari</i> (adult)	0.32	0.036*	<i>Paraleptophlebia submarginata</i>	0.41	0.002**
<i>Polycelis nigra/tenius</i>	0.30	0.049*	<i>Erpobdella octoculata</i>	0.35	0.003**
<u><b>Ranunculus sp.</b></u>			<i>Piscicola geometra</i>	0.34	0.007**
Simuliidae	0.93	0.001***	<i>Lymnaea peregra</i>	0.32	0.017*
Chironomidae	0.85	0.001***	<i>Limnephilus lunatus</i>	0.30	0.048*
Hydracarina	0.83	0.001***	<i>Hydroptila</i> sp.	0.29	0.006**
<i>Baetis</i> sp.	0.82	0.001***	<i>Physa fontinalis</i>	0.27	0.011*
<i>Seratella ignita</i>	0.80	0.001***	<i>Calopteryx splendens</i>	0.24	0.02*

## References

De Caceres, M. and Jansen, F. (2015). Package “indicspecies” Relationship Between Species and Groups of Sites Version 1.7.5, 1–31.