- **Development of a biotic index of stream macroinvertebrates to**
- 2 deposited fine-grained sediment
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- 4 John F. Murphy¹, Jones, J. Iwan^{1*}, Pretty, James L.¹, Duerdoth, Chas P.¹,
- 5 Hawczak, Adrianna^{1†}, Arnold, Amanda¹, Blackburn, John H.¹, Naden, Pamela
- 6 S.², Old, Gareth², Sear, David A.³, Hornby, Duncan⁴, Clarke, RalphT.² and
- 7 Collins, Adrian L.⁵
- 8
- ¹ School of Biological and Chemical Sciences, Queen Mary University of London, Mile End
 Road, London, E1 4NS, UK.
- ² Centre for Ecology and Hydrology, Maclean Building, Benson Lane, Crowmarsh Gifford,
 Wallingford, Oxfordshire, OX10 8BB, UK.
- ³ Geography and Environment, University of Southampton, Highfield, Southampton, SO17
 1BJ, UK.
- ⁴ GeoData Institute, University of Southampton, Highfield, Southampton, SO17 1BJ, UK.
- ⁵ Sustainable Soils and Grassland Systems Department, Rothamsted Research, North
 Wyke, Okehampton, Devon, EX20 2SB, UK.
- [†] present address: Freshwater Habitats Trust, North Place, Headington, Oxford, OX3 9HY,
 UK.
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- 24 *Corresponding Author:
- J. Iwan Jones, School of Biological and Chemical Sciences, QMUL, The River Laboratory,
- East Stoke, Wareham, BH20 6BB, UK. j.i.jones@qmul.ac.uk

1 Abstract

- Detrimental impacts of excessive fine-grained sediment inputs to streams and rivers
 are well established. What is less well understood is the susceptibility of different
 elements of the freshwater biota to such perturbations and how such knowledge of
 their susceptibility could aid in identifying where excessive fine-grained sediment is
 impairing ecological condition.
- Following the collection of biological and sediment data from 179 streams across
 England and Wales, representative of a range of river types over a gradient of fine
 sediment loading, objective statistical approaches were applied to establish
 relationships between the macroinvertebrate assemblage and fine-grained sediment
 inputs to river channels.
- Having factored out that portion of the biological variation associated with natural environmental gradients, a model comprising mass of organic sediment in erosional areas of the stream bed (predominantly associated with the first axis of the partial canonical correspondence analysis (pCCA)), and mass of fine-grained sediment in the surface drape of depositional areas and % organic content in erosional areas (associated with the second axis of the pCCA) as explanatory variables best accounted for the residual variation in the macroinvertebrate assemblage.
- The relative position of taxa along both axes of the pCCA, provided a ranking of taxa
 in relation to the two gradients of fine-grained sediment and provided the basis for a
 new empirically-derived diagnostic index for fine-grained sediment stress in rivers.
 Two sub-indices were derived to capture the assemblage responses to both the
- gradient of organic sediment in erosional areas and the gradient of total fines in
 depositional areas. The two sub-indices were then combined to derive the new
 combined fine sediment index (CoFSI_{sp}).
- 5. The index was tested on an independent test dataset (comprising 127 samples from 83 sites) and was found to provide a robust indication of benthic fine-grained sediment conditions (Spearman rank correlations $\rho = -0.519$ to -0.703). The strength of correlation with the total fine-grained sediment gradient was always greater than that for other routinely used indices, confirming that CoFSI_{sp} offered additional explanatory power when assessing this stressor of aquatic environments.

1 Introduction

2 While, historically, organic pollution from domestic sewage was considered the dominant 3 threat to water quality, recent decades have seen a drive to assess and manage the impact of a wider variety of stressors that affect the ecological condition of freshwaters (Jones et al., 4 5 2010). In Europe, for instance, much of this work has been driven by the over-arching water 6 management policy embodied in the Water Framework Directive (WFD; European 7 Parliament, 2000). It has long been noted that excessive amounts of fine-grained sediment 8 (defined here as mineral and organic particles < 2 mm) can have a detrimental effect on 9 aquatic ecosystems (e.g. Ellis, 1936, Waters, 1995). Although the delivery of fine-grained sediment to rivers, and its retention and transport downstream, are natural and essential 10 processes, the consequences of disruption to these processes are multifaceted. Recent 11 decades have seen an increase in sediment loading to freshwaters, threatening the integrity 12 of these ecosystems and the services they provide (Foster et al., 2011). The increase has 13 largely come from agricultural land where more intensive land management practices lead to 14 elevated levels of delivery to watercourses (Zhang et al., 2014). The challenge for society is 15 16 to balance the necessity for increased food production with the maintenance of freshwater ecosystem integrity (Tilman et al., 2011, Quinn et al., 2013). 17

18 A sound evidence base is therefore critical to understanding the impact of excessive finegrained sediment on stream biota, particularly as the biological impact of fine sediment is 19 likely to be a function of its source, quantity, rate and timing of delivery and retention, as well 20 as the susceptibility of the resident biota to any impact. Fine-grained sediment influences all 21 22 components of the biological community of freshwaters (Collins et al., 2011, Kemp et al., 23 2011, Jones et al., 2012a, Jones et al., 2013), and thus has both direct and indirect impacts 24 on the macroinvertebrate assemblage (Jones et al., 2012b). Different components of the macroinvertebrate assemblage are likely to respond to different aspects of the sediment 25 26 pressure as, for example, certain taxa are likely to be susceptible to the chemical changes 27 associated with the amount of organic matter deposited on the river bed (Von Bertrab et al., 28 2013), whereas others may be more susceptible to the physical impacts of mineral finegrained sediment (Townsend, Uhlmann & Matthaei, 2008). The response of 29 macroinvertebrates to the oxygen stress associated with organic matter are well 30 documented, with a particular focus on sewage effluent (Walley & Hawkes, 1996; Walley & 31 Trigg, 1997; Jones et al., 2009), although taxa are unlikely to distinguish between the 32 various sources of organic matter that cause such oxygen stress. Certain macroinvertebrate 33 taxa are likely to be susceptible to abrasion from mineral particles either saltating or 34 suspended in the flow, which could cause dislodgement or damage to their body parts (Culp 35 36 et al., 1986). Furthermore, community composition may respond to changes in habitat

1 availability induced both directly or indirectly (e.g. through changes in the availability of 2 macrophyte habitat) by increased fine-grained sediment inputs (Pardo & Armitage, 1997). 3 Notwithstanding these complexities, there have been a number of previous attempts to use 4 the quantified or assumed assemblage response of macroinvertebrates to deposited finegrained sediment stress to derive diagnostic biotic indices (e.g. Zweig & Rabeni, 2001; 5 6 Relyea, Minshall & Danehy, 2012) including the recently-developed Proportion of Sediment-7 sensitive Invertebrates (PSI) index developed for UK fauna (Extence et al., 2013). PSI was 8 developed by assigning taxa to one of four sensitivity groups based on an expert review of 9 existing literature and an assessment of biological traits. The index works by producing an abundance-weighted proportion of fine sediment-sensitive taxa present in a sample as an 10 indication of the extent of fine sediment cover on the stream bed. A subsequent evaluation 11 of the relationship between PSI and visually estimated percent cover of fines (sand, silt and 12 clay) on a spatially-extensive dataset found a significant negative relationship (Turley et al., 13 2014). However, the authors noted large variances around the relationship, especially at the 14 15 high-stress end of the gradient, which limited its ability to indicate fine sediment conditions effectively. They suggested that visual estimates of fine sediment cover are perhaps an 16 inadequate measure of the stressor. 17

Deciding the best approach to quantifying the pressure from fine-grained sediment is 18 19 complicated. To date there is no consensus as to which aspect(s) of fine sediment the biota respond to and, hence, which is the most appropriate measure of fine sediment to quantify 20 this pressure (Collins & Anthony, 2008, Collins et al., 2011). Von Bertrab et al. (2013) have 21 22 shown that the chemical composition of the deposited fine sediment can be more important to biota than just the quantity of deposited material on/in the stream bed. Turley et al. (2014) 23 also advocated that our understanding of the effects of fine sediment on river biota would be 24 improved by a more objective, qualitative reach-scale measure, incorporating particle size 25 and geochemical composition. 26

As the scale of investigations into the impact of fine-grained sediment on biota can influence 27 the outcome (Larsen, Vaughan & Ormerod, 2009; Jones et al., 2012b), evidence must be 28 29 acquired at an appropriate scale to determine the outcome of the various potential 30 responses. Since the management of both rivers and fine-grained sediment run-off must eventually take place at the reach or sub-catchment scale (Collins & Anthony, 2008, Collins 31 32 et al., 2011), it is at this scale that investigations must take place. Investigations at this scale 33 avoid the difficulties associated with extrapolating from the patch to the reach scale that have hampered previous works (e.g. Larsen et al., 2009). Critically, appropriate data that 34 describe the extent of disturbance from fine-grained sediment on rivers, particularly sediment 35 36 derived from agricultural activity, and the response of the macroinvertebrate assemblage at

- 1 the catchment scale, do not exist. Previous assumptions of the response of
- 2 macroinvertebrates to fine-grained sediment have been derived from expert opinion based
- 3 on smaller scale experiments and case studies, which can be contradictory when compared
- 4 across scales (Jones *et al.*, 2012b).

5 To address this gap, it has been necessary to collect new data in a structured manner, 6 where potentially confounding impacts are controlled, in order to establish relationships 7 between the macroinvertebrate assemblage and fine-grained sediment pressure. To avoid the potential pitfalls of expert opinion (Walley & Hawkes, 1996), objective statistical 8 9 approaches have been used to derive new relationships. As the mechanism(s) by which fine-grained sediment affects macroinvertebrates are not known at the reach/sub-catchment 10 scale, various measures of delivery and retention of fine-grained sediment have been 11 applied, with the response of the biota determining which is the most appropriate. In 12 addition, the ranking of biota according to their relative sensitivity to fine-grained sediment 13 deposition provides the basis for a diagnostic biotic index. The objectives of this study were 14 to: (i) obtain robust evidence of the impact of fine-grained sediment on aquatic invertebrate 15 communities at an appropriate management scale; (ii) develop a diagnostic biotic index 16 based on this newly-established relationship and (ii) test the performance of the new index 17 18 on an independent dataset

1 Methods

2 Site selection

3 To achieve the study objectives required an assessment of the macroinvertebrate

- 4 assemblage in a sample of replicate streams representative of a range of river types over a
- 5 gradient of pressure from fine sediment sources (the calibration dataset). As agriculture is
- 6 by far the main anthropogenic source of fine sediment being delivered to watercourses
- 7 (Collins & Anthony, 2008; Zhang *et al.*, 2014) we focussed our current study on rural
- 8 streams. A series of catchment-scale filtering criteria were used to identify a pool of
- 9 potential sites to be surveyed, such that the sites selected: (i) were representative of a range
- 10 of river types; (ii) were experiencing a wide range of fine-grained sediment loading; (ii) were
- 11 not affected by confounding disturbances and (iv) where they were experiencing fine-grained
- 12 sediment pressure, this was primarily from agricultural sources.
- 13 Environmental details for 12,447 stream sites across England and Wales were extracted
- 14 from the Environment Agency River Habitat Survey database (Raven *et al.*, 1997). A
- catchment shape file was derived for each site from GIS, and the modelled total fine-grained
- 16 sediment load characteristics were derived from a combination of national layers and outputs
- 17 (Collins & Anthony, 2008) including PSYCHIC (Phosphorus and Sediment Yield
- 18 CHaracterisation In Catchments), a process-based model of fine-grained sediment
- 19 mobilisation in surface run-off or drain flow from agricultural land and subsequent delivery to
- watercourses (Collins *et al.*, 2007; Davison *et al.* 2008; Stromqvist *et al.*, 2008; Collins *et al.*,
- 21 2009a,b). Using the modelled estimates of cross sector total fine-grained sediment inputs
- 22 (Collins & Anthony, 2008; Collins *et al.*, 2009a,b) from agriculture, diffuse urban areas,
- eroding channel banks and sewage treatment works (STWs), sites were rejected that were
- 24 downstream of: (i) major STWs or had monitored STW sediment inputs from their catchment
- $> 0.5 \text{ kg ha}^{-1} \text{ yr}^{-1}$; (ii) lakes/reservoirs or (iii) urban areas or had modelled diffuse urban
- sediment inputs > 2.0 kg ha⁻¹ yr¹. This filtering reduced the original 12,447 sites to 2,610.
- 27 To focus our survey effort on those sites where fine-grained sediment inputs were dominated
- by agricultural sources, a threshold was set at 75% for the proportion of the total sediment
- input (kg ha⁻¹ yr⁻¹; Collins & Anthony, 2008; Collins *et al.*, 2009a,b) that was from agriculture.
- 30 Once those sites that failed to achieve this threshold had been removed, 1,800 potential
- 31 survey sites remained. To establish a range of fine-grained sediment pressures, each of the
- 32 1,800 sites was assigned to one of six sediment pressure categories based on their
- 33 modelled total sediment inputs (Table 1).
- To ensure that the sampled invertebrate communities came from as wide a range of natural river types as possible, within the limits set by the other site selection criteria, each site was

1 allocated to one of four approximate stream types based on four map-based physical 2 variables, namely catchment geology, distance from source (km), altitude (m a.s.l.) and river 3 slope (m km⁻¹). The boundary values for this guideline stream typology were loosely based on the physical characteristics associated with the seven RIVPACS IV super end groups 4 summarising the range of biological river types found in the UK (Davy-Bowker et al. 2008; 5 Table S1 in Supplementary Material). The fundamental aim was to aid the selection of sites 6 7 to be visited during the field survey such that, as far as possible, there was equal sampling effort across the gradient of fine-grained sediment stress for each broad stream type. Thus, 8 9 by ensuring that a representative sample of streams was included in the study where fine 10 sediment pressure was the main difference among sites, we could better attribute any difference in species occurrence to the effects of pressure from fine-grained sediment rather 11 than other site differences or uneven sampling effort. 12

The selection procedure produced a matrix of 24 stream/sediment pressure types from 13 which sites to be sampled were selected (Table S2 in Supplementary Material). To ensure 14 that all sampled sites were on independent watercourses, given a choice of sites within the 15 same watercourse the site that was furthest downstream was selected, although sites were 16 preferentially selected if they were of stream/sediment pressure types that were not well 17 represented in the dataset, i.e. stream types 3 and 5 (Table S2). The resultant matrix 18 19 included a pool of 568 independent sites that potentially could be sampled. Sites were selected from the pool of 568 potential sites to give, as far as possible, an even distribution 20 21 of sites across the range of sediment pressure within each river type. Sample collection was 22 distributed over the spring and autumn of 2010 and 2011, with the sites distributed over the 23 two years of sampling in a stratified random manner. Within each period, efforts were made 24 to distribute the sites sampled evenly both across the site selection matrix (Table S2) and 25 geographically (Fig. 1).

- Data from 179 sites (those sampled in spring 2010, autumn 2010 and spring 2011) formed
 the calibration dataset from which a new diagnostic biotic index was developed. Data from a
- further 26 sites (sampled in autumn 2011) were retained to form part of an independent test
- 29 dataset (more details below). Each site was visited once, and a sample of the
- 30 macroinvertebrate assemblage and deposited sediment was collected.

31 Macroinvertebrate sampling

- 32 The macroinvertebrates were sampled using the RIVPACS method (Furse *et al.* 1981;
- 33 Murray-Bligh *et al.* 1997), which comprised a standard three-minute kick/sweep and one
- 34 minute search sample with a pond net (1 mm mesh-size). Samples were preserved with 4%
- 35 formaldehyde and returned to the laboratory for subsequent identification and quantification

1 to the lowest practicable taxonomic level. Prior to data analysis the taxonomic resolution of

2 the full macroinvertebrate dataset was standardised to ensure that it only contained discrete

- 3 taxa (as described in Chinnayakanahalli *et al.*, 2011). Before collecting the invertebrate
- 4 sample, a spot sample of water chemistry was determined using a daily-calibrated dip probe
- 5 for pH and conductivity (Hanna Instruments Combo HI98129, Leighton Buzzard,
- 6 Bedfordshire, UK). Associated RIVPACS environmental variables were recorded either at
- 7 the site (stream width and depth, velocity, substratum composition), or from map-based data
- 8 (discharge category, altitude, distance from source and slope; Murray-Bligh *et al.*, 1997).

9 Fine-grained sediment sampling

10 Fine-grained sediment deposits on the stream bed were quantified immediately upstream of 11 the macroinvertebrate sampling area using the sediment re-suspension technique described in Duerdoth et al. (2015) and adapted from Collins and Walling (2007a,b). At each site, 12 areas with either a propensity to erode or to deposit fine-grained sediment were identified 13 within the main channel, thus representing the extremes of the range of fine-grained 14 15 sediment retention. In broad terms, patches with a propensity to erode fine sediment (hereafter erosional) were defined as those higher velocity areas in or close to the thalweg, 16 whereas patches with a propensity to deposit fine sediment (hereafter depositional) were in 17 eddies or areas of lower flow velocity such as pools or backwaters. To sample the deposited 18 19 sediment, an open-ended, stainless steel cylinder (height 75 cm, diameter 48.5 cm) was 20 inserted at least 10 cm into undisturbed patches of each type. Once in position, the depth of water within the cylinder was measured. Water within the cylinder was then vigorously 21 22 agitated for 60 seconds with an auger without touching the river bed, but sufficient to bring 23 fine-grained sediment from the surface of the bed into suspension. The water and suspended sediment was then immediately sampled by plunging an inverted 50 ml vial to 24 the bottom of the cylinder which then filled as it was turned upright and brought to the 25 surface. Subsequently, a further 60 seconds of agitation was undertaken, this time including 26 an initial 30 seconds of digging/stirring the top 10 cm of the bed substratum with the auger to 27 raise any sub-surface/interstitial fine-grained sediment into suspension. Again, immediately 28 29 following agitation, a sample of the suspended material was collected by drawing an inverted 30 50 ml vial up through the water column. In this way it was possible to collect samples of both the surface and the total (i.e. combined surface and sub-surface) deposited fine-grained 31 32 sediment from the patch. Four such sets of water samples (surface, and combined surface 33 and subsurface) were collected from each site, two from erosional patches and two from depositional patches. The samples were refrigerated and kept in the dark, and returned to 34 the laboratory within five days, where each 50 ml sample was independently processed for 35 36 dry mass and organic content. Our focus was on the fine-grained (< 2mm) fraction so the

1 samples were passed through a 2 mm sieve prior to filtration using pre-ashed, washed and 2 dried 90 mm Whatman Glass Microfibre GF/C filters. The filtered samples were then dried in 3 a pre-heated oven at 105 °C overnight and cooled in a desiccator for 1 hr before weighing. They were then ashed in a pre-heated muffle furnace at 500° C for 30 minutes and cooled in 4 5 a desiccator for one hour before weighing. The mass of organic matter (volatile sediment) 6 was calculated by subtraction of non-volatile fine sediment mass from fine sediment mass. The depth of water within the stilling well was used to convert the laboratory weights to a 7 mass of fine-grained sediment per m² of river bed sampled. A reach scale average for 8 9 surface and total deposited fine sediment was derived using the geometric mean of the four 10 sampled patches (two erosional and two depositional patches) collected at each site. Similarly, an erosional average and a depositional average were calculated from the two 11 12 sampled patches in each habitat type. Recent research has confirmed that this resuspension technique performs as well as visual estimates of fine sediment cover in its 13 14 ability to discriminate between rivers but, unlike visual estimates, is not affected by operator bias and provides an objective quantification of both surface and total fine-grained sediment 15 16 (Duerdoth et al., 2015).

17 Index development

The specific objective of the field survey was to quantify the association between variation in the macroinvertebrate assemblage and the fine-grained sediment stressor gradient having

20 first factored out that portion of the biological variation correlated with natural background

variation between streams. From such an analysis, the relative sensitivity of a range of

22 macroinvertebrates to fine-grained sediment stress could be quantified and would form the

23 empirical basis for a new diagnostic biotic index.

24 Multivariate ordination was used to first quantify variation in the macroinvertebrate

assemblage, and then to determine which set of natural environmental variables best

described the pattern (see Table S3 in Supplementary Material). Of the 313 taxa recorded

in the calibration dataset, 208 occurred in fewer than 10% of samples and therefore were

28 excluded to ensure that inferences about sensitivities to fine-grained sediment were based

29 on a reasonable number (>18) of replicate occurrences.

30 Canonical Correspondence Analysis (CCA) was used to relate variation in the biotic data

31 with seven natural environmental variables: discharge category, catchment area, slope,

32 altitude, distance from source, surface velocity and local channel bank erosion fine-grained

33 sediment inputs (Table S3; Collins & Anthony, 2008; Collins *et al.*, 2009a,b). The CCA was

34 undertaken with Hill's scaling of ordination scores, with focus on inter-species distances, and

manual forward selection (n = 999 permutations, P < 0.01 as the significance threshold for

1 inclusion in the model) to determine the optimal subset of variables that accounted for the

2 natural gradients in the sampled macroinvertebrate assemblage.

3 Variables with excessive co-linearity (inflation factor > 6) with other more powerful predictor

4 variables were also excluded. Macroinvertebrate abundance data were log (x+1)

5 transformed prior to analysis to reduce the influence of dominant taxa. Environmental

6 variables were also transformed where necessary; to either normalise their distributions or to

7 ensure that relative changes in their value were more biologically meaningful (Table S3).

8 A partial CCA (pCCA) was then carried out with those variables selected in the previous

9 CCA as co-variables in the analysis. Residual variation in the sampled macroinvertebrate

10 assemblage, having factored out that associated with the co-variables, was then related to

11 the 27 measured and modelled fine-grained sediment variables, with the forward selection

12 procedure (n = 999 permutations, P < 0.01 as the significance threshold for inclusion in the

13 model and inflation factor <6) again being used to derive the most parsimonious explanatory

14 model. The relative position of taxa (their pCCA species scores, which indicate the centre of

their distribution along the axes of the pCCA ordination space) provided a robust ranking and

16 basis for the development of a new diagnostic biotic index. Species scores were converted

to a percentage of the range of species scores along an axis (% *Dist*) where:

18

 $\% Dist = \frac{(Highest species score-Species score)}{(Highest species score-Lowest species score)} \times 100$ (1)

19 The % Dist values were then categorised into 10-percentile bands such that each taxon was

assigned an index score of zero (100%) to 10 (0-9%), where zero was the most sediment-

21 tolerant taxon and 10 was the most sediment-sensitive taxon. All ordinations were

22 undertaken using CANOCO 4.5 software (ter Braak & Šmilauer, 2002).

23 Independent testing

24 An independent test dataset was compiled from 26 samples retained from the current survey and 101 samples from 57 stream sites in Wales (Fig. 1), sampled between 2009 and 2011 25 26 as part of a study investigating the environmental impacts of agri-environment schemes 27 (Anthony et al. 2012), where the macroinvertebrate assemblage and deposited fine-grained 28 sediment were sampled, using the same methodology as described above, in spring and autumn at 44 sites and in only one of the two seasons at 13 sites. Modelled fine-grained 29 30 sediment inputs from the catchment were also derived for the 57 sites using the cross sector layers cited above. We correlated (Spearman rank correlation) the calculated scores for the 31 new diagnostic index against modelled total and agricultural fine-grained sediment inputs, 32 33 total reach-scale fine-grained sediment mass, total reach-scale organic sediment mass and 34 organic sediment mass in erosional areas (the latter three averaged across seasons and

1 with season considered separately) to test its relationship with the fine-grained sediment

- 2 stress gradient. These five variables were chosen from the large number of measured
- 3 sediment variables (Table S3) to represent the fine-grained sediment stress gradient to
- 4 reduce the chance of finding spurious significant relationships when repeatedly relating
- 5 many variables to one another. These five variables were judged most likely to capture the
- 6 key aspects of the stressor gradient. Furthermore, we assessed the performance of the new
- 7 index relative to six other established biotic indices to determine whether it offered additional
- 8 explanatory power. The six established indices were: number of BMWP-scoring taxa
- 9 present (NTAXA), average-BMWP score of scoring taxa present (ASPT), family-level and
- 10 species-level lotic invertebrate flow index (LIFE_{fam} and LIFE_{sp}; Extence, Balbi & Chadd,
- 11 1999), family-level and species-level PSI (PSI_{fam} and PSI_{sp}; Extence *et al.*, 2013).

As well as correlating various measures of the fine-grained sediment stress gradient against 12 calculated index scores, we also correlated them against the ecological quality index (EQI) 13 of each, where the observed score is presented as a ratio relative to the value expected for 14 15 that site were it not impacted by anthropogenic stress. This is the routine format in which all bioassessment indices are applied to assess ecological condition in compliance with the EU 16 WFD. In this way, comparisons of index values across watercourses of different 17 18 environmental character are possible, as the confounding influence of natural background variability is factored out (Clarke et al., 2003). Ordinarily, for any stream site the standard 19 UK WFD-compliant River Invertebrate Classification Tool (RICT) (Davy-Bowker et al., 2008) 20 21 predicts the reference value for a given biotic index based on physicochemical 22 characteristics of the stream site (stream width, depth, substratum composition, average 23 annual stream discharge category, altitude, slope, distance from source, average alkalinity 24 and average temperature conditions at the site). This 'expected' value for the biotic index is 25 compared with the observed value from a macroinvertebrate sample taken at the site and 26 the ratio of the two (observed:expected or ecological quality index (EQI)) gives an indication of the biological condition of the site (Murphy & Davy-Bowker, 2006). However, when 27 assessing fine-grained sediment stress in streams, the RICT prediction of the 'expected' 28 29 value needs to be generated without the use of environmental variables likely to be affected 30 by the stressor. In this case, substratum composition, width and depth are likely to be linked to sediment stress (the latter two through their influence of water velocity and hence 31 32 propensity for fine-grained sediment to deposit or erode) and were removed from the RICT model. EQIs were therefore calculated for the new index and the LIFE and PSI indices for 33 the 127 independent test samples using a modified version of the RICT model where 34 35 predictions were not influenced by stress from fine-grained sediment (Clarke et al., 2011). EQIs for NTAXA and ASPT were calculated using the standard version of RICT. 36

- 1 As we assessed the statistical significance of 210 rank correlations, of which 10 would be
- found to be significant by chance at α = 0.05, we corrected for the family-wise error rate
- 3 using the Holm-Bonferroni method (Holm, 1979) to reduce the chance of Type I errors.

1 Results

2 Index development

3 In total, 205 stream sites were sampled over the spring and autumn of 2010 and 2011 (Fig.

4 1, see Supplementary Material for site details), from which 326 taxa were identified to the

5 most resolved taxonomic level possible; this was most often at species or genus level, but

6 for some of the groups that were more difficult to identify it was sub-family, family or order

7 level (see Supplementary Material for full taxon list).

8 An initial detrended correspondence analysis (DCA) on the calibration dataset (n=179) found

9 that taxa turnover (a measure of change in taxonomic composition across the calibration

10 dataset) was sufficiently great (DCA axis 1 gradient length = 3.16) to meet the unimodal

11 response assumption of CCA (ter Braak, 1995). The initial CCA found that a model

12 incorporating catchment area, slope, altitude, distance from source, surface velocity and

13 local bank erosion fine-grained sediment inputs best explained the natural and non-

14 agriculture-related background variation in the dataset.

15 A subsequent pCCA with these six variables included as co-variables found that a model comprising mass of organic sediment in erosional areas, mass of fine sediment in the 16 surface drape of depositional areas and % organic content in erosional areas as explanatory 17 variables best accounted for the residual biological variation (see Fig. S1 in Supplementary 18 Material). The addition of any of the other 24 measures of fine-grained sediment made no 19 significant improvement to the model. Axis 1 and 2 of the pCCA were found to contribute 20 substantially to the model (see Table S4 in Supplementary Material) and, therefore, were 21 both included in the development of the new index. Axis 1 was predominantly related to 22 mass of organic sediment in erosional areas, while axis 2 was related mostly to a 23 24 combination of total mass of surface fines in depositional areas and, to a lesser extent, % organic content in erosional areas. Whilst axis 2 may appear to encompass two distinct 25 characteristics of fine-grained sediment, the total mass of deposited fine sediment was 26 27 largely determined by the mineral component, with low masses typically comprising a high % organic matter. 28

The relative position of taxa along axes 1 and 2 of the pCCA, provided a ranking of taxa according to the centre of their distributions in relation to the two gradients of fine-grained sediment pressure. Along axis 1, the ranking distinguished those most associated with high masses of organic sediment in erosional areas (e.g. the stonefly *Nemoura cinerea* (Retzius, 1783)), from those associated with low masses of organic sediment in erosional areas (e.g. the net-spinning caddis fly *Hydropsyche pellucidula* (Curtis, 1834). Along axis 2, the ranking separated those taxa associated with high masses of fine-grained sediment in the surface 1 drape of depositional areas (e.g. the burrowing mayfly *Ephemera danica* Müller, 1764) from

- 2 those associated with low masses of fine-grained sediment in the surface drape of
- 3 depositional areas and a high % content of organic fines in erosional areas (e.g. the stonefly
- 4 *Chloroperla tripunctata* (Scopoli, 1763).

5 Axis species scores were converted to a percentage of the range of species scores along 6 each axis (% Dist), which were then categorised into 10-percentile bands and each one 7 assigned an index score of zero to 10, where zero was the most sediment-tolerant taxon and 8 10 was the most sediment-sensitive taxon (Table 2). For each sample in the calibration 9 dataset, the axis 1 (organic sediment in erosional areas = species level organic Fine Sediment Index (oFSI_{sp})) and axis 2 (total fine sediment in surface drape of depositional 10 areas = species level Total Fine Sediment Index (ToFSI_{sp})) index scores were calculated as 11 12 the mean index score for those taxa present in the sample. In order to combine the two mean scores into one, these values were then offered as explanatory variables in separate 13 regressions against measured total mass of organic sediment or measured total fine 14 15 sediment mass. Of the two, the regression with organic sediment as the dependent variable explained more of the variance and hence this equation (having subtracted the intercept) 16 was used to produce a combined species-level fine sediment index (CoFSI_{sp}): 17

18

CoFSIsp = 0.349oFSIsp + 0.569ToFSIsp

(2)

19
$$(F = 208.9, P < 0.001, R^2 = 70\%)$$

20 In order to produce a more intuitive and conventional range of values for CoFSI_{sp} the

- 21 intercept value (6.80) was subtracted from the returned value to provide a range of
- approximately 3.0 6.5, rather than the uncorrected range of 10.0 13.0. This 'cosmetic'

23 alteration did not affect the performance of the index in any way.

24 Independent Testing

25 The independent test dataset covered a wide range of deposited fine-grained sediment (total fine sediment mass: 32-32,445 g.m⁻²) within the bounds of the calibration dataset (total fine 26 sediment mass: 8-69,664 g.m⁻²) (See Fig S.2 in Supplementary Material). There was a 27 significant negative correlation between CoFSI_{sp} and total reach-scale fine-grained sediment 28 29 mass, total reach-scale organic sediment mass and organic sediment mass in erosional areas for both the autumn and averaged seasons datasets (Table 3, Fig 2). The index was 30 also negatively correlated with fine-grained sediment mass and organic sediment mass in 31 erosional areas in the spring test dataset (Table 3, Fig 2). Across the three test datasets, 32 the correlation was consistently strongest with total fine-grained sediment mass (Table 3, 33 Fig. 2). There was no relationship between CoFSI_{sp} and modelled sediment inputs (Table 34 35 3).

- 1 PSI and LIFE indices were also found to be significantly negatively correlated with measures
- 2 of benthic deposited fine-grained sediment, in particular with total fine sediment mass, but in
- 3 most cases, with weaker associations than CoFSI_{sp} (Table 3). ASPT was only found to be
- 4 significantly correlated with total fine-grained sediment mass and organic sediment mass in
- 5 the autumn dataset, and with a much less pronounced association than $CoFSI_{sp}$. NTAXA
- 6 was not correlated with any measure of fine-grained sediment stress for any of the datasets.
- 7 Strength of correlation between the measures of fine-grained sediment stress and indices
- 8 declined markedly when the latter were presented as EQI (Table 3). Despite this, EQI for
- 9 CoFSI_{sp} was significantly negatively correlated with the three measures of benthic fine-
- 10 grained sediment mass in the autumn dataset, with the strongest correlations being with total
- 11 fine sediment mass. In the autumn dataset, EQI for PSI_{fam} , PSI_{sp} and $LIFE_{fam}$ were
- 12 significantly negatively correlated with benthic fine sediment mass also but almost always
- 13 with a weaker association than EQI for CoFSIsp (Table 3). EQI for NTAXA, ASPT and
- 14 $LIFE_{sp}$ were not correlated with any measures of fine-grained sediment stress. No significant
- 15 correlations were found between EQI for any index and modelled sediment inputs (Table 3).
- 16 All three fine sediment indices (PSI_{fam}, PSI_{sp} and CoFSI_{sp}) were significantly positively
- 17 correlated with ASPT, $LIFE_{fam}$ and $LIFE_{sp}$, both in their raw form and as EQI (Table 4), with
- the strongest correlations being between the PSI and LIFE indices ($\rho = 0.680-0.900$). Of the
- 19 three fine sediment indices, CoFSI_{sp} was almost always the least significantly correlated with
- ASPT, LIFE_{fam} and LIFE_{sp} (ρ = 0.574-0.833; Table 4). NTAXA and EQI NTAXA were not
- 21 correlated with any of the fine sediment indices.
- 22 Overall, independent testing has established that CoFSIsp provides a robust indication of
- benthic fine-grained sediment conditions and it does so with more confidence than otheravailable indices.
- 25

1 Discussion

2 A new empirically-derived diagnostic index to fine-grained sediment stress in rivers has been 3 developed from a unique and spatially extensive, calibration dataset specifically designed to 4 maximise the sediment stress gradient whilst allowing other confounding factors to be 5 controlled. A dataset of this nature provides more confidence in the derived inferences of macroinvertebrate sensitivities to fine-grained sediment than expert opinion. Such an 6 7 approach to index development has been successfully applied to other stressors, e.g. acidity 8 (Davy-Bowker et al., 2005) and organic pollution (Jones et al., 2009). However, for fine-9 grained sediment it was apparent that the macroinvertebrate assemblage was responding to two separate aspects of sediment stress: the quantity of organic fine sediment as well as 10 total fine sediment. Hence, it was necessary to derive two sub-indices to capture the 11 assemblage responses to both the gradient of organic sediment in erosional areas (oFSI_{sp}) 12 and the gradient of total fines in depositional areas (ToFSI_{sp}). The two sub-indices were 13 then combined to derive the combined fine sediment index (CoFSI_{sp}). The inclusion of both 14 sub-indices lends support to the arguments to take account of both the mineral and organic 15 16 components of sediment stress on the aquatic environment (Collins et al., 2009c, 2011) and 17 properly addresses the definition of sediment stress in the EU WFD. Organic material can 18 be introduced into the fine-grained sediment load of river systems from a variety of sources 19 and recent studies have demonstrated fingerprinting procedures for apportioning such inputs (Collins et al., 2014). 20

21 Many taxa exhibited a different association with organic sediment mass than with total 22 sediment mass. The mayflies E. danica and Serratella ignita (Poda, 1761) and the caddis 23 flies Agapetus sp. and Ithytrichia sp. were found to be very sensitive to deposited organic 24 sediments but very tolerant of total sediment mass (Table 2). In contrast, the diving beetle 25 Agabus sp., caddis fly Limnephilus lunatus Curtis, 1834 and stonefly Protonemura meyeri (Pictet, 1841) were more tolerant of deposited organic sediments but sensitive to total 26 sediment mass (Table 2). Taxa such as the stonefly *N. cinerea* and the phantom cranefly 27 Ptychoptera sp. were tolerant of both sources of fine-grained sediment stress, while the 28 caddis fly Hydroptila sp and the mayfly Caenis rivulorum Eaton, 1884 appeared to be equally 29 sensitive to both stress gradients (Table 2). Nevertheless, most of the scoring taxa were 30 similarly sensitive to both organic and total sediment stress; %Dist of 64 of the 105 taxa 31 were within 20% of each other for the two gradients used to derive oFSIsp and ToFSIsp 32 scores (Table 2). 33

Other studies have attempted to quantify macroinvertebrate responses to sediment stress using a variety of methods (Jones *et al.*, 2012b). Larsen & Ormerod (2010) found that the

1 experimental addition of sand to an upland stream system led to increased drift in Baetis 2 rhodani and Ecdyonurus spp. In our study, these taxa were found to be moderately 3 sensitive to both organic and total sediment mass. Angradi (1999) manipulated fine sediment levels in colonisation trays in forest streams and found that densities of the mayfly 4 Paraleptophlebia and the relative abundance of Chironomini midge larvae decreased with 5 increasing fine sediment levels, while relative abundances of Orthocladiinae increased. This 6 7 broadly concurs with our findings where we also found Paraleptophlebia to be sensitive to fine-grained sediment, though our index ranks do not indicate that Orthocladiinae, 8 9 Chironomini or Tanytarsini are particularly sensitive to either measure of sediment (Table 2). 10 Relyea et al. (2012) ranked macroinvertebrate taxa according to their relative abundance among streams varying in fine sediment cover in north-western USA, to create a stressor-11 specific biomonitoring index. Of the limited number of genera in common with UK 12 assemblages, Rhithrogena and Rhyacophila tended to be classified as sediment-sensitive 13 14 by both indices. Serratella and Agapetus were considered 'slightly fine sediment sensitive' by the American index while we found that they were very sensitive to organic fine sediment 15 but tolerant of the total mass of fines. Recently, Extence et al. (2013) assigned an 16 17 exhaustive list of macroinvertebrate taxa recorded in the UK to one of four fine sediment-18 sensitivity classes using expert opinion. When the ranking of taxa in oFSI_{sp} and ToFSI_{sp} are 19 compared with that for PSI_{sp}, we find that there is broad agreement with oFSI_{sp}, with taxa 20 classified as highly tolerant by PSI_{sp} having oFSI_{sp} scores ranging from 0-5. There was 21 much less agreement with ToFSI_{sp} with the full range of possible ToFSI_{sp} scores (0-10) being 22 assigned to taxa in the most fine sediment-sensitive PSI group (see Fig. S3 in Supplementary Material). This adds support to the view that CoFSI_{sp}, by being composed of 23 24 two separate gradients describing different constituents of fine-grained sediment, uniquely captures an additional aspect of the macroinvertebrate assemblage response to fine 25 26 sediment pressure in streams. Von Bertrab et al. (2013) also found that the quality (as C:N) 27 of deposited fine sediment was a more important factor than the quantity in a study of macroinvertebrate assemblage composition across 29 sites and a gradient of 10-90% 28 visually-assessed fine sediment cover. 29 30 The CoFSI_{sp} index has been shown to perform well in independent tests and is capable of

31 indicating fine-grained sediment conditions across a wide range of stream types. The

32 strength of correlation with the total fine sediment gradient was always greater than that for

other indices including PSI, confirming that, compared with indices already routinely used by

34 the UK environment agencies, CoFSI_{sp} offered additional explanatory power when assessing

35 this stressor. The strength of the relationship between CoFSI_{sp} and the total fine sediment

36 gradient (as measured using the re-suspension technique) was also greater than

1 relationships reported by Turley *et al.* (2014) in their testing of the associations between PSI,

2 LIFE and ASPT indices and visually-assessed percent-cover of sand, silt and clay.

3 Duerdoth et al. (2015) have shown that although visual estimates, similarly to the re-

4 suspension technique, are good at discriminating between sites, unlike the re-suspension

5 technique, the person making the visual estimate affects the results to a much greater

6 extent; accounting for 40% of within-site variance as opposed to 5% for the re-suspension

7 technique. Furthermore, visual estimates do not provide information on the quality (organic

8 $\,$ content) of the fine-grained sediment. When we correlated CoFSI_{sp} values in our

9 independent dataset to visual estimates of fines recorded at the same time as biological

sampling, we found that $CoFSI_{sp}$ was marginally better correlated ($\rho = -0.559$ to -0.683) than

11 PSI_{sp} ($\rho = -0.573$ to -0.633). These correlations were weaker than those for either index

12 against the re-suspension technique estimates of total deposited fine sediment mass (Table

13 3).

14 Similarly to Turley *et al.* (2014), we found that PSI_{sp} was better correlated with the fine

15 sediment gradient than PSI_{fam}. Better-resolved taxonomic data does not always provide a

16 more reliable bioassessment of environmental quality (Bennett *et al.*, 2014) but for

macroinvertebrates it would appear to be the case (Monk *et al.*, 2012, Murphy *et al.*, 2013).

18 This has been recognised by UK environment agencies; who are now quantifying routine

19 monitoring macroinvertebrate assemblage samples beyond family-level to a pragmatic

20 mixed-taxonomic level where the taxa are identified to genus or species level where

21 practicable (Davy-Bowker *et al.*, 2010). Ultimately, the costs associated with acquiring more

resolved data have to be set against the gains in confidence or the power to discriminate

between sites that are meeting their environmental objectives and those that are failing

24 (Jones, 2008).

25 We found that the fine sediment indices (PSI and $CoFSI_{sp}$) were positively correlated with

the low-flow (LIFE) and organic pollution (ASPT) indices. Turley *et al.* (2014) also found a lack of independence between PSI, LIFE and ASPT indices (ρ = 0.74-0.89). It would appear

that those taxa sensitive to fine sediment deposition also tend to be sensitive to low-flow and

29 organic pollution stress. More diagnostic indices are being demanded and developed in

30 response to societal pressure to protect and enhance freshwater ecosystems (Friberg *et al.*,

2011). However, it is insufficient to just quantify the strength of the relationship with the

32 stressor of interest: to be uniquely diagnostic, indices must be shown to be independent of

33 other stressors. Extreme care must be taken in the development of such compositional

34 indices to ensure that they can extract the maximum information available describing the

unique effects of fine-grained sediment, low-flows or organic pollution; all three stressors

36 result in diminished oxygen supply, acting to varying extent as the proximal stress on the

biota. Adequate separation of the unique aspects of the stressors is required to assign
confidently a cause for failure based on biological monitoring and derived diagnostic index
scores. Manipulative experimentation can also help disentangle the individual and combined
effects of multiple stressors (Matthaei, Piggott & Townsend, 2010; Jones et al., 2015).
Where this is not possible, additional supporting evidence may be required to ascertain, with
confidence, which of these three stressors is suppressing ecological condition.

7 It was expected that presenting indices as an EQI would lead to an improved relationship 8 between index and stressor gradient, with the removal of the confounding influence of 9 natural background variation. We found the opposite to be the case. It is likely that the reference condition approach (use of EQI) removed that portion of the index response to 10 deposited sediment that was attributable to natural variation in river type, leaving only the 11 stress attributable to excess fine-grained sediment. Fine-grained sediment input to streams 12 and rivers is a natural process and as such the deposited load of fine-grained sediment 13 tends to increase with distance downstream (Vannote et al., 1980). This natural gradient in 14 15 fine-grained sediment presents a challenge to the assessment of anthropogenic fine-grained sediment stress, as such any assessment should be focussed on the effect of the additional 16 fine sediment found at a site over and above what would be expected were the site less or 17 18 completely unimpacted (Foster et al., 2011). It is this excess fine sediment to which diagnostic indices, such as CoFSI_{so}, ideally need to be responding, as opposed to the 19 natural fine-grained sediment gradient, especially as management interventions should only 20 21 be targeting excess rather than natural sediment inputs (Collins et al., 2012). In the 22 development of CoFSI_{sp} we have attempted to address this by factoring out the biological 23 variation associated with measures of natural background variation before ranking taxa 24 along the fine sediment gradients. However, more research is required to define better site-25 specific benthic fine-grained sediment thresholds beyond which ecological condition is 26 affected. Incorporating CoFSI_{sp} into a future version of RICT (Clarke *et al.*, 2011) to more 27 accurately generate EQIs would further ensure that the index is diagnosing actual sediment 28 stress as opposed to underlying natural variability reflecting catchment-scale sediment 29 dynamics.

Fundamentally, this study has been correlative in nature and further experimental manipulations would be required to fully understand the proximal causative factors determining the distribution of species. Jones *et al.* (2012b) reviewed the multiple direct and indirect ways that fine-grained sediment stress can affect macroinvertebrate taxa. We do not know for sure which aspect of increased deposited fine-grained sediment the macroinvertebrate assemblage in our datasets was responding to, be it physical clogging of interstices, depleted oxygen concentrations in the benthos, abrasive damage from

- 1 suspended or saltating mineral sediment or modifications to other components of the community or habitat (Jones et al., 2012b). While we have measured sequestered fine-2 grained sediment as part of the study, we have only an estimate of sediment delivery to the 3 river channel (from Collins & Anthony, 2008; Collins et al., 2009a,b) and no actual 4 measurements of turbidity at each site, or any indication of the temporal variability of 5 6 turbidity. Hence, we do not know the temporal scale of stress (in terms of sediment load) to 7 which the invertebrates are responding, either pulsed events or chronic long term stress. 8 Manipulative experiments in artificial streams carried out in tandem with the current work
- 9 have sought to address this issue by quantifying the biological response to altered flow and
- 10 fine sediment colmation both individually and in combination (Jones *et al.* 2015). Despite
- 11 these apparent shortcomings, the correlative approach demonstrably produces a reliable
- 12 ranking of taxa in terms of their aggregated response to the measured fine-grained sediment
- variables and, as such, can be a powerful tool in better understanding community-level
- 14 responses to fine sediment stress over large spatial scales.

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- 24

1 Tables

Table 1. Sediment pressure categories used to identify stream sites. Total sediment inputs to the river

4 channel in each contributing catchment derived from Collins & Anthony, 2008; Collins *et al.*, 2009a,b.

Fine-grained Sediment Pressure Category	Range (kg ha ⁻¹ yr ⁻¹)
A	0-29.99
В	30-179.99
С	180-329.99
D	330-479.99
E	480-629.99
F	630+

Table 2. The assignment of $oFSI_{sp}$ and $ToFSI_{sp}$ scores for 105 calibration dataset taxa. Also presented are the pCCA axis 1 and 2 species scores that form the basis for the ranking of taxa, and the percentile data that were used to divide the gradients of response into bands.

Tayon	Avie 1	%Diet	oFSI _{sp}	Avie 2	%Diet	ToFSI _{sp}
Heptagenia sp.	0.6932	0	10	-0.0462	59	5
Ithytrichia sp.	0.4244	18	9	-0.3071	82	2
Nemoura cambrica group	0.3662	21	8	0.0315	52	-
Drusus annulatus (Stephens, 1837)	0.3653	21	8	-0.0723	61	4
Baetis muticus (Linnaeus, 1758)	0.3613	22	8	-0.0129	56	5
Serratella ignita (Poda, 1761)	0.3517	22	8	-0.413	91	1
Leuctra nigra (Olivier, 1811)	0.3379	23	8	-0.0848	62	4
Ancylus fluviatilis (O.F. Müller, 1774)	0.3232	24	8	-0.2475	77	3
Polycentropus flavomaculatus (Pictet, 1834)	0.3177	25	8	0.1332	43	6
Halesus sp.	0.2915	26	8	0.086	47	6
Agapetus sp.	0.2913	26	8	-0.3175	83	2
Oreodytes sanmarkii (C.R. Sahlberg, 1826)	0.2865	27	8	0.0933	47	6
Orectochilus villosus (O.F. Müller, 1776)	0.2861	27	8	-0.1021	64	4
Caenis rivulorum Eaton, 1884	0.2824	27	8	0.1999	37	7
Athripsodes sp.	0.2679	28	8	-0.1858	71	3
Lepidostoma hirtum (Fabricius, 1775)	0.266	28	8	-0.0742	61	4
Simulium (Simulium) ornatum group	0.2579	28	8	-0.1011	64	4
Ephemera danica Müller, 1764	0.2542	29	8	-0.4062	90	1
Limnius volckmari (Panzer, 1793)	0.2495	29	8	-0.0168	56	5
Paraleptophlebia submarginata (Stephens, 1835)	0.2479	29	8	0.0633	49	6
Hemerodrominae	0.2469	29	8	-0.1722	70	3
Sericostoma personatum (Spence in Kirby &	0.2421	29	8	-0.1978	72	3
Spence, 1826) Silo pallipes (Fabricius, 1781)	0.2303	30	7	-0.1301	66	4
Pedicia sp.	0.2057	32	7	-0.0783	62	4
Hydropsyche pellucidula (Curtis, 1834)	0.1985	32	7	0.1413	43	6
Hvdraena gracilis Germar. 1824	0.1957	33	7	0.1025	46	6
Odontocerum albicorne (Scopoli, 1763)	0.1899	33	7	-0.2729	79	3
Hydroptila sp.	0.1736	34	7	0.2163	36	7
Dicranota sp.	0.169	34	7	-0.018	56	5
<i>Centroptilum luteolum</i> (Müller, 1776)	0.1688	34	7	-0.1857	71	3
Chaetopteryx villosa (Fabricius, 1798)	0.1475	36	7	-0.2792	79	3
Ecdyonurus sp.	0.1461	36	7	0.1392	43	6
Leuctra hippopus Kempny, 1899	0.1375	36	7	0.295	29	8
Brachyptera risi (Morton, 1896)	0.1335	37	7	0.1252	44	6
Elmis aenea (Müller, 1806)	0.1308	37	7	-0.0077	56	5
Isoperla grammatica (Poda, 1761)	0.1221	37	7	0.1709	40	6
Polycelis felina (Dalyell, 1814)	0.1176	38	7	0.023	53	5
Rhithrogena sp.	0.1149	38	7	0.1423	42	6
Baetis rhodani (Pictet, 1843-1845)	0.1144	38	7	0.0916	47	6
Potamophylax cingulatus group	0.1114	38	7	-0.2686	78	3
Siphonoperla torrentium (Pictet, 1841)	0.0949	39	7	0.3191	27	8
Glossosoma sp.	0.0813	40	6	0.3784	22	8

continued

Taxon	Axis 1	%Dist	oFSI _{sp} Score	Axis 2	%Dist	ToFSI _{sp} Score
Habrophlebia fusca (Curtis, 1834)	0.08	40	6	-0.2141	74	3
Oulimnius sp.	0.0796	40	6	0.0219	53	5
Simulium (Nevermannia) angustitarse group	0.0785	40	6	-0.133	67	4
Rhyacophila sp.	0.0781	40	6	0.1554	41	6
Hydropsyche siltalai Döhler, 1963	0.0702	41	6	0.1486	42	6
Leuctra fusca (Linnaeus, 1758)	0.0339	43	6	-0.036	58	5
Calopteryx sp.	0.0258	44	6	-0.4056	90	1
Sialis fuliginosa Pictet, 1836	0.0243	44	6	0.1975	38	7
Eloeophila sp.	0.011	45	6	-0.1324	66	4
Philopotamus montanus (Donovan, 1813)	0.0089	45	6	0.3248	27	8
Simulium (Simulium) argyreatum group	-0.0087	46	6	0.4148	19	9
Simulium (Eusimulium) aureum group	-0.0338	48	6	0.1979	38	7
Potamopyrgus antipodarum (J.E.Gray, 1843)	-0.0395	48	6	-0.235	75	3
Hydropsyche instabilis (Curtis, 1834)	-0.0536	49	6	0.2052	37	7
Hydracarina	-0.0624	49	6	-0.0175	56	5
Esolus parallelepipedus (Müller, 1806)	-0.0687	50	5	0.2809	30	7
Gammarus pulex (Linnaeus, 1758)	-0.0739	50	5	-0.1316	66	4
Amphinemura sulcicollis (Stephens, 1836)	-0.0783	50	5	0.6031	2	9
Simulium (Nevermannia) cryophilum-vernum	-0.0792	51	5	0.1871	39	7
Clinocerinae	-0.0912	51	5	0.3975	20	8
Plectrocnemia sp.	-0.0952	52	5	0.1117	45	6
Tubificidae	-0.1049	52	5	-0.1535	68	4
Orthocladiinae [sub-family]	-0.1136	53	5	0.0742	48	6
Lumbriculidae	-0.1155	53	5	-0.0711	61	4
Chironomini [tribe]	-0.1185	53	5	-0.2941	81	2
Ceratopogonidae	-0.1248	53	5	-0.0115	56	5
Lumbricidae	-0.1328	54	5	-0.0769	62	4
Baetis scambus group	-0.1332	54	5	-0.5157	100	0
Leuctra inermis Kempny, 1899	-0.1354	54	5	0.3475	25	8
Platambus maculatus (Linnaeus, 1758)	-0.1373	54	5	-0.4524	94	1
Tanytarsini [tribe]	-0.1439	55	5	-0.0501	59	5
Perlodes microcephalus (Pictet, 1833)	-0.159	56	5	0.3306	26	8
Electrogena lateralis (Curtis, 1834)	-0.1851	57	5	0.6053	2	9
Diamesinae [sub-family]	-0.1871	58	5	0.0094	54	5
Naididae	-0.198	58	5	0.0932	47	6
<i>Elodes</i> sp.	-0.2064	59	5	0.0582	50	5
Nemoura avicularis Morton, 1894	-0.2104	59	5	-0.2289	75	3
Enchytraeidae	-0.2122	59	5	0.2167	36	7
Prodiamesinae [sub-family]	-0.2269	60	4	-0.3586	86	2
<i>Velia</i> sp.	-0.238	61	4	0.0776	48	6
Erpobdella octoculata (Linnaeus, 1758)	-0.2438	61	4	0.1234	44	6
Helobdella stagnalis (Linnaeus, 1758)	-0.2499	62	4	-0.1457	68	4
Tipula (Yamatotipula) montium group	-0.2642	63	4	-0.0189	57	5
Pericoma group	-0.2659	63	4	0.1209	44	6
Protonemura meyeri (Pictet, 1841)	-0.2687	63	4	0.4777	13	9

Table 2. continued

Taxon	Axis 1	%Dist	oFSI _{sp} Score	Axis 2	%Dist	ToFSI Score
Pisidium sp.	-0.2825	64	4	-0.2803	79	3
Chloroperla tripunctata (Scopoli, 1763)	-0.3002	65	4	0.6283	0	10
Tanypodinae [sub-family]	-0.3134	66	4	-0.1152	65	4
Glossiphonia complanata (Linnaeus, 1758)	-0.3155	66	4	-0.2676	78	3
Dinocras cephalotes (Curtis, 1827)	-0.3222	66	4	0.3035	28	8
<i>Dixa maculata</i> complex	-0.3392	67	4	-0.2289	75	3
<i>Sialis lutaria</i> (Linnaeus, 1758)	-0.3453	68	4	-0.363	87	2
<i>Lype</i> sp.	-0.357	69	4	-0.3415	85	2
<i>Asellus aquaticus</i> (Linnaeus, 1758)	-0.4004	72	3	-0.118	65	4
<i>Ptychoptera</i> sp.	-0.4392	74	3	-0.2687	78	3
<i>Radix balthica (</i> Linnaeus, 1758)	-0.4928	78	3	-0.0806	62	4
Limnephilus lunatus Curtis, 1834	-0.5416	81	2	0.2383	34	7
<i>Pilaria</i> sp.	-0.6383	87	2	0.0456	51	5
Anacaena globulus (Paykull, 1829)	-0.6392	87	2	-0.2147	74	3
<i>Micropterna sequax</i> McLachlan, 1875	-0.6598	88	2	-0.077	62	4
<i>Nemoura cinerea</i> (Retzius, 1783)	-0.6936	91	1	-0.2761	79	3
<i>Agabus</i> sp.	-0.7808	96	1	0.2623	32	7
Proasellus meridianus (Racovitza, 1919)	-0.8363	100	0	0.0225	53	5

Table 3. Spearman rank correlations (ρ) between the combined species-level Fine Sediment Index (CoFSI_{sp}) and six other established biotic indices and three measures of benthic deposited sediment (g.m⁻²): fine-grained sediment mass in the stream bed, organic sediment mass in the stream bed and organic sediment mass in erosional areas of the stream bed and two modelled estimates of fine-grained sediment inputs (kg ha⁻¹ yr⁻¹), from autumn samples, spring samples and autumn and spring averaged data. The correlations between the ecological quality index (EQI) of the indices and the five measures fine-grained sediment stress are also shown. Correlation coefficients in bold were significant at *P* < 0.05 after correcting for the family-wise error rate using the Holm-Bonferroni method (Holm, 1979).

Autumn (n=78)	NTAXA	ASPT			PSI _{fam}			EQI NTAXA	EQI ASPT	EQI LIFE _{fam}	EQI LIFE _{sp}	EQI PSI _{fam}	EQI PSI _{sp}	EQI CoFSI _{sp}
Total fine-grained sediment mass	0.162	-0.420	-0.578	-0.607	-0.627	-0.647	-0.703	-0.010	-0.253	-0.412	-0.353	-0.412	-0.426	-0.497
Organic sediment mass Organic sediment mass in	0.172	-0.398	-0.560	-0.559	-0.598	-0.611	-0.667	0.015	-0.248	-0.416	-0.338	-0.402	-0.400	-0.473
erosional areas Total Fine-grained sediment	0.162	-0.327	-0.539	-0.532	-0.578	-0.555	-0.593	0.055	-0.209	-0.412	-0.350	-0.440	-0.390	-0.438
inputs Agricultural fine-grained sediment	-0.112	0.255	0.341	0.245	0.375	0.348	0.350	-0.086	0.108	0.143	-0.034	0.174	0.138	0.033
inputs	-0.118	0.198	0.312	0.219	0.341	0.281	0.281	-0.121	0.088	0.164	0.000	0.210	0.127	0.039
Spring (n=49)	-													
Total fine-grained sediment mass	0.181	-0.246	-0.453	-0.421	-0.501	-0.637	-0.670	0.028	-0.085	-0.336	-0.264	-0.318	-0.455	-0.471
Organic sediment mass	0.209	-0.088	-0.240	-0.203	-0.307	-0.372	-0.412	0.118	0.033	-0.146	-0.079	-0.141	-0.215	-0.269
erosional areas	0.102	-0.214	-0.395	-0.405	-0.409	-0.538	-0.526	0.018	-0.167	-0.357	-0.265	-0.291	-0.417	-0.445
Total fine-grained sediment inputs Agricultural fine-grained sediment	-0.102	0.248	0.185	0.096	0.294	0.219	0.244	-0.068	0.090	0.071	-0.047	0.117	0.067	0.042
inputs	0.030	0.194	0.107	0.027	0.230	0.112	0.099	0.030	0.099	0.047	-0.068	0.137	0.040	-0.010
Aut-Spr averaged (n=44)	-													
Total fine-grained sediment mass	0.133	-0.349	-0.619	-0.580	-0.607	-0.662	-0.673	0.007	-0.158	-0.503	-0.388	-0.402	-0.439	-0.467
Organic sediment mass	0.157	-0.216	-0.455	-0.370	-0.462	-0.466	-0.468	0.067	-0.084	-0.378	-0.234	-0.293	-0.281	-0.335
erosional areas	0.096	-0.255	-0.535	-0.484	-0.523	-0.559	-0.516	0.041	-0.206	-0.502	-0.415	-0.449	-0.486	-0.489
Total fine-grained sediment inputs Agricultural fine-grained sediment	-0.126	0.151	0.153	0.102	0.253	0.211	0.237	-0.085	-0.006	0.048	-0.093	0.062	0.020	-0.072
inputs	-0.097	0.064	0.080	0.039	0.210	0.127	0.133	-0.090	-0.026	0.056	-0.095	0.094	0.017	-0.087

Table 4. Spearman rank correlations (ρ) between the fine sediment indices (PSI_{fam}, PSI_{sp} and CoFSI_{sp}) and other routinely-used indices. Correlation coefficients in bold were significant at *P* < 0.05 after correcting for the family-wise error rate using the Holm-Bonferroni method (Holm, 1979).

Autumn (n=78)	ΝΤΑΧΑ	ASPT				EQI NTAXA	EQI ASPT	EQI LIFE _{fam}	EQI LIFE _{sp}
PSI _{fam}	-0.113	0.737	0.897	0.838	EQI PSI _{fam}	-0.019	0.698	0.857	0.680
PSI _{sp}	-0.117	0.732	0.883	0.861	EQI PSI _{sp}	0.076	0.696	0.813	0.712
CoFSI _{sp}	-0.104	0.701	0.833	0.800	EQI CoFSI _{sp}	-0.014	0.574	0.707	0.607
Spring (n=49)									
PSI _{fam}	-0.256	0.694	0.850	0.803	EQI PSI _{fam}	-0.255	0.690	0.834	0.830
PSI _{sp}	-0.239	0.641	0.800	0.819	EQI PSI _{sp}	-0.217	0.643	0.802	0.851
CoFSI _{sp}	-0.263	0.667	0.756	0.732	EQI CoFSI _{sp}	-0.270	0.623	0.795	0.806
Aut-Spr averaged (n=44)									
PSI _{fam}	-0.110	0.769	0.890	0.901	EQI PSI _{fam}	-0.057	0.689	0.804	0.786
PSI _{sp}	-0.081	0.768	0.873	0.900	EQI PSI _{sp}	0.021	0.662	0.801	0.831
CoFSI _{sp}	-0.070	0.749	0.825	0.795	EQI CoFSI _{sp}	-0.003	0.578	0.759	0.739

1 Figure Legends

- 2
- 3 Figure 1. The distribution of 262 stream sites across England and Wales sampled for
- 4 macroinvertebrates and deposited fine sediment; 179 of which formed the calibration dataset (white
- 5 circles) and 83 of which formed the independent test dataset (black circles).

6

- 7 Figure 2. Relationship between the combined species-level Fine Sediment Index (CoFSI_{sp}) and three
- 8 measures of benthic deposited sediment (g.m⁻²): fine sediment mass in the stream bed, organic
- 9 sediment mass in the stream bed and organic sediment mass in erosional areas of the stream bed,
- 10 from (a) autumn samples, (b) spring samples and (c) autumn and spring averaged data. The
- 11 relationship between ecological quality index (EQI) of CoFSI_{sp} and the three measures of benthic
- deposited sediment, from (d) autumn samples, (e) spring samples and (f) autumn and spring
- 13 averaged data is also shown.

14



Figure 1.





1 Supplementary Material

- 2
- **Table S1.** Physical characteristics of the four stream types in the present study with their approximate relation to the
 RIVPACS IV super end groups (Davy-Bowker *et al.* 2008).

Stream type	Distance from source (km)	Altitude (m)	Slope (m.km ⁻¹)	RIVPACS IV Super End Group ¹	General description
2	4 - 13	> 170 (Calcareous)	> 6	2	Upland streams in N England
		> 140 (Siliceous)			
3	> 13	75 - 170 (Calcareous)	2 – 6 (Calcareous)	3	Intermediate rivers in Wales, N and SW England
		35 - 140 (Siliceous)	3 - 6 (Siliceous)		
4	0 - 4	75 - 170 (Calcareous)	> 6	4	Small steep streams
		35 - 140 (Siliceous)			
5	> 13	< 75 (Calcareous)	< 2 (Calcareous)	5	Intermediate size lowland streams, including chalk, SE
		< 35	< 3		England
		(Siliceous)	(Siliceous)	6	small lowland streams, including chalk, SE England
				7	Larger lowland streams, SE England, finer bed sediment

5 ¹all representatives of RIVPACS IV super end group 1 were excluded as this biological river type is not represented in

6 England and Wales.

- **Table S2.** Matrix of potential sites used for site selection, covering a range of stream types and fine-grained sediment
- 2 pressures (see Tables 1 & S1 for definitions).

Stream Type	A	В	С	D	Е	F	Total
2	2	33	35	29	18	15	132
3	6	14	17	5	2	4	48
4	13	127	92	49	15	16	312
5	4	32	22	12	2	4	76
Total	25	206	166	95	37	39	568

Fine-grained Sediment Pressure Category

Table S3. Environmental variables used to account for variation in the sampled stream macroinvertebrate community across the calibration sites.

	Description and data transformations applied						
Natural	Discharge category						
environmental	Surface velocity category						
variables	log Distance from source (km)						
	log Altitude of site (m)						
	log Slope of site (m.km ⁻¹)						
	log(x+1) estimate of local bank erosion fine sediment load to site from catchment (kg.ha ⁻¹ .yr ⁻¹ ; Collins & Anthony, 2008; Collins <i>et al.</i> , 2009a,b)						
	log Catchment area (km ²)						
Measured fine-	log geometric mean Total sediment mass (g.m ⁻²)						
grained sediment	log range Total sediment mass (g.m ⁻²)						
variables	log geometric mean Depositional area sediment mass (g.m ⁻²)						
	log geometric mean Erosional area sediment mass (g.m ⁻²)						
	log geometric mean Total organic sediment mass (g.m ⁻²)						
	log range Total organic sediment mass (g.m ⁻²)						
	log geometric mean Depositional area organic sediment mass (g.m ⁻²)						
	log geometric mean Erosional area organic sediment mass (g.m ⁻²)						
	log arithmetic mean % Organic sediment content						
	log range % Organic sediment content						
	log arithmetic mean Depositional area % organic sediment content						
	log arithmetic mean Erosional area % organic sediment content						
	log geometric mean Surface sediment mass (g.m ⁻²)						
	log range Surface sediment mass (g.m ⁻²)						
	log geometric mean Depositional area surface sediment mass (g.m ⁻²)						
	log geometric mean Erosional area surface sediment mass (g.m ⁻²)						
	log geometric mean Surface organic sediment mass (g.m ⁻²)						
	log range Surface organic sediment mass (g.m ⁻²)						
	log geometric mean Depositional area surface organic sediment mass (g.m ⁻²)						
	log geometric mean Erosional area surface organic sediment mass (g.m ⁻²)						
	log arithmetic mean Surface % organic sediment content						
	log range Surface % organic sediment content						
	log arithmetic mean Depositional area surface % organic sediment content						
	log arithmetic mean Erosional area surface % organic sediment content						
Modelled fine- grained sediment	log(x+1) PSYCHIC 2010 estimate of agricultural fine-grained sediment load to site from catchment (kg.ha ⁻¹ .yr ⁻¹)						
inputs	log(x+1) estimate of total fine-grained sediment load to site from catchment (kg.ha ⁻ ¹ .yr ⁻¹ ; Collins & Anthony, 2008; Collins <i>et al.</i> , 2009a,b)						
	% of fine-grained sediment load estimated to be coming from agricultural sources						

Table S4. Results from the partial canonical correspondence analysis (pCCA) showing the eigenvalues for each environmental variable if it were the only variable in the pCCA model (marginal effect), the additional contribution (as eigenvalues) of each successive variable to the forward selected model (conditional effect) with associated Monte Carlo permutation test results and the inflation factors associated with the final model. Correlation coefficients between the selected environmental variables and the first two pCCA axes are also presented. Eigenvalues measure the contribution of each variable to the explanatory power of the overall pCCA model. If all variables were added to the pCCA model the sum of all conditional effect eigenvalues would be 0.35.

Variable	Marginal effect eigenvalue	Conditional effect eigenvalue	Р	F	Final inflation factor	Interset correlatio Axis 1	ons Axis 2
Organic sediment mass in erosional areas (g.m ⁻²)	0.043	0.043	0.001	4.08	2.2	-0.554	-0.333
Fine-grained sediment mass in surface drape of depositional areas (g.m ⁻²)	0.037	0.038	0.001	3.69	1.6	0.180	-0.579
% organic content in erosional areas	0.029	0.018	0.001	1.71	2.4	-0.171	0.441

Table S5. The 326 taxa recorded across the 179 calibration and 26 independent test sites.

MAJOR GROUP	TAXON NAME	No. of occurrences
Coelenterata	Coelenterata	10
Microturbellaria	Microturbellaria	6
Tricladida	Tricladida	2
	Planaria torva (Müller, 1774)	1
	Polycelis felina (Dalyell, 1814)	83
	Polycelis nigra group ¹	12
	Phagocata vitta (Duges, 1830)	4
	Crenobia alpina (Dana, 1766)	17
	Dugesia polychroa group ²	5
	Dendrocoelum lacteum (O.F.Müller, 1774)	9
Nemertea	Nemertea	2
Nematomorpha	Nematomorpha	14
Nematoda	Nematoda	3
Gastropoda	Theodoxus fluviatilis (Linnaeus, 1758)	1
	Valvata (Valvata) cristata O.F. Müller, 1774	2
	Valvata (Cincinna) piscinalis (O.F. Müller, 1774)	1
	Potamopyrgus antipodarum (J.E.Gray, 1843)	111
	Physa fontinalis (Linnaeus, 1758)	4
	Physella sp.	1
	Lymnaea stagnalis (Linnaeus, 1758)	1
	Galba truncatula (O.F. Müller, 1774)	5
	Stagnicola palustris (O.F. Müller, 1774)	8
	Radix balthica (Linnaeus, 1758)	55
	Planorbis (Planorbis) sp.	6
	Anisus (Anisus) leucostoma (Millet, 1813)	4
	Anisus (Disculifer) vortex (Linnaeus, 1758)	7
	Bathyomphalus contortus (Linnaeus, 1758)	2
	Gyraulus (Gyraulus) albus (O.F. Müller, 1774)	5
	Gyraulus (Armiger) crista (Linnaeus, 1758)	5
	Ancylus fluviatilis (O.F. Müller, 1774)	83

MAJOR GROUP	TAXON NAME	No. of occurrences
	Acroloxus lacustris (Linnaeus, 1758)	1
	Succineidae	3
Bivalvia	Sphaerium sp.	6
	Pisidium sp.	152
Oligochaeta	Lumbriculidae	177
	Haplotaxidae	5
	Enchytraeidae	91
	Naididae	113
	Tubificidae	154
	Lumbricidae	120
Hirudinea	Piscicola geometra (Linnaeus, 1761)	22
	Theromyzon tessulatum (O.F.Müller, 1774)	6
	Glossiphonia complanata (Linnaeus, 1758)	72
	Helobdella stagnalis (Linnaeus, 1758)	26
	Haemopis sanguisuga (Linnaeus, 1758)	1
	Erpobdella octoculata (Linnaeus, 1758)	50
	Trocheta bykowskii Gedroyc, 1913	3
	Trocheta subviridis Dutrochet, 1817	5
Hydracarina	Hydracarina	123
Oribatei	Oribatei	3
Cladocera	Cladocera	4
Ostracoda	Ostracoda	25
Copepoda	Copepoda	5
	Cyclopoida	1
Decapoda	Austropotamobius pallipes (Lereboullet, 1858)	2
	Pacifastacus leniusculus (Dana, 1858)	8
Isopoda	Asellus aquaticus (Linnaeus, 1758)	59
	Proasellus meridianus (Racovitza, 1919)	25
Amphipoda	Crangonyx pseudogracilis Bousfield, 1958	16
	Gammarus lacustris Sars, 1863	1
	Gammarus pulex (Linnaeus, 1758)	172

MAJOR GROUP	TAXON NAME	No. of occurrences
	Gammarus zaddachi Sexton, 1912	1
	Niphargus aquilex Schiodte, 1855	1
Ephemeroptera	Baetis rhodani (Pictet, 1843-1845)	174
	Baetis vernus Curtis, 1834	20
	Baetis scambus group ³	25
	Centroptilum luteolum (Müller, 1776)	36
	Cloeon dipterum (Linnaeus, 1761)	8
	Procloeon bifidum (Bengtsson, 1912)	1
	Procloeon pennulatum (Eaton, 1870)	2
	Alainites muticus (Linnaeus, 1758)	90
	Nigrobaetis digitatus (Bengtsson, 1912)	1
	Nigrobaetis niger (Linnaeus, 1761)	18
	Rhithrogena sp.	121
	Heptagenia sp.	23
	Ecdyonurus sp.	135
	Electrogena lateralis (Curtis, 1834)	45
	Ameletus inopinatus Eaton, 1887	1
	Leptophlebia marginata (Linnaeus, 1767)	3
	Paraleptophlebia submarginata (Stephens, 1835)	104
	Paraleptophlebia werneri Ulmer, 1919	1
	Habrophlebia fusca (Curtis, 1834)	45
	Ephemera danica Müller, 1764	77
	Ephemera vulgata Linnaeus, 1758	1
	Serratella ignita (Poda, 1761)	44
	Caenis rivulorum Eaton, 1884	44
	<i>Caenis luctuosa</i> group ⁴	6
Plecoptera	Taeniopteryx nebulosa (Linnaeus, 1758)	7
	Brachyptera risi (Morton, 1896)	56
	Protonemura meyeri (Pictet, 1841)	76
	Protonemura praecox (Morton, 1894)	10
	Amphinemura standfussi Ris, 1902	9

MAJOR GROUP	TAXON NAME	No. of occurrences
	Amphinemura sulcicollis (Stephens, 1836)	38
	Nemurella picteti Klapálek, 1900	19
	Nemoura avicularis Morton, 1894	72
	Nemoura cinerea (Retzius, 1783)	19
	<i>Nemoura cambrica</i> group ⁵	48
	<i>Leuctra fusca</i> (Linnaeus, 1758)	56
	Leuctra geniculata (Stephens, 1836)	16
	<i>Leuctra hippopus</i> Kempny, 1899	76
	Leuctra inermis Kempny, 1899	55
	Leuctra moselyi Morton, 1929	2
	Leuctra nigra (Olivier, 1811)	36
	Capnia bifrons (Newman, 1839)	9
	Capnia vidua Klapálek, 1904	1
	Perlodes microcephalus (Pictet, 1833)	70
	Diura bicaudata (Linnaeus, 1758)	1
	Isoperla grammatica (Poda, 1761)	103
	Dinocras cephalotes (Curtis, 1827)	20
	Perla bipunctata Pictet, 1833	15
	Chloroperla tripunctata (Scopoli, 1763)	26
	Siphonoperla torrentium (Pictet, 1841)	86
Odonata	Pyrrhosoma nymphula (Sulzer, 1776)	4
	Ischnura elegans (Vander Linden, 1820)	1
	Coenagrion puella group ⁶	1
	Calopteryx sp.	26
	Cordulegaster boltonii (Donovan, 1807)	16
Heteroptera	Hydrometra stagnorum (Linnaeus, 1758)	2
	<i>Velia</i> sp.	27
	Gerridae	10
	Nepa cinerea Linnaeus, 1758	5
	Notonecta glauca Linnaeus, 1758	2
	Notonecta maculata Fabricius, 1794	2

MAJOR GROUP	TAXON NAME	No. of occurrences
	Notonecta viridis Delcourt, 1909	1
	Micronecta sp.	4
	Hesperocorixa sahlbergi (Fieber, 1848)	1
	Sigara (Sigara) dorsalis (Leach, 1817)	2
	Sigara (Subsigara) falleni (Fieber, 1848)	1
	Sigara (Subsigara) scotti (Douglas & Scott, 1868)	1
	Sigara (Vermicorixa) lateralis (Leach, 1817)	1
	Sigara (Pseudovermicorixa) nigrolineata (Fieber, 1848)	1
	Paracorixa concinna (Fieber, 1848)	1
Coleoptera	Brychius elevatus (Panzer, 1793)	4
	Haliplus fluviatilis Aubé, 1836	1
	Haliplus ruficollis group ⁷	1
	Haliplus lineatocollis (Marsham, 1802)	13
	Hydroporus discretus Fairmaire & Brisout, 1859	1
	Hydroporus gyllenhalii Schiødte, 1841	1
	Hydroporus incognitus Sharp, 1869	1
	Hydroporus palustris (Linnaeus, 1761)	1
	Hydroporus planus (Fabricius, 1782)	1
	Hydroporus tessellatus (Drapiez, 1819)	2
	Deronectes latus (Stephens, 1829)	1
	Nebrioporus depressus group ⁸	7
	Stictotarsus duodecimpustulatus (Fabricius, 1792)	1
	Oreodytes davisii (Curtis, 1831)	1
	Oreodytes sanmarkii (C.R. Sahlberg, 1826)	59
	Oreodytes septentrionalis (Gyllenhal, 1826)	4
	Platambus maculatus (Linnaeus, 1758)	48
	Agabus sp.	30
	<i>Ilybius</i> sp.	20
	Gyrinus substriatus Stephens, 1828	1
	Gyrinus natator group ⁹	2
	Orectochilus villosus (O.F. Müller, 1776)	84

Helophorus (Meghelophorus) aequalis Thomson, 1868	1	
Helophorus (Meghelophorus) grandis Illiger, 1798	7	
Helophorus (Rhopalohelophorus) brevipalpis Bedel, 1881	16	
Helophorus (Helophorus) flavipes Fabricius, 1792	5	
Helophorus (Helophorus) griseus Herbst, 1793	1	
Helophorus (Helophorus) minutus Fabricius, 1775	1	
Helophorus (Helophorus) obscurus Mulsant, 1844	3	
Paracymus sp.	2	
Anacaena globulus (Paykull, 1829)	17	
Anacaena lutescens (Stephens, 1829)	4	
Laccobius sp.	2	
Laccobius (Macrolaccobius) bipunctatus (Fabricius, 1775)	1	
Cercyon marinus Thomson, 1853	1	
Ochthebius bicolon Germar, 1824	1	
Ochthebius dilatatus Stephens, 1829	1	
Ochthebius exsculptus (Germar, 1824)	1	
Ochthebius marinus (Paykull, 1798)	1	
Ochthebius minimus (Fabricius, 1792)	1	
Hydraena gracilis Germar, 1824	111	
Hydraena pygmaea Waterhouse, 1833	1	
Hydraena riparia Kugelann, 1794	6	
Hydraena rufipes Curtis, 1830	2	
Hydraena testacea Curtis, 1831	1	
Limnebius truncatellus (Thunberg, 1794)	10	
Elodes sp.	94	
Cyphon sp.	2	
Hydrocyphon deflexicollis (Müller, 1821)	12	
Scirtes sp.	4	
Dryops sp.	6	
Elmis aenea (Müller, 1806)	166	
Esolus parallelepipedus (Müller, 1806)	67	

MAJOR GROUP	TAXON NAME	No. of occurrences
	Limnius volckmari (Panzer, 1793)	135
	Oulimnius sp.	81
	Riolus cupreus (Müller, 1806)	3
	Riolus subviolaceus (Müller, 1817)	9
Megaloptera	Sialis fuliginosa Pictet, 1836	27
	Sialis lutaria (Linnaeus, 1758)	27
Neuroptera	Osmylus fulvicephalus (Scopoli, 1763)	3
	Sisyra sp.	1
Trichoptera	Rhyacophila sp.	135
	Glossosoma sp.	25
	Agapetus sp.	68
	Agraylea sp.	3
	Hydroptila sp.	34
	Oxyethira sp.	6
	Ithytrichia sp.	23
	Philopotamus montanus (Donovan, 1813)	30
	Wormaldia sp.	15
	Lype sp.	46
	Psychomyia pusilla (Fabricius, 1781)	9
	Tinodes sp.	12
	Cyrnus trimaculatus (Curtis, 1834)	12
	Plectrocnemia sp.	70
	Polycentropus flavomaculatus (Pictet, 1834)	49
	Polycentropus irroratus (Curtis, 1835)	11
	Polycentropus kingi McLachlan, 1881	2
	Hydropsyche angustipennis (Curtis, 1834)	5
	Hydropsyche fulvipes (Curtis, 1834)	1
	Hydropsyche instabilis (Curtis, 1834)	27
	Hydropsyche pellucidula (Curtis, 1834)	41
	Hydropsyche saxonica McLachlan, 1884	3
	Hydropsyche siltalai Döhler, 1963	108

MAJOR GROUP	TAXON NAME	No. of occurrences
	Diplectrona felix McLachlan, 1878	10
	Brachycentrus subnubilus Curtis, 1834	4
	Crunoecia irrorata (Curtis, 1834)	9
	Lasiocephala basalis (Kolenati, 1848)	12
	Lepidostoma hirtum (Fabricius, 1775)	43
	Drusus annulatus (Stephens, 1837)	60
	Ecclisopteryx guttulata (Pictet, 1834)	21
	Allogamus auricollis (Pictet, 1834)	1
	Halesus sp.	74
	Hydatophylax infumatus (McLachlan, 1865)	10
	Melampophylax mucoreus (Hagen, 1861)	1
	Micropterna lateralis (Stephens, 1837)	3
	Micropterna sequax McLachlan, 1875	30
	Potamophylax cingulatus group ¹⁰	81
	Stenophylax permistus McLachlan, 1895	2
	Chaetopteryx villosa (Fabricius, 1798)	28
	Anabolia nervosa (Curtis, 1834)	12
	Glyphotaelius pellucidus (Retzius, 1783)	11
	Limnephilus auricula Curtis, 1834	1
	Limnephilus centralis Curtis, 1834	1
	Limnephilus extricatus McLachlan, 1865	6
	Limnephilus hirsutus (Pictet, 1834)	1
	Limnephilus lunatus Curtis, 1834	25
	Limnephilus marmoratus Curtis, 1834	2
	Limnephilus rhombicus (Linnaeus, 1758)	1
	Goera pilosa (Fabricius, 1775)	5
	Silo nigricornis (Pictet, 1834)	11
	Silo pallipes (Fabricius, 1781)	85
	Beraea maurus (Curtis, 1834)	8
	Beraea pullata (Curtis, 1834)	4
	Beraeodes minutus (Linnaeus, 1761)	7

MAJOR GROUP	TAXON NAME	No. of occurrences
	Sericostoma personatum (Spence in Kirby & Spence, 1826)	115
	Odontocerum albicorne (Scopoli, 1763)	69
	Athripsodes sp.	27
	Mystacides sp.	22
	Adicella reducta (McLachlan, 1865)	10
	Oecetis sp.	7
	Apatania muliebris McLachlan, 1866	1
Lepidoptera	Elophila nymphaeata (Linnaeus, 1758)	1
Diptera	Tipula (Yamatotipula) montium group	47
	Tipula (Tipula) paludosa Meigen, 1830	1
	Tipula (Acutipula) vittata Meigen, 1804	2
	Tipula (Acutipula) maxima group ¹¹	17
	Nephrotoma sp.	3
	Limonia sp.	5
	Antocha (Antocha) vitripennis (Meigen, 1830)	12
	Helius (Helius) sp.	1
	Austrolimnophila sp.	7
	Pseudolimnophila sp.	1
	Limnophila sp.	2
	Eloeophila sp.	86
	Phylidorea sp.	5
	Neolimnomyia (Brachylimnophila) sp.	1
	Neolimnomyia (Neolimnomyia) sp.	13
	Pilaria sp.	25
	Hexatoma sp.	2
	Rhypholophus sp.	1
	Molophilus sp.	6
	Paradelphomyia sp.	1
	Pedicia sp.	39
	Dicranota sp.	152
	Tricyphona sp.	2

MAJOR GROUP	TAXON NAME	No. of occurrences
	Psychoda group ¹²	12
	Pericoma group ¹³	103
	Ptychoptera sp.	36
	Dixa nebulosa Meigen, 1830	22
	Dixa puberula Loew, 1849	19
	<i>Dixa maculata</i> complex ¹⁴	31
	Dixella sp.	1
	Anopheles sp.	8
	Culiseta sp.	2
	Culex sp.	2
	Thaumalea sp.	6
	Ceratopogonidae	134
	Prosimulium hirtipes (Fries, 1824)	11
	Prosimulium latimucro (Enderlein, 1925)	1
	Prosimulium tomosvaryi (Enderlein, 1921)	1
	Simulium (Nevermannia) costatum Friederichs, 1920	4
	Simulium (Nevermannia) cryophilum-vernum group ¹⁵	122
	Simulium (Nevermannia) angustitarse group ¹⁶	29
	Simulium (Eusimulium) aureum group ¹⁷	41
	Simulium (Wilhelmia) sp.	6
	Simulium (Simulium) morsitans Edwards, 1915	1
	Simulium (Simulium) noelleri Friederichs, 1920	1
	Simulium (Simulium) reptans (Linnaeus, 1758)	1
	Simulium (Simulium) tuberosum (Lundström, 1911)	1
	Simulium (Simulium) argyreatum group ¹⁸	84
	Simulium (Simulium) ornatum group ¹⁹	99
	Tanypodinae [sub-family]	150
	Diamesinae [sub-family]	48
	Prodiamesinae [sub-family]	61
	Orthocladiinae [sub-family]	200
	Chironomini [tribe]	93

MAJOR GROUP	TAXON NAME	No. of occurrences
	Tanytarsini [tribe]	171
	Oxycera sp.	10
	Vanoyia tenuicornis (Macquart, 1834)	1
	Odontomyia sp.	2
	Chrysophilus erythrophthalmus Loew, 1840	1
	Chrysops sp.	11
	Hybomitra sp.	2
	Tabanus sp.	7
	Atherix ibis (Fabricius, 1798)	6
	Ibisia marginata (Fabricius, 1791)	10
	Clinocerinae	77
	Hemerodrominae	91
	Dolichopodidae	9
	Syrphidae	3
	Sciomyzidae	3
	Ephydridae	11
	Muscidae	12

¹ Polycelis nigra (Müller, 1774) and P. tenuis Ijima, 1884

² *Dugesia polychroa* (Schmidt, 1861) and *D. lugubris* (Schmidt, 1861)

³ Baetis scambus Eaton, 1870 and B. fuscatus (Linnaeus, 1761)

⁴ Caenis luctuosa (Burmeister, 1839) and C. macrura Stephens, 1835

⁵ Nemoura cambrica Stephens, 1836 and *N. erratica* Claassen, 1936

⁶ *Coenagrion puella* (Linnaeus, 1758) and *C. pulchellum* (Vander Linden, 1825)

⁷ Haliplus apicalis C.G. Thomson, 1868, H. fluviatilis Aubé, 1836, H. furcatus Seidlitz, 1887, H. heydeni Wehncke, 1875, H. *immaculatus* Gerhardt, 1877, H. *lineolatus* Mannerheim, 1844 and H. *ruficollis* (DeGeer, 1774)

⁸ Nebrioporus depressus (Fabricius, 1775) and N. elegans (Panzer, 1794)

9 Gyrinus natator (Linnaeus, 1758) and G. substriatus Stephens, 1828

¹⁰ Potamophylax cingulatus (Stephens, 1837) and P. latipennis (Curtis, 1834)

¹¹ *Tipula (Yamatotipula) montium* Egger, 1863, *T. (Yamatotipula) couckei* Tonnoir, 1921 and *T. (Yamatotipula) lateralis* Meigen, 1804

¹² *Psychoda* sp., *Tinearia alternata* (Say, 1824) and *Feuerborniella* sp.

¹³ Pericoma sp., Szaboiella sp., Bazarella sp. and Tonnoiriella pulchra (Eaton, 1893)

¹⁴ *Dixa maculata* Meigen, 1818, *D. nubilipennis* Curtis, 1832 and *D. submaculata* Edwards, 1920

¹⁵ Simulium (Nevermannia) cryophilum (Rubtsov, 1959), S. (Nevermannia) armoricanum Doby & David, 1961, S. (Nevermannia) dunfellense Davies, 1966, S. (Nevermannia) urbanum Davies, 1966, S. (Nevermannia) vernum Macquart, 1826, S. (Nevermannia) juxtacrenobium Bass & Brockhouse, 1990 and S. (Nevermannia) naturale Davies, 1966

¹⁶ Simulium (Nevermannia) angustitarse (Lundström, 1911) and S. (Nevermannia) lundstromi (Enderlein, 1921)

¹⁷ Simulium (Eusimulium) aureum Fries, 1824, S. (Eusimulium) angustipes Edwards, 1915 and S. (Eusimulium) velutinum (Santos Abreu, 1922)

 ¹⁸ Simulium (Simulium) argyreatum Meigen, 1838 and S. (Simulium) variegatum Meigen, 1818
 ¹⁹ Simulium (Simulium) ornatum Meigen, 1818, S. (Simulium) trifasciatum Curtis, 1839 and S. (Simulium) intermedium Roubaud, 1906

Table S6 Details of the 205 sites comprising the calibration dataset and part of the independent test dataset. Eastingand Northing apply to the British National Grid (Geographic Coordinate System OSGB_1936).

DATASET	RIVER NAME	SITE NAME	EASTING	NORTHING
Calibration	Hareshaw Burn	u/s Hareshaw Linn	384110	585522
Calibration	Gelt	Talkin Head	355474	555943
Calibration	Unnamed (Hart)	u/s Hart	446604	534978
Calibration	Lune	d/s Kelleth	365399	505330
Calibration	Smelt Mill Beck	Gilling Wood	415680	505211
Calibration	Annas	u/s Foldgate Farm	312230	492201
Calibration	Hartoft Beck	Birch Farm	475670	495389
Calibration	Unnamed	Hale Hall	345805	435300
Calibration	Unnamed (Cae Mawr)	Tyn-y-coed	242730	385506
Calibration	Hamps	u/s Pethill Farm	406675	352588
Calibration	Erch	Llwyndyrns Farm	238703	341039
Calibration	Llafar	u/s Tal y Bont	285371	335400
Calibration	Ceiriog	d/s Dolwen Farm	314661	333614
Calibration	Gam	Nant-y-Teira	296225	305774
Calibration	Alconbury Brook	d/s Brook Farm (lower farm)	511122	283153
Calibration	Hazeley Brook	Upper Langley	365090	274193
Calibration	Dulas Brook	nr. Brynsadwrn	304487	255674
Calibration	Sor Brook	Poplurs Fram	438090	246483
Calibration	Tyweli	Abergwen Mill	244208	235905
Calibration	Usk	Cwm-Hydfer	285588	226499
Calibration	Unnamed (Poodle Brook)	Poodle Gorse	462115	225846
Calibration	Afon Llia	d/s Aber Llia	293428	214695
Calibration	Lower Clydach	Clydach	267662	203382
Calibration	, Ginge Brook	, d/s West Ginge	444482	187004
Calibration	St Catherine's Brook	Great Moody's Wood	376202	172597
Calibration	Unnamed	Meade Farm	355132	165023
Calibration	Brue	u/s Brewham Lodge Farm	375402	136518
Calibration	Mere	Suddon Farm	246622	113778
Calibration	Mully Brook	Handsford Plantation	265081	115498
Calibration	, Unnamed	u/s Heifer Mill Cottages	344764	104800
Calibration	Rampisham Brook	u/s Uphall	355383	103412
Calibration	Unnamed (Wonston)	u/s Hazelbury Bryan	374282	107382
Calibration	Sid	Plyford Farm	314217	94491
Calibration	Lynher	, u/s North Hill	226723	76698
Calibration	, Common/Carey Burn	d/s Commonburn House	393509	626739
Calibration	Elsdon Burn	d/s High Carrick	392419	595398
Calibration	Kirk Beck	Bush	357468	574803
Calibration	Langley Beck	Raby Castle	412404	520743
Calibration	Trout Beck	d/s Limefitt	341448	502846
Calibration	Staindale Beck	West Worsall	437507	506535
Calibration	Ribble/Gayle Beck	Ingman Lodge	378399	478433
Calibration	Stainfield Beck	Panton	517408	379221
Calibration	Ceirw	Ty-isa-cwm	292491	347242
Calibration	Rhaeadr	, Tyn-y-Wern	307969	328853
Calibration	Rhiw	fish ponds	303482	301039
Calibration	Trannon	Nant y Glyn	294577	290926
Calibration	Blue Lins Brook	u/s Pen y cwm Bridge	307236	281236
Calibration	Camddwr	Lower Crosseynon	312979	272788
Calibration	Unnamed (Hill Farm)	d/s footbridge	568723	257870
Calibration	Unnamed (Bromham)	d/s Firs Farm	498475	248329
Calibration	Cheney Water	Steeple Morden	529317	241963
Calibration	Blackwater/Pant	d/s pumping station	562386	236295
Calibration	Dwr Cleifon	East of Trecenny	177071	225500
Calibration	Dalch	, nr Lapford	274099	108051
Calibration	Unnamed (Emlett)	Kennerleigh Wood	281521	106558
Calibration	Unnamed (Luppit)	u/s Stonehayes Farm	316877	103427

DATASET	RIVER NAME	SITE NAME	EASTING	NORTHING
Calibration	Wash	d/s Whiteway Barn	281012	55410
Calibration	Buckland Stream	d/s Buckland Park	268696	44301
Calibration	North Low/Allerdeanmill Burn	Pump Wood	399084	647082
Calibration	Black Lyne	Sorbies	351234	576949
Calibration	Roe Beck	Roe Farm	340081	540201
Calibration	Caldew	u/s Mosedale	335189	532003
Calibration	Potto Beck	u/s Swainby	447892	501811
Calibration	Bluewath Beck	Lamb Fold Hill	474573	500014
Calibration	Hodge Beck	u/s Tilehouse Bridge	467968	485261
Calibration	Pocklington Beck	d/s Woodhouse Lane	481022	450311
Calibration	Unnamed	Langham	601300	340776
Calibration	Teirw	Ty'n-y-pistyll	317266	336854
Calibration	Ithon	nr. Hafod Fach Farm	308379	281409
Calibration	Whilton Nene	d/s Washbrook Spinney	462384	271011
Calibration	Chwerfri	u/s Dol-v-felin	297526	255385
Calibration	Unnamed	Brook Farm	596990	258036
Calibration	Bourn	u/s sewage works	558275	243180
Calibration	Dulais	Troed v rhiw	270068	235022
Calibration	Honddu	Cwmfforch	301553	237533
Calibration	Alton Water	Hubbard's Hall Farm	613435	239476
Calibration	Unnamed	u/s Skenfrith	343292	219533
Calibration	Yeo	u/s Brockham Bridge	260415	141001
Calibration	Little Silver Stream	South Varde	277006	120857
Calibration	Barle	d/s Mounsey Castle	289013	129501
Calibration	Piddle	u/s Piddletrenthide	370319	101005
Calibration	Bratley Water	Bratley Inclosure	422226	101005
Calibration	Veo (Binneford)	Millmoor Conse	276005	07212
Calibration	Cara	Washwalk Mill	270333	40049
Calibration	Gala		275517	49940 240505
Calibration	Caletwi	Plas Ocilai Dont Abor Coirw	203737	220017
Calibration	Bidne	Point Abel-Gellw	270805	326917
Calibration	Biulio		20/00/	202177
Calibration	Greec		2/1/00	207473
Calibration	Groes	I dili dili - Isal	209037	259889
Calibration	Liwyd		287189	290511
Calibration	Aman	u/s Rhosaininan	273502	214204
Calibration	Gledd		279118	212811
Calibration	Wissey		591499	308836
Calibration		u/s sewage works	586142	251161
Calibration		widgnam wood	567212	254900
Calibration	Unnamed (Nan Trues Hole)	u/s Nan True's Cottage	601822	255928
Calibration	lud	u/s Riverside Farm (poultry)	601051	311459
Calibration	Unnamed	Glebe Farm	474330	272551
Calibration	Unnamed (Wollaston)	Greenfield Lodge	491488	260508
Calibration	Unnamed (Whorne Wood)	Whorne Wood	579439	121040
Calibration	Unnamed	Whiteland Wood	580670	114714
Calibration	East Sour River	Postling	614313	138911
Calibration	Unnamed	Lodge House	608310	139403
Calibration	Unnamed (Minepit Wood)	Minepit Wood	536691	134984
Calibration	Cynon	Llygad Cynon	295251	207732
Calibration	Ruan River	Ruan Lanihorne	189913	41820
Calibration	West Looe	Cliver Wood	221593	62597
Calibration	Yeo	nr Lower Hampson Farm	270923	100951
Calibration	Shobrooke Lake	u/s Moor Farm	286966	101962
Calibration	Dart (Exe trib.)	Ashilford	292494	109214
Calibration	Haddeo	d/s Cuckolds Combe	300109	129992
Calibration	Unnamed (Membury)	u/s Membury Court	326782	103862
Calibration	Unnamed	Unnamed	388018	172723
Calibration	Avon (East)	Anvill's Farm	417084	161372
Calibration	Dipple Water	d/s bridge	235060	117472

DATASET	RIVER NAME	SITE NAME	FASTING	NORTHING
Calibration	Rainsford Brook	Lodge Farm	346096	402795
Calibration	Windle Brook	Woodside Farm	346191	397423
Calibration	Brefi	Cae Fforest	267994	254606
Calibration	Afon Cwerchyr	Abervant	236985	243192
Calibration	Afon Bedw	Nant-goch	235423	250448
Calibration	Unnamed (Champernhaves)	d/s Bowshott Farm	335077	95612
Calibration	Win	Winfrith Newburgh	380561	84618
Calibration	Wigglesworth Beck	d/s Wigglesworth Hall Farm	381694	457532
Calibration	Tarnbrook Wyre	Larpet Wood	356368	454654
Calibration	, Unnamed (Tregurno)	u/s ford	187556	51065
Calibration	Gwydderig	Halfway	283749	232404
Calibration	Unnamed (Perranwell)	d/s Tresamble	175469	39165
Calibration	Unnamed (Trewindle)	d/s Trewindle Farm	214150	62557
Calibration	Barbon Beck	u/s Barbon Manor	364096	482689
Calibration	Kingsdale Beck	Keld Head	369561	476226
Calibration	Birrel Sike	nr. Laverack How	306392	506492
Calibration	Swarth Beck	Boat Haw	309389	510288
Calibration	Cholwell Brook	nr Mary Tavy Church	250825	78584
Calibration	Rathmell Beck	Layhead Farm	380336	459387
Calibration	Swanside Beck	d/s Middop Hall	382881	445403
Calibration	Unnamed (Canworthy)	u/s Canworthy Water	222281	91291
Calibration	Hart Burn	Oakford Bridge	403613	587193
Calibration	Vanycrooks Beck	near Threapland	316235	539827
Calibration	Gill Gooden	d/s Beck House	318295	538800
Calibration	Greengill Beck	Hill Farm	311830	537137
Calibration	Rose Gill	Tallentire	310408	536309
Calibration	Unnamed	Medhone Copse	499795	124488
Calibration	Avon	Horton Farm	405140	163083
Calibration	Unnamed (Stanton)	Stanton Dairy	408547	160186
Calibration	Unnamed (Woodborough)	Ford Wood	410804	160695
Calibration	Wylye	Brixton Deverill	386311	138807
Calibration	Camel	d/s Slaughterbridge	210913	85411
Calibration	Lockholme Beck/Jackson Gill	Ellergill	372701	500993
Calibration	Wyegarth Gill	Shawmire	371476	502665
Calibration	Thackthwaite Gill	Banks	371906	502089
Calibration	Heck Gill	Brunt Hill	374496	502407
Calibration	Hilton beck	Stoneriggs	372692	520521
Calibration	Heltondale Beck	d/s Beckfoot House	351269	520256
Calibration	Swindale Beck	u/s Big Bridge	352703	515028
Calibration	Popping Beck/Redgate Gill	Redgate Farm	381568	510361
Calibration	College Burn	u/s Hethpod	389392	627774
Calibration	Unnamed (Silver Hill)	Little Iridge Farm	574054	126590
Calibration	Unnamed (Coulsey Wood)	d/s cottage	565692	133403
Calibration	Unnamed (Old Soar Manor)	d/s Old Soar Cottages	561906	153940
Calibration	Westworth Stream	Burrows Farm	408306	110299
Calibration	Valency	Boscastle	210194	91232
Calibration	Dockens Water	Linwood Bog	417918	109660
Calibration	The Cam	Hunters Bridge Coppice	365965	110999
Calibration	Unnamed (Droop)	Lower u/s Lower Fifehead Farm	376884	109324
Calibration	Croasdale Brook	Tenter Hill	370653	452945
Calibration	Lodden	Bloomers Farm	382485	128376
Calibration	Leam	Sky Larke Farm	452561	260987
Calibration	Unnamed (Kellinch)	nr Burne Cottage	2/9996	/1046
Calibration	Unnamed	Polford Cottage	276083	92798
Calibration	Unnamed		276000	91378
Calibration	Unnamed (Woodbrooke)	nr Woodbrooke	27/588	90/97
Calibration		KOIOFA PARK	283449	88521
Calibration	Colacove GIII	Deepaale Dotford Earm	338/0/	513/03
Calibration	PIALL BLOOK	Pottora Farm	303013	322009

DATASET	RIVER NAME	SITE NAME	EASTING	NORTHING
Calibration	Unnamed (Hincknowle)	d/s Elcombe Farm	349315	96337
Calibration	Og	d/s Bay Bridge	418889	170822
Calibration	Cunsey Beck/Black Beck	The Croft Campsite	335448	498194
Calibration	Dugoed	d/s road bridge	285890	312646
Calibration	Nant Gochen	Cynwyl Elfed	237134	227579
Test	Heddon	u/s Higher Bumsley	265631	145393
Test	Croglin Water	Scarrowmanwick Fell	361282	547661
Test	Rye	Brewster Hill	452520	492574
Test	Healam Beck	Well	427480	481446
Test	Unnamed (Limebrook)	Arthur Ridges Wood	335575	269456
Test	Quarme	d/s Quarme Bridge	291739	136238
Test	Umborne Brook	d/s Cotleigh Mill	321243	102357
Test	Cardinham Water	Cardinham Woods	210530	67494
Test	Settrington Beck	u/s Kirk Hall	484431	469201
Test	Tresillian	d/s bridge	189790	51906
Test	Unnamed (Little Comfort)	Trevozah Barton	233354	80600
Test	Ash Brook	Ash Bullayne	277425	103666
Test	Unnamed (Rodbourne)	u/s Bottom Farm	393071	182530
Test	Waldon	d/s Old Wood	241253	108220
Test	Whiteleigh Water	Lashbrook Wood	243805	106199
Test	Unnamed (Lashbrook)	near Bason Farm	241069	107806
Test	Small Brook	d/s Pancrasweek	229923	105589
Test	Fflur	Hafod-Rhyd Farm	272403	264057
Test	Warslow Brook	Stoneyfold Farm	406990	357795
Test	Lyd	Lydford Forest	249034	84030
Test	Hindburn	Cragg Wood	363070	467276
Test	Unnamed (Prior Scales)	u/s High Prior Scales Bridge	306385	507505
Test	Penberth River	u/s Treen	139286	23430
Test	Roeburn	u/s Kitten Bridge	360471	467054
Test	Coldkeld Beck	Arras Close	382884	510898
Test	Divelish	u/s Southley Farm	377587	109665



Figure S1. The direction of influence of the three explanatory variables included in the partial canonical correspondence analysis model and the position of taxa in ordination space. The relative contribution of each variable to the model is given by length of the arrows, while their direction indicates the gradient of increasing value. The percentage contribution of each axis to the explanatory power of the pCCA model is also given.





Figure S2. Frequency histogram of total reach-scale benthic fine-grained sediment mass (g.m⁻²) measurements in the calibration and independent test datasets. Note that the x-axis categories are on a log₁₀-scale.



(b)



Figure S3. Relationship between PSI_{sp} and the constituent indices of CoFSI_{sp} (oFSI_{sp} and ToFSI_{sp}) in their assignment of fine sediment-sensitivity scores to taxa common to both indices (n=85). The number of taxa in each combination of index scores is indicated by the size of the circles. PSI_{sp} groups 1-4 equate to 'Highly insensitive', 'Moderately insensitive', 'Moderately sensitive', 'Highly sensitive', respectively. The lower the oFSIsp score assigned to a taxon, the more it is associated with high masses of organic fines in the stream bed. The lower the ToFSI_{sp} score assigned to a taxon, the more it is associated with a high mass of surficial fines and a low % content of organic fines.