

A biomonitoring tool to identify and quantify the impacts of
fine sediment in river and stream ecosystems

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Abstract

In rivers and streams, fine sediment pollution is one of the most commonly attributed causes of ecological degradation. As fine sediment is also an important natural component of these ecosystems, and is variable both temporally and spatially, setting environmental targets and using conventional monitoring to determine where fine sediment is causing ecological degradation, is a complex challenge. An alternative approach is the use of biomonitoring tools, which utilise biota to gauge and track changes in the environment. This thesis examines the current state of sediment-biomonitoring, considers the numerous theoretical and methodological decisions made during tool development, and applies a best practice approach to the development and testing of a sediment-specific tool. An existing biological index, which utilises knowledge of biological and ecological traits, is used as a basis for this development, following an evaluation of its performance over 835 minimally impacted temperate river and stream sites. The index is further developed using empirical data to assign indicator weights to individual taxa. The resulting index, the Empirically-weighted Proportion of Sediment-sensitive Invertebrates (E-PSI) index had moderate to strong correlations with fine sediment in independent test datasets of minimally ($n = 84$) (family-level: $r_s = -0.72$; mixed-level: $r_s = -0.70$; $p < 0.01$) and agriculturally impacted sites ($n = 754$) (family-level: $r_s = -0.68$, $p < 0.01$). The E-PSI index has retained a biological basis, is easily integrated into regulatory agency protocols, and can be applied retrospectively to historic datasets. Despite the improved performance, a high amount of variation was observed between E-PSI and fine sediment cover. This is likely the result of the presence of multiple pressures, uncertainties concerning sediment-biota interactions, and the relatively low precision of sediment quantification techniques.

Due to the prevalence of freshwater non-native invasive species, the effects of North American signal crayfish (*Pacifastacus leniusculus*) invasions on sediment-

biomonitoring and fine sediment are also investigated. Additionally, given the subjective nature and lack of representativeness of many existing sediment quantification techniques, a novel, more objective, verifiable, and non-destructive method is developed and presented as “proof of concept”.

This thesis contributes significantly to the literature concerning biomonitoring tool development and testing, and has resulted in a sediment-specific index that has a stronger correlation with its specific pressure, than the average biomonitoring tool used throughout Europe. The E-PSI index can be used alongside a suite of other biomonitoring tools, to identify the most likely causes of ecological degradation, and inform more targeted monitoring.

Table of contents

Abstract.....	iii
Table of Contents.....	v
List of Figures.....	xiii
List of Tables.....	xvi
List of Equations.....	xviii
List of Supplementary Material	xviii
Acknowledgements.....	xix
Abbreviations and acronyms.....	xx
Author's Declaration	xxi
Chapter 1 Introduction	1
1.1 Background	1
1.1.1 Sources of fine sediment	2
1.1.2 Impacts of elevated fine sediment loads	5
1.1.3 Ecosystem monitoring and management.....	6
1.1.3.1 Conventional monitoring of fine sediment.....	8
1.1.3.2 Biomonitoring.....	9
1.2 Thesis aim and objectives	10
1.2.1 Aim.....	10
1.2.2 Objectives.....	11
Chapter 2 Biomonitoring of rivers and streams	13
2.1 Introduction	14
2.2 The biomonitoring approach	15
2.2.1 Developing a biomonitoring tool.....	19
2.2.2 Biomonitoring and ecological theory	21
2.2.3 Biotic elements used for biomonitoring tools.....	21
2.2.3.1 Biomonitoring approaches using macroinvertebrates.....	23

2.2.3.2	Biomonitoring approaches using fish.....	24
2.2.3.3	Biomonitoring using diatoms	25
2.2.4	Reference-based approach in the UK – RIVPACS/RICT	26
2.2.5	Sampling procedures.....	29
2.2.6	Taxonomy-based indices.....	30
2.2.6.1	Taxonomic resolution of biomonitoring tools	30
2.2.7	Trait-based indices	32
2.2.8	Pressure-specific biomonitoring tools	35
2.2.9	Confounding pressures.....	36
2.2.10	Sediment-specific biomonitoring	41
2.2.10.1	Sediment-specific indices developed globally.....	42
2.2.11	Uncertainties	47
2.3	Conclusion.....	48
Chapter 3 Evaluating the performance of sediment-specific biomonitoring		
tools across a wide range of temperate river and stream ecosystems 51		
3.1	Abstract.....	51
3.2	Introduction	52
3.2.1	Aims and objectives.....	54
3.2.2	Ephemeroptera, Plecoptera and Trichoptera metrics	55
3.2.3	The Proportion of Sediment-sensitive Invertebrates (PSI) index	55
3.2.3.1	Evaluating the theoretical basis and development of the PSI index....	56
3.2.3.2	Uncertainties associated with the PSI index.....	58
3.3	Methods.....	59
3.3.1	Data.....	59
3.3.1.1	Macroinvertebrate data	60
3.3.1.2	Fine sediment data	61
3.3.2	Statistical analyses	62

3.4	Results	63
3.4.1	Biological indices and fine sediment metrics.....	63
3.4.2	Taxonomic resolution.....	66
3.4.3	Discrimination ability of the best performing index	66
3.4.4	Comparisons between the biological indices	67
3.5	Discussion	69
3.5.1	The ability of the best-performing index to indicate fine sediment conditions	69
3.5.2	Effect of taxonomic resolution on the performance of the index	71
3.5.3	Effect of substratum particle size on the performance of the index	72
3.5.4	Independence of the biological indices	72
3.6	Conclusion.....	74
 Chapter 4 Developing an improved biomonitoring tool for fine sediment:		
	Utilising expert knowledge and empirical data.....	76
4.1	Abstract.....	76
4.2	Introduction	78
4.2.1	Biological basis.....	78
4.2.2	Easily integrated into standardised biological sampling and recording methods	80
4.2.3	Developed and tested over the full range of different rivers and streams	82
4.2.4	Strong relationship with pressure of interest	82
4.2.5	Aims	83
4.3	Methods.....	84
4.3.1	Data.....	84
4.3.2	Developing the Empirically-weighted PSI (E-PSI) index.....	89
4.3.2.1	Developing the family level E-PSI index	90
4.3.2.2	Adjustments for family-level sensitivity variations and indicator	

suitability	90
4.3.3 Developing the mixed level E-PSI index	92
4.3.4 Testing the E-PSI index	93
4.3.5 Ability of the E-PSI index to discriminate between fine sediment conditions	93
4.3.6 Comparisons with other biomonitoring tools	93
4.4 Results	95
4.4.1 Biological indices and fine sediment	95
4.4.2 Assigning sediment indicator weightings	97
4.4.3 Ability of the E-PSI to discriminate between fine sediment conditions....	97
4.4.4 Comparisons between biomonitoring tools	98
4.5 Discussion	99
4.5.1 Identifying fine sediment pressure	99
4.5.2 Statistically based sediment-specific tools	101
4.5.3 Sources of uncertainty and natural variation	103
4.5.4 Taxonomic resolution.....	105
4.5.5 Application of the E-PSI index	107
4.6 Conclusion.....	108
 Chapter 5 Testing the Empirically-weighted Proportion of Sediment-sensitive Invertebrates (E-PSI) index with consideration of longitudinal gradients, and in the presence of agricultural pressures.	 110
5.1 Abstract.....	110
5.2 Introduction	111
5.3 Methods.....	113
5.3.1 Data.....	113
5.3.1.1 Agriculturally impacted sites.....	113
5.3.1.2 Minimally impacted sites	116

5.3.2	Testing of the E-PSI index over agriculturally impacted sites.....	116
5.3.3	Fine sediment covariates.....	116
5.3.4	Comparisons with other biomonitoring tools	118
5.4	Results	119
5.4.1	Biological indices and fine sediment	119
5.4.2	Restricting the collinearities between fine sediment and longitudinal gradients.....	120
5.5	Discussion	123
5.5.1	Identifying fine sediment pressure	123
5.5.2	Collinearity between longitudinal gradients and fine sediment	123
5.5.3	Sources of variation.....	124
5.5.3.1	Confounding pressures	124
5.6	Conclusion.....	126
Chapter 6	The impacts of non-native signal crayfish (<i>Pacifastacus leniusculus</i>) on sediment-biomonitoring and fine sediment.....	128
6.1	Abstract.....	128
6.2	Introduction	130
6.3	Methods.....	134
6.3.1	Site selection	134
6.3.2	Substrate composition data	136
6.3.3	Macroinvertebrate data and calculation of sediment biomonitoring indices	136
6.4	Statistical analysis.....	136
6.4.1	Subgroup analyses.....	138
6.4.2	Habitat heterogeneity.....	139
6.4.3	Percentage of coarse substrate	139
6.4.4	Shifts in community composition.....	139

6.5	Results	140
6.5.1	Sediment-biomonitoring and fine sediment	140
6.5.2	Habitat heterogeneity.....	142
6.5.3	Coarse substrate	142
6.5.4	Shifts in community composition.....	143
6.6	Discussion	148
6.6.1	Fine sediment.....	148
6.6.2	Biomonitoring tool outputs	149
6.6.3	Habitat heterogeneity.....	150
6.6.4	Coarse substrate	151
6.6.5	Community composition	151
6.6.6	Fine sediment quantification	152
6.6.7	Limitations	153
6.6.8	Reliability of biomonitoring in the presence of invasive species	153
6.7	Conclusion.....	154
Chapter 7	A novel technique for quantifying submerged deposited fine sediment in rivers and streams	156
7.1	Abstract.....	156
7.2	Introduction	157
7.3	Methods.....	164
7.3.1	Site selection	164
7.3.2	Visual estimates	168
7.3.3	Digital image collection	169
7.3.4	Image processing	171
7.3.5	Estimate-based Digital Image Analysis (eDIA).....	171
7.3.6	Software-based Digital Image Analysis (sDIA).....	172
7.3.7	Precision of Digital Image Analysis techniques.....	173

7.3.8	Fine sediment spatial variation	174
7.3.9	Biological relevance.....	174
7.3.10	Statistical analysis	175
7.4	Results	176
7.4.1	Comparison of methods.....	176
7.4.2	Precision of software-based and estimate-based Digital Image Analysis	178
7.4.3	Fine sediment spatial variation	179
7.4.4	Biological relevance.....	179
7.5	Discussion	180
7.5.1	Comparison of methods.....	180
7.5.2	Precision of software-based and estimate-based Digital Image Analysis	183
7.5.3	Fine sediment spatial variation	184
7.5.4	Biological relevance.....	185
7.6	Conclusion.....	185
Chapter 8	Synthesis and Conclusions	187
8.1	The rise of biomonitoring for environmental assessment.....	187
8.2	Developing biomonitoring tools: Expert opinion versus statistical approaches.....	187
8.3	Best-practice evaluation of biomonitoring tools.....	189
8.4	Uncertainties in the development and testing of biomonitoring tools .	190
8.5	Biomonitoring tools are only as good as their reference-condition models	191
8.6	The future of biomonitoring.....	191
8.7	Further research	194
	References.....	196

Appendices	221
Appendix A. Indicator weights (W) for the family-level Empirically-weighted Proportion of Sediment-sensitive Invertebrates (E-PSI) index.	221
Appendix B. Indicator weights (W) for the mixed-level Empirically-weighted Proportion of Sediment-sensitive Invertebrates (E-PSI) index.	224
Appendix C. Relationship between % fine sediment and a) $E-PSI_{fam69}$ b) $E-PSI_{mixed}$ and c) CoFSI, across 84 minimally impacted river and stream sites.	243
Appendix D. Coefficients, p -values and confidence intervals for gradual step change linear mixed effect models and linear quantile mixed models of PSI_{fam} , $E-PSI_{fam69}$ and fine sediment, for all sites, and sites split into subgroups based on their (i) habitat heterogeneity and (ii) percentage of coarse substrate.	245
Appendix E. Plots of linear mixed effects models for time series data of the Proportion of Sediment-sensitive Invertebrates (PSI) index	247
Appendix F. Plots of linear mixed effects models for time series data of Empirically-weighted Proportion of Sediment-sensitive Invertebrates (E-PSI) index.	251
Appendix G. Plots of linear mixed effects models for time series data of percentage fine sediment.	255

List of Figures

Figure 1.1. A conceptual diagram highlighting the sources of fine sediment delivery to surface waters.	3
Figure 1.2. Proportion of classified river and lake water bodies in different river basin districts holding less than good ecological status for rivers and lakes in 2009.....	7
Figure 2.1. Proportion of biotic elements used in riverine biomonitoring schemes throughout Europe.	22
Figure 2.2. Taxonomic resolution utilised by biomonitoring tools in rivers in Europe..	31
Figure 2.3. “Wedge-shaped” relationship often observed between a single pressure and response.....	37
Figure 2.4. Consideration of uncertainties in 107 riverine biomonitoring tools used throughout the EU.....	47
Figure 3.1. The process of calculating the observed (O) and expected (E) Proportion of Sediment-sensitive Invertebrates (PSI) index scores for a site using the “River Invertebrate Classification Tool” approach, highlighting areas of uncertainty.	58
Figure 3.2. Distribution of RIVPACS ‘reference condition’ sites.....	60
Figure 3.3. Relationship between PSI_{sp} score (combined seasons) and (a) % sand, silt and clay substratum (based on visual estimates) (b) suspended solids (mean; $n \geq 12$), derived from RIVPACS data.	64
Figure 3.4. Boxplots showing the relationship between grouped PSI_{sp} scores and % sand, silt and clay substratum (based on visual estimates) derived from minimally impacted RIVPACS sites, using combined seasons data.....	67
Figure 4.1. Distribution of minimally impacted sites throughout the UK that formed the training dataset for development of the E-PSI index.	87
Figure 4.2. Distribution of minimally impacted sites throughout the UK that formed the independent dataset for testing of the E-PSI index.	88
Figure 4.3. Relationship between (a) $E-PSI_{fam69}$ and fine sediment, and (b) $E-PSI_{mixed}$	

and fine sediment, across the independent minimally impacted test dataset.....	96
Figure 4.4. Boxplot showing the relationship between percentage fine sediment (based on visual estimates) and grouped E-PSI _{fam69} and PSI _{fam} scores for the minimally impacted test dataset.....	98
Figure 5.1. Location of sites from the agriculturally impacted test dataset.....	115
Figure 5.2. Relationship between E-PSI _{fam69} and fine sediment across agriculturally impacted river and stream sites	119
Figure 5.3. Relationship between E-PSI _{fam69} and fine sediment across minimally impacted river and stream sites	120
Figure 6.1. Locations of the selected river sites throughout England with records of signal crayfish (<i>Pacifastacus leniusculus</i>).	135
Figure 6.2. Conceptual diagram of the gradual step change modelling approach used in this study	137
Figure 6.3a-g. Non-metric dimensional scaling centroid plots of benthic macroinvertebrate community composition for all sites, and sites split into subgroups based on their (i) habitat heterogeneity and (ii) percentage of coarse substrate	144
Figure 7.1. Schematic diagram of the stratified and systematic site selection process, highlighting the criteria applied and the ten selected sites.....	166
Figure 7.2. Locations of the ten sampling sites throughout the UK, which were selected using a stratified and systematic process.....	168
Figure 7.3. Schematic diagram of the sample design (five equidistant transects perpendicular to the flow direction, each with three equidistant survey points) used for collecting digital images of deposited fine sediment on streambeds.	169
Figure 7.4. Examples of DIA images (top row) and these images with fine sediment highlighted (bottom row)	172
Figure 7.5. Comparison between a) visual estimates and software-based DIA, b) software-based DIA and estimate-based DIA, and c) visual estimates and estimate-based DIA, at ten sites sampled over three seasons	177

Figure 7.6. Differences between fine sediment values from two operators using a) software-based DIA, and b) estimate-based DIA techniques.	178
Figure 7.7. Mean percentage fine sediment values from software-based Digital Image Analysis of five transects (each comprising three images), from ten sites over three seasons	179
Figure 7.8. Relationship between % fine sediment (estimate-based Digital Image Analysis) and the mixed-level Empirically-weighted PSI (E-PSI _{mixed}) index.	180

List of Tables

Table 2.1. Typology based approaches to biomonitoring specified by the Water Framework Directive using obligatory and optional environmental characteristics.	17
Table 2.2. Characteristics of the River InVertebrate Prediction And Classification System sites.	27
Table 2.3. List of biological and ecological traits	33
Table 2.4. Confounding pressures (physical, chemical and biological) commonly associated with fine sediment in rivers and streams globally, and their effect on macroinvertebrates.	38
Table 2.5. Modified from Bonada et al. (2006) to include criteria relevant to pressure-specific biomonitoring tools and with additional criteria added.	46
Table 3.1. The Fine Sediment Sensitivity Ratings (FSSRs) and relevant abundance related weights that form the basis of the PSI index.....	56
Table 3.2. Spearman's correlation coefficients between combined seasons of biological indices and fine sediment metrics at minimally impacted RIVPACS sites.	65
Table 3.3. Spearman's correlation coefficients for relationships between biological indices from combined seasons, at minimally impacted streams in RIVPACS.	68
Table 4.1. Characteristics of the 835 RIVPACS sites.....	85
Table 4.2. Sensitive macroinvertebrate families removed from E-PSI _{fam69} calculations, based on a 33% threshold being applied to their corresponding sediment values.....	92
Table 4.3. Spearman's rank correlation coefficients (2 d.p.) between versions of PSI, E-PSI, LIFE, CoFSI and fine sediment, in the minimally impacted test dataset.....	95
Table 4.4. Spearman's rank correlation coefficients (2 d.p.) between biological indices, in the minimally impacted test dataset ($n = 84$).	99
Table 5.1. Discharge categories used by the Environment Agency.....	117
Table 5.2. Spearman's rank correlation coefficients between the best performing versions of the E-PSI index, LIFE and fine sediment, in the minimally impacted dataset,	

using all sites with discharge data.....	121
Table 5.3. Spearman’s rank correlation coefficients between the E-PSI index, LIFE and fine sediment, in the agriculturally impacted dataset.	122
Table 6.1. Coefficients, <i>p</i> -values and confidence intervals for gradual step change linear mixed effect models of PSI_{fam} , $E-PSI_{fam69}$ and fine sediment, for all sites, and sites split into subgroups based on their (i) habitat heterogeneity and (ii) percentage of coarse substrate	141
Table 6.2. Analysis of Similarities (ANOSIM) of community composition pre-invasion versus post-invasion for all sites based on NMDS centroid plots using Bray-Curtis similarity coefficients (R), and sites split into subgroups based on their (i) habitat heterogeneity and (ii) percentage of coarse substrate	145
Table 6.3. Results of SIMPER analyses of community composition pre-invasion versus post-invasion, based on NMDS centroid plots using Bray-Curtis similarity coefficients, for all sites, and sites split into subgroups based on (i) habitat heterogeneity and (ii) percentage of coarse substrate.....	147
Table 7.1. Various approaches used globally to quantify submerged, surficial fine sediments in rivers and streams.	159
Table 7.2. Characteristics of the ten stream sites selected for their deposited fine sediment conditions, and suspended solids concentrations based on data from the RIVPACS IV database.....	167

List of Equations

Equation 3.1. Formula used to calculate PSI scores using abundance-weighted scores	56
Equation 4.1. Formula used to calculate E-PSI scores.....	89
Equation 6.1. Formula to calculate the Shannon Diversity Index (H) for substrate composition	139
Equation 7.1. Formula used to calculate the length of streambed (L ; metres) captured at a known camera height.....	171
Equation 7.2. Formula to calculate the overall percentage cover of fine sediment in digital images.....	173

List of Supplementary Material

Supplementary Material A. Data of biomonitoring tool scores and fine sediment from the RIVPACS database utilised in Chapter 3.

Supplementary Material B. Independent test dataset of biomonitoring tool scores and fine sediment utilised in Chapter 4.

Supplementary Material C. Dataset of agriculturally impacted sites, and minimally impacted sites (with stream power) utilised in Chapter 5.

Supplementary Material D. Data of signal crayfish (*Pacifastacus leniusculus*) invasions utilised in Chapter 6.

Supplementary Material E. Digital Image Analysis, visual estimate, and biomonitoring tool scores utilised in Chapter 7.

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Abbreviations and acronyms

DIA – Digital Image Analysis

EA – Environment Agency

E-PSI – Empirically-weighted Proportion of Sediment-sensitive Invertebrates

eDIA – estimate-based Digital Image Analysis

fam – family taxonomic level

fam69 – family taxonomic level using 69 macroinvertebrate families

FSSR – Fine Sediment Sensitivity Rating

ITS – Interrupted Time Series

LIFE – Lotic invertebrate Index for Flow Evaluation

lme – linear mixed effect

lqmm – linear quantile mixed model

PSI - Proportion of Sediment-sensitive Invertebrates

RICT – River Invertebrate Classification Tool

RIVPACS – River Invertebrate Prediction and Classification System

sDIA – software-based Digital Image Analysis

mixed – mixed taxonomic level using 415 taxa (family-, genus-, or species-level)

SS – suspended solids

WFD – Water Framework Directive

Author's Declaration

I declare that the research contained in this thesis, unless otherwise formally indicated within the text, is the original work of the author. The thesis has not been previously submitted to this or any other university for a degree, and does not incorporate any material already submitted for a degree.

Signed:

Dated: 27/10/2017

Chapter 1 Introduction

1.1 Background

Freshwater ecosystems are considered some of the most impacted ecosystems globally (Sala et al. 2000, Dudgeon et al. 2006). The growing global human population is likely to increase the pressures on freshwater ecosystems, particularly as humans disproportionately inhabit areas close to freshwaters, significantly altering riparian zones and land cover (Sala et al. 2000). With increased demand for food, energy, water and other natural resources, activities such as agricultural intensification, energy production, forestry, mining, water abstraction and waste disposal, are likely to put further strains on these ecosystems. According to analyses of global biodiversity loss, freshwater ecosystems are significantly impacted (Dudgeon 2014, Gatti 2016) and perhaps to a greater extent than terrestrial ecosystems (Sala et al. 2000). The main threats to freshwater ecosystems can be categorized as overexploitation, water pollution, flow modification, destruction or degradation of habitat and the introduction of invasive species (Dudgeon et al. 2006).

Fine sediment is one of the most commonly attributed causes of ecological impairment globally (Richter et al. 1997, USEPA 2000). Fine sediment is often considered to comprise of both inorganic and organic materials less than 2mm in size, and is (i) integral to global denudation (Walling and Fang 2003, Bilotta et al. 2012a), (ii) an important component of global biogeochemical cycles (Schlesinger and Melack 1981, Ittekkot and Zhang 1989, Mainstone and Parr 2002), and (iii) an essential component of freshwater ecosystems (Wood and Armitage 1997, Owens et al. 2005, Yarnell et al. 2006). Although sediment is essential for habitat heterogeneity and ecological functioning (Wood and Armitage 1997, Owens et al. 2005, Yarnell et al. 2006), when anthropogenic activities cause a significant deviation in the dynamics of fine sediment

from 'natural' or 'reference' conditions, changes to the physical, chemical and biological conditions in freshwater ecosystems can occur and result in ecological degradation (Cordone and Kelley 1961, Gammon and Office 1970, Peddicord 1980, Alabaster and Lloyd 1982, Newcombe and Macdonald 1991, Ryan 1991, Wood and Armitage 1997, Owens et al. 2005, Bilotta and Brazier 2008). These changes can also lead to a significant decline in the ecosystem services provided by freshwater ecosystems, which have been valued at over \$2.5 trillion per annum globally (rivers and lakes only) (Costanza et al. 2014). It is therefore essential that fine sediment pressure is monitored and managed in order to minimise these impacts.

The European Environment Agency promotes the use of the Driver, Pressure, State, Impact, Response (DPSIR) framework to assist environmental reporting and communication with policy makers. In this thesis, the term 'pressure' is used as intended by the DPSIR framework (Borja et al. 2006), and following the interpretation by Friberg et al. (2010). Pressures are the ways that drivers (e.g. land use, industry and agriculture) are expressed and the specific ways that ecosystems are perturbed (Borja et al. 2006). Although applied scientists often use the term pressure and stressor interchangeably, here, a "stressor" or "stress" is used to describe one aspect of a pressure. Following these definitions, organic pollution is considered a "pressure", whereas the resulting oxygen depletion (one aspect of the pressure; due to microbial decomposition of the organic matter) is considered a stressor (Friberg 2010).

1.1.1 Sources of fine sediment

Sediment loads vary both spatially and temporally (Bilotta et al. 2012a, Grove et al. 2015), with the amount of fine sediment delivered to, and mobilised in rivers, depending largely on catchment land cover, catchment geology, riparian vegetation, topography, hydro-geomorphology, hydro-meteorology, and human interventions (Allan

2004, Vercruyse et al. 2017).

Non-natural sources of fine sediment are often categorized as either point or diffuse sources (Figure 1.1). Point sources, for example sewage treatment works or industrial effluent outputs, are often relatively easy to isolate and control, but only if legislative requirements or financial incentives exist. In contrast, diffuse sediment pollution sources (such as those resulting from agricultural activities) are difficult to isolate, as they occur over large areas, making them challenging to monitor and control (Collins and McGonigle 2008).

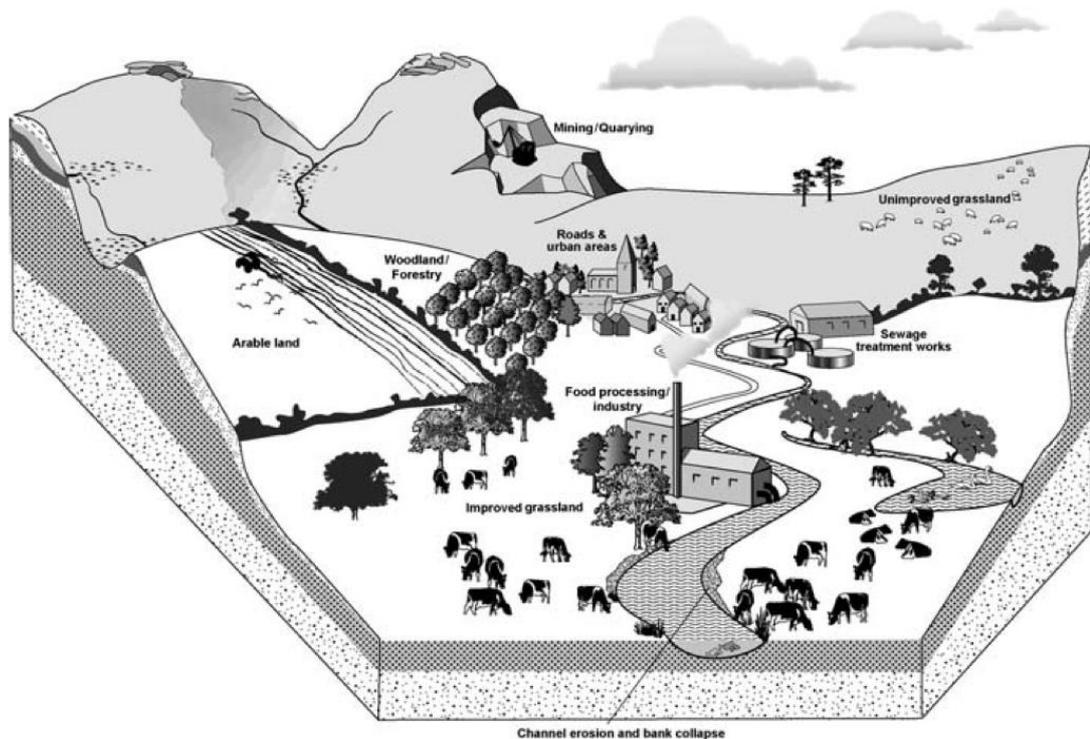


Figure 1.1. A conceptual diagram highlighting the sources of fine sediment delivery to surface waters (Bilotta et al. 2010).

Typically, precipitation and the subsequent erosion and surface run-off result in stream discharge and suspended sediment concentrations having similar seasonal variations (in the absence of point source pollution; e.g. wastewater treatment plants), albeit

displaying some hysteresis (Collins and Walling 2004). In addition to external sources of fine sediment, in-stream processes can result in fine sediment suspension and deposition (Wood and Armitage 1997). For example, both natural (low flows, droughts, floods etc.) and unnatural (abstraction, channelization etc.) flow regimes can lead to increased channel erosion or deposition, resulting in downstream effects (Simon and Rinaldi 2006, Church and Ferguson 2015). In UK rivers and streams, suspended sediment concentrations at minimally impacted sites have mean background concentrations of between 1 mg L⁻¹ and 26 mg L⁻¹ (Bilotta et al. 2012a). However, suspended sediment concentrations can be substantially higher at impacted sites and during storm events when concentrations can exceed 1000 mg L⁻¹ (Walling 1977, Old et al. 2003). Stream sediment dynamics are predominantly influenced by particle size and the amount of energy in the stream (i.e. stream power), with coarser particles being preferentially deposited, often resulting in a downstream fining (Walling et al. 2000). If the rate of sediment supply or the sediment particle size exceeds the streams transport capacity at a given point, sediment will settle out, becoming deposited or being transported via bedload (Einstein 1950). This deposited or bedload sediment can be resuspended and entrained if exposed to turbulence that provides sufficient bed shear stress (Lisle and Hilton 1992, Lopez and Garcia 2001). Longitudinal gradients and reach scale variations in a streams transport capacity result in spatial variation of substrate composition and the formation and perpetuation of depositional zones and erosional zones. At the patch scale, streambed roughness (including substrate and in-stream objects; e.g. woody debris, leaf litter) influences lift and drag forces and introduces hiding effects, resulting in complex spatial patterns of sediment entrainment and entrapment (Brayshaw et al. 1983, Garcia and Parker 1991, Niño et al. 2003).

Certain species can act as ecosystem engineers, and are important agents of geomorphological change (Johnson et al. 2010, 2011, Harvey et al. 2014, Rice et al. 2016). Anthropogenic alterations to flow and other hydromorphological characteristics

are widespread and can lead to increased channel erosion, but also exacerbate existing fine sediment pressures locally (e.g. through impoundment) (Wood and Armitage 1997). Studies of source apportionment for fine sediment in the UK show that the dominant sources vary by catchment (Walling 2005, Davis and Fox 2009, Walling 2013, Smith and Blake 2014). Nevertheless, throughout England and Wales, recent studies utilising predictive models have shown that agriculture is typically the largest source of fine sediment to rivers and streams followed by in-stream sources (channel banks), diffuse urban pollution and wastewater treatment plants (Zhang et al. 2014). However, these models neglect the potential influence of biogeomorphic agents on sediment dynamics, such as the activities of native (Petticrew and Albers 2010) and invasive species (Harvey et al. 2011, Fei et al. 2014).

1.1.2 Impacts of elevated fine sediment loads

The implications of elevated suspended and deposited sediments for aquatic biota have been extensively studied and reviewed (Bilotta and Brazier 2008, Kemp et al. 2011, Jones et al. 2012). Deviations in fine sediment dynamics can impact all trophic levels, including periphyton, macrophytes, invertebrates and fish. Periphyton and macrophytes have shown reduced primary productivity (Nieuwenhuys and LaPerriere 1986, Lloyd 1987), damage due to abrasion/scour (Francoeur and Biggs 2006), and reduced biomass (Birkett et al. 2007), when exposed to fine sediment in various concentrations and durations. These impacts can affect higher trophic levels in the ecosystem, for example through a reduction in food availability. Invertebrates are impacted by fine sediment as a result of increased mortality and drift (Rosenberg and Wiens 1978, Shaw and Richardson 2001) following the direct impacts of physical abrasion, clogging/damage of filter-feeding mechanisms or other sensitive appendages (Alabaster and Lloyd 1982), and burial of taxon with low motility (Wood et al. 2005). In addition, the indirect effects of fine sediment can impact invertebrates, through light

limitation (increased turbidity), habitat modification (filling of interstitial spaces thus reducing refugia, reducing habitat heterogeneity and creating a surface drape of fines), decreased oxygen (as a result of increased decomposition), introduction of toxic substances, and changes to predator-prey interactions and feeding habits (reviewed in Bilotta and Brazier 2008, Jones et al. 2012). Fish are also subjected to similar stressors from elevated levels of fine sediments (Kemp et al. 2011). However, in addition, it has also been shown to result in increased physiological stress (Redding and Schreck 1983), lower disease resistance, and disrupt development and growth (Shaw and Richardson 2001). In particular, the impacts on salmonids have received a large amount of interest, both in terms of research and management (Soulsby et al. 2001, Suttle et al. 2004, Greig et al. 2005, Sear et al. 2008), largely due to the economic importance of salmonid fisheries, and the cultural importance of these species.

1.1.3 Ecosystem monitoring and management

Monitoring has three main functions; (i) to identify when a system is deviating from the desired condition, (ii) to measure the effectiveness of management initiatives (e.g. restoration), and (iii) to detect the impacts of perturbations and disturbances (Legg and Nagy 2006). In the past, environmental monitoring has largely consisted of isolated evaluations that consider a limited number of parameters (Brack et al. 2009). However, in Europe, a more holistic approach to monitoring has been adopted as a result of the EU Water Framework Directive (Directive 2000/60/EC) (WFD). The directive requires that member states monitor and manage their surface waters to achieve good ecological status (Brack et al. 2009). Ecological status is a measure of the quality of the structure and functioning of an ecosystem. The most recent reporting of Europe's surface waters (Figure 1.2), shows that the majority of river basin districts have between 30% and 90% of water bodies with an ecological status below "good" (European Environment Agency 2015). Member states are under a legal obligation to

monitor, classify, and manage its surface waters by considering biological elements along with supporting chemical, hydrological and morphological parameters (Bennett et al. 2011). The WFD recognises the need for an integrated approach to water management and places a firm emphasis on the use of bioassessment techniques to determine the ecological quality of aquatic ecosystems (Logan and Furse 2002).

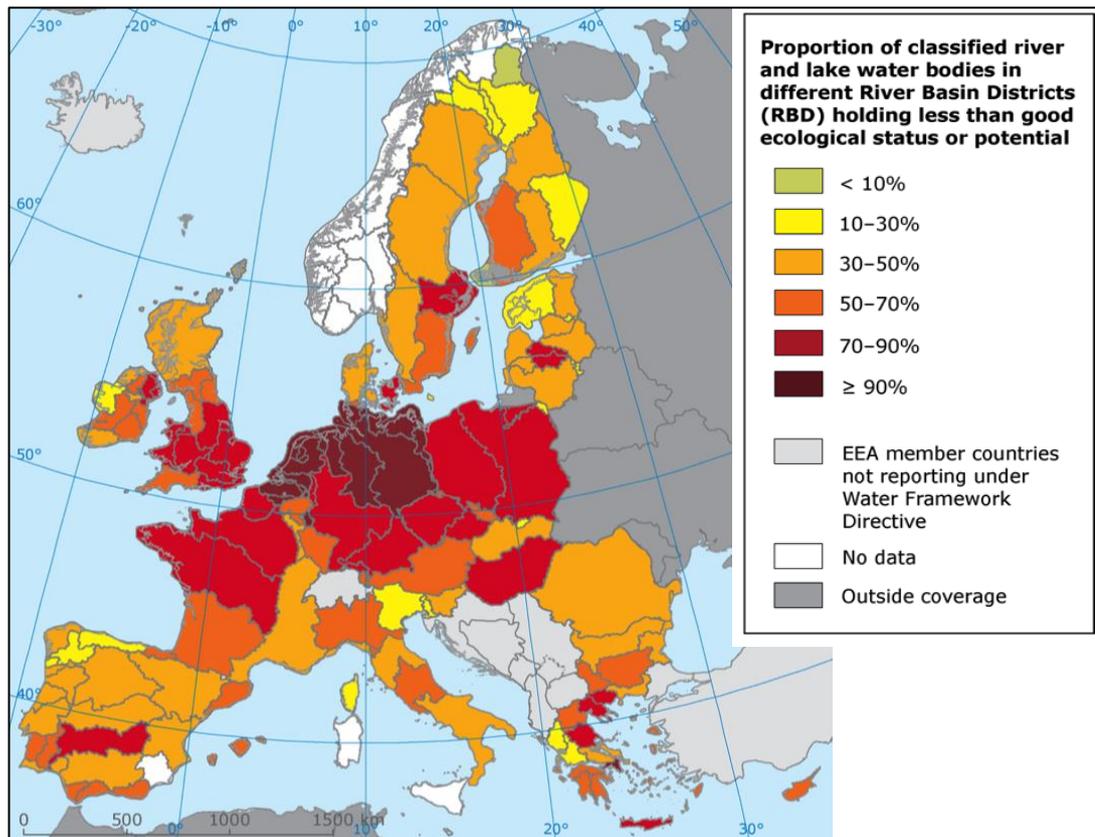


Figure 1.2. Proportion of classified river and lake water bodies in different river basin districts holding less than good ecological status for rivers and lakes in 2009 (European Environment Agency 2015).

In 2016, the UK voted to leave the European Union, and triggered Article 50 of the Lisbon Treaty, in March 2017; commencing a two-year period of negotiation. Part of these negotiations will involve decisions concerning the future of the UK’s environmental legislation (Reid 2016). Therefore, at the time of writing, the future of

legislation relating to the UK's freshwater ecosystems is uncertain. Due to EU law being deeply embedded within UK law, and given the complexity of processes involved in law-making, the most likely short-term outcome in the event of the European Communities Act being repealed, is that a "savings" clause would ensure that existing legislation remains, until any decisions are made to repeal a particular act (Rodgers 2016). Despite this, in the long term it is likely that leaving the EU will have major implications for land and water resource management, as well as the conservation of biodiversity within Europe, with opportunities for improving aspects of existing legislation, as well as risks of legislation being weakened (Rodgers 2016).

1.1.3.1 Conventional monitoring of fine sediment

Previous attempts to address the degradation caused by elevated sediment loads in the EU, resulted in a suspended solids (SS) blanket guideline of 25 mg L⁻¹, initially under the Freshwater Fish Directive and subsequently transposed into the WFD. However, the guideline was removed, partly due to a number of inherent problems: (i) Monitoring SS requires an estimation of mean annual SS which involves a substantial resource investment particularly given the frequency of data required to capture the high variability of SS loads (Walling 2005); (ii) it is not only the concentration but also the duration, geochemical composition and particle size distribution that are important (Bilotta and Brazier 2008, Collins et al. 2011); (iii) previous research has shown SS concentrations of less than 25 mg L⁻¹ can have negative biological impacts on all trophic levels (Bilotta and Brazier 2008, Bilotta et al. 2012b); (iv) a study of 638 'reference condition' sites in the UK found mean background SS concentrations of less than 12.5 mg L⁻¹ at 78% of the sites, with one ecosystem type having mean background concentrations of higher than the 25 mg L⁻¹ guideline (Bilotta et al. 2012a). For these reasons the guideline is recognised as being incapable of indicating good ecological status with regard to sediment pressure (D'Arcy and Frost 2001, Collins et

al. 2011, Bilotta et al. 2012a).

Kefford et al. (2010) suggested that setting targets for deposited sediments may be more ecologically meaningful as many of the impacts on aquatic biota are from sedimentation. However, the limited understanding of deposited sediment dynamics, the lack of an accepted, standardised method, and the subjective and/or semi-quantitative methods used to quantify deposition (Naden et al. 2003), has caused problems for determining a biologically relevant Environmental Quality Standard (EQS). In addition, sediment deposition is a natural process, which varies both temporally and spatially, so any EQS for deposited sediment would need to be not only environment-specific but also habitat-specific (e.g. accounting for pools, glides, riffles). Alternatively, a reference-based approach could be used, with observed substrate compositions being compared to the expected substrate compositions at a site with similar environmental characteristics. The problem with these approaches is determining the frequency of sampling that is sufficient, and also whether the standards are biologically relevant (Crane 2003).

1.1.3.2 Biomonitoring

Recently, partly as a result of the WFD, there has been an increase in the use of biomonitoring approaches, that focus on low-frequency (lower-cost) biomonitoring techniques, defined broadly as “the use of biota to gauge and track changes in the environment” (Wright et al. 1993, Gerhardt 2000, Friberg et al. 2011). Biomonitoring relies on organisms to indicate the effect of a pressure through their presence/absence, abundance or behaviour. The most commonly used groups of indicator organisms are macroinvertebrates, fish and algae (Resh 2008). Depending on the taxonomic group that is utilised, biota can provide a number of advantages over conventional monitoring approaches. For example, biota have a wide range of ecological

requirements/sensitivities, different lifecycle lengths/strategies, and spatial distributions that make them useful as indicators of different types of pressure, and allow them to integrate the effects of a pressure over space and time. For this reason biomonitoring is particularly useful when the pressure or stressors are intermittent or exhibit high temporal variability. Monitoring such pollutants is extremely challenging and costly using conventional techniques, and may not provide a biologically relevant metric (Bonada et al. 2006, Friberg et al. 2011). By monitoring biotic responses directly, biomonitoring can help to evaluate the ecological condition of a water body (Bonada et al. 2006), and is considered a vital tool for assessing the extent of impacts to freshwater ecosystems.

One common criticism of biomonitoring tools is that often the cause of a deviation in biotic community composition/index scores is unclear, and cannot be assigned to a single pressure (Clews and Ormerod 2009). The difficulties of linking cause and effect is a common challenge for monitoring and management, which can hinder the decision-making process (Harris and Heathwaite 2012, Bilotta et al. 2015), and may be exacerbated by the use of poorly tested biomonitoring tools. As such, the development and testing of pressure-specific biomonitoring tools, able to diagnose the cause of deviations in biotic community composition, is highly desirable.

1.2 Thesis aim and objectives

1.2.1 Aim

To develop a sediment-specific biomonitoring tool, able to identify and quantify the impacts of fine sediment across a wide range of temperate rivers and streams.

1.2.2 Objectives

i) Conduct an evaluation of the performance of existing, published, sediment-specific biomonitoring tools.

Independent testing of biomonitoring tools over the range of environments to which they will be applied, is commonly lacking in the literature (Birk et al. 2012). Therefore, current biomonitoring approaches will be assessed in terms of their theoretical, technical, and empirical basis. Using existing large datasets with macroinvertebrate and fine sediment data, those indices that have potential for application in the UK will be evaluated in terms of their relationship with fine sediment metrics, and their predictive capabilities. This evaluation will allow for best practice to be followed in the development of a new or modified tool, which is able to meet the aims of this project.

ii) Using empirical data and modelling, further develop the best performing tool identified as having the greatest potential for application in temperate rivers and streams.

Following the evaluation of current biomonitoring tools (Objective 1), the tool deemed to have the greatest potential for application as a sediment-specific tool, will be further developed. This process will involve consideration of the technical choices that may affect the performance of a biomonitoring tool, for example the treatment of macroinvertebrate abundance, taxonomic resolution and sampling method (Gayraud et al. 2003). The tool will be further developed using the previous evaluation of the theoretical basis for the tool and existing ecological knowledge combined with empirical data to assign taxon-specific indicator weights. In addition to the development of the new/modified tool, a best practice approach will be identified and described.

iii) Test the biomonitoring tool over a wide range of unimpacted and impacted river and stream sites throughout the UK.

Any potential improvements made to the performance of a tool will be assessed by comparisons with the index in its original form, in terms of correlations to a biologically relevant fine sediment metric (Feio et al. 2009). It is important in this type of performance testing that the data/sites are independent of the development stages of the model (Elith et al. 2005). This will be made possible using opportunistic data analysis in the form of monitoring datasets, enabling the model to be tested across the full range of ecosystem ‘types’ and sediment pressure gradients to which it will be applied (Vaughan et al. 2009). This includes testing the index over sites that are subjected to multiple pressures, such as the presence of invasive species, or those pressures associated with agricultural activities, and attempting to disentangle the numerous confounding pressures associated with fine sediment.

iv) Establish a measurement technique for deposited fine sediments to feed into better calibration of sediment-specific biomonitoring tools.

Data from bespoke monitoring of a set of systematically selected field sites, which represent a range of river and stream ecosystems, will be used to provide “proof of concept” for a novel technique of quantifying fine sediment that is verifiable, non-destructive, and crucially, more objective than many existing techniques. Comparisons will be made to the visual estimate technique, one of the most commonly used methods of quantifying fine sediment (Sennatt et al. 2006, Descloux et al. 2010). The biological relevance of the technique will also be considered by evaluating the techniques correlation with the newly developed biomonitoring tool.

Chapter 2 Biomonitoring of rivers and streams

Part of the content of this chapter is reproduced from the following:

Turley, M., G. Bilotta, G. Arbocicute, R. Chadd, C. Extence, and R. Brazier. 2017. Quantifying Submerged Deposited Fine Sediments in Rivers and Streams Using Digital Image Analysis. *River Research and Applications* DOI: 10.1002/rra.3073.

Turley, M. D., G. S. Bilotta, R. P. Chadd, C. A. Extence, R. E. Brazier, N. G. Burnside, and A. G. Pickwell. 2016. A sediment-specific family-level biomonitoring tool to identify the impacts of fine sediment in temperate rivers and streams. *Ecological Indicators* 70:151-165.

Turley, M. D., G. S. Bilotta, C. A. Extence, and R. E. Brazier. 2014. Evaluation of a fine sediment biomonitoring tool across a wide range of temperate rivers and streams. *Freshwater Biology* 59:2268-2277.

Contributions: MT led the analysis and writing of this chapter (and publications), supported and supervised by GB (primary supervisor), CE & RB (external supervisors). RC helped with the identification of macroinvertebrates and gave advice on invertebrate ecology. GA was the independent operator for Digital Image Analyses. NB assisted with obtaining data and matching sampling locations using GIS. AP provided assistance obtaining data and advice on macroinvertebrate identification.

2.1 Introduction

Increasingly, freshwater managers and policy-makers require conservation measures that protect and improve biodiversity, whilst minimising the costs and societal impacts on users and inhabitants of catchments (Turak and Linke 2011). This includes minimising the costs associated with conventional monitoring of water quality parameters, such as suspended and deposited sediment. Monitoring of physico-chemical water quality parameters can be relatively expensive and time-consuming; there are tens of parameters that could be analysed, and sampling must be frequent enough to ensure that the values obtained are representative of long-term exposure. Biomonitoring is seen as an alternative or complementary approach to the conventional monitoring of surface waters, due to its use of biota, which are often able to indicate and integrate the effects of a pressure over space and time (Bonada et al. 2006). Furthermore, often the reason for monitoring is to assess the ecological condition of a site (Noges et al. 2009, Reyjol et al. 2014), and so monitoring the biota directly is a logical step for this assessment.

Biomonitoring has been practiced to some extent for ca. 100 years (e.g. Kolkwitz and Marsson 1909), receiving substantial resource investment, with research building up a large body of knowledge. The best examples take ecological theory and knowledge, and apply it to the challenge of protecting biodiversity by monitoring ecological status in surface waters (Friberg 2014). However, biomonitoring is still in its infancy, with most indices having little or no predictive or diagnostic capabilities (Clews and Ormerod 2009, Friberg 2014). It is thought that this is partly due to the disconnect between current ecological theory and new methodologies (Friberg et al. 2011). In many cases the pressure-impact-response relationship for which an index was designed has not been assessed (Birk et al. 2012). This represents a significant flaw in the development of biomonitoring tools, particularly as they are used to support legislators and water managers. Therefore, tools should be based on sound science, given the

consequences of incorrect assignment of ecological status (Friberg et al. 2011). Under the EU Water Framework Directive (WFD), it is necessary for water managers to distinguish between multiple pressures, make accurate predictions of the level of impact, and cost-effectively tackle these pressures. As such, this bio-diagnostic capability is a significant goal for biomonitoring tools (Clews and Ormerod 2009) particularly as anthropogenic influences on these ecosystems increase, and environmental legislation becomes more stringent.

The lack of a best practice approach to the development of biomonitoring tools represents a significant knowledge gap, which hinders the development of effective biomonitoring tools. It is also unclear as to how pressure-specific biomonitoring tools perform over a range of environments and alongside other confounding pressures. The following review considers the various broad approaches to biomonitoring, and critically discusses the processes used to develop and test current biological indices. Rather than an exhaustive list of biomonitoring tools, the review focuses on those that meet the following criteria: (i) are specific to riverine environments, (ii) are sediment-specific, or are used as indicators of fine sediment. The review provides an insight into the current state of biomonitoring tool development and evaluation, which will form the basis for the development of a biomonitoring tool that is able to identify fine sediment pressures.

2.2 The biomonitoring approach

Biomonitoring relies on organisms to indicate the effects of a pressure, through their presence/absence, abundance or behaviour (Bonada et al. 2006). The approach attempts to improve the information and communication as to the state of the environment (Walz 2000), and is recognised as an alternative to high frequency conventional monitoring, due to the ability of biological indicators to integrate their

responses over space and time, allowing for less frequent sampling (Friberg et al. 2011). Where conventional monitoring of physico-chemical parameters is often used to infer an impact on the biological community, biomonitoring measures any biotic response directly and is therefore able to indicate the health and function of an ecosystem, which is often ultimately the reason for monitoring (Friberg et al. 2011). Conversely, blanket guidelines for physico-chemical parameters are often arbitrary, based on a weak evidence base and with little ecological relevance (Bilotta and Brazier 2008). The approach is also relatively simple and cheap to implement, particularly as newly created biological indices can simply be applied to biological data that is already being routinely collected by water managers. For these reasons biomonitoring is recognised as a necessary tool and is used extensively in freshwater management (Bonada et al. 2006).

Whilst some whole community-based indices may provide an indication of the ecological quality in rivers and streams, they typically do not describe the causes of biodiversity loss (Feld et al. 2014). The occurrence of taxa in reference/un-impacted conditions is an important consideration when selecting indicator species, and it is often argued that common taxa are the most useful in terms of developing a biomonitoring tool that is widely applicable, has less variance, and so is able to make more confident predictions (Marchant 2002). However, some argue that the inclusion of rare taxa can provide important early indications of an impact (Cao et al. 1998). Other tools such as Ephemeroptera, Plecoptera, Trichoptera metrics (Lenat 1988) that respond to a multitude of pressures, use only certain orders/families that are thought to be 'sensitive', and were designed to infer general water quality or habitat degradation. As such, these indices of general degradation are unlikely to be suitable for identifying specific pressures, which may require analysis of taxa at a higher resolution (e.g. family, genus or species), to identify more fine scale pressure responses (Clews and Ormerod 2009). In order for water managers to manage freshwater ecosystems

effectively, and given the penalties for member states non-compliant with the WFD, it is increasingly necessary for biomonitoring tools to be established that have the ability to identify specific pressures. Another important feature of a biomonitoring tool is the ability for it to distinguish the extent of an impact, rather than simply impacted or un-impacted (Woodward et al. 2013).

In the EU, the WFD places a firm emphasis on the use of biomonitoring. The directive requires member states to monitor and report on the ecological status of their surface waters, which includes physico-chemical, biological and hydromorphological parameters (Hering et al. 2010). Member states are required to take a reference-based approach using river typologies (Logan and Furse 2002). The typologies can be defined using either: System A, based on eco-regions and three obligatory environmental characteristics, or System B, using five obligatory environmental characteristics and numerous optional factors (Table 2.1) (Logan and Furse 2002).

Table 2.1. Typology based approaches to biomonitoring specified by the Water Framework Directive using obligatory (bold) and optional environmental characteristics.

System A	System B
Altitude, geology, size	Altitude, geology, size, latitude, longitude, distance from source, energy of flow (function of flow and slope), mean water width, mean water depth, mean water slope, form and shape of main river bed, river discharge (flow) category, valley shape, transport of solids, acid neutralizing capacity, mean substratum composition, chloride, air temperature range, mean air temperature, precipitation.

Although System B allows the possibility of using more predictor variables (potentially leading to greater predictive power) a drawback of using this system is that some of the optional factors can be influenced by anthropogenic activities, which could lead to false predictions of community composition (Logan and Furse 2002). For example, currently the UK uses the River Invertebrate Classification Tool (RICT), a predictive model to determine the expected macroinvertebrate community composition of a site. The current version of RICT relies on mean substrate composition as a predictor variable, and therefore the tool's predictions of community composition are influenced by the potentially modified fine sediment conditions at a site.

Other member states take a fixed typology-based approach to these systems (Davy-Bowker et al. 2006). The fixed typology-based approach resembles the outdated ecological theory of the stream zonation concept (Illies and Botosaneanu 1963) with rivers being compartmentalised into "types" based on environmental characteristics, each hosting a distinct biotic community (Woodward et al. 2010). The problem with this approach is that it lacks a biological basis, and as such a 'river type' could be assigned that has significant biological differences within it (leading to under sampling), or conversely, that different 'types' cannot be separated in terms of their biology (which leads to excessive sampling) (Logan and Furse 2002). Modern ecology now recognises non-equilibrium processes, species turnover and the continuous variations in community structure along environmental gradients (Friberg et al. 2011). As such, some member states have developed predictive models based on a gradient of environmental characteristics (Davy-Bowker et al. 2006). This continuous typology-based approach relies on being able to predict the expected fauna and/or flora for a site if it were in, or close to reference condition (minimal anthropogenic disturbance). Where the observed community composition does not deviate significantly from the expected community, no major monitoring or mitigation programmes are required. If the biological community composition does deviate significantly from the expected

composition, then the presence or abundance of certain species or assemblages of species can provide information on the likely causes of the deviation from reference condition, allowing for monitoring and management resources to be targeted.

2.2.1 Developing a biomonitoring tool

When developing a biomonitoring tool, there are a number of initial considerations and decisions that must be made:

i) On which biotic assemblage or combinations of assemblages will the tool be based? Ideally utilising those that are likely to respond in a measurable way to the pressure(s) of concern (Marzin et al. 2012).

ii) In what context will the tool be used? Taking a reference-based approach using observed/expected scores, or as a stand-alone score of impact?

iii) At which spatial scale will the tool be applied (e.g. local, regional, national or international scale)?

iv) Which method will be used to sample the assemblage (e.g. quantitative, qualitative, subsampling)? This can impact the effectiveness of monitoring and the output from predictive models (Doberstein et al. 2000, Ostermiller and Hawkins 2004).

v) The level of identification that is necessary, and how abundance is recorded/used (e.g. raw abundances, weighted, ln-transformed, presence/absence) (Gayraud et al. 2003).

vi) How will the tool be tested? For example, using data of both the biotic assemblage and the pressure of concern. This should involve consideration of the most useful, or biologically-relevant techniques for quantifying and monitoring the pressure of concern.

The following sections will investigate the theory that underpins these decisions that form the basis of current biomonitoring tools, in order to identify a best practice approach to developing a pressure-specific biomonitoring tool.

Bonada et al. (2006) defined the 'ideal' biomonitoring tool as one based on sound ecological theory, allowing for *a priori* predictions and assessment of ecological functions across large geographic areas. The sampling, sorting and identification process required to use the tool should ideally be low cost and simple, and aligned with the other biomonitoring tools and surveys in existence (Bonada et al. 2006). In order to aid the interpretation of assessments, a linear scale output is desirable (Bonada et al. 2006), and there should be a robust calibration against the physical, chemical, or hydromorphological parameters that the tool is designed to assess (Friberg et al. 2011, Birk et al. 2012).

A recent study of 297 biomonitoring tools used throughout Europe, found that 31% of those applied to rivers had no documented empirical testing of their respective pressure-impact relationship (Birk et al. 2012). This testing of biotic indices is an essential step in the development stages of a biotic index (Borja et al. 2011). Without this testing it is unclear as to what exactly the index is indicating. Similarly, this uncertainty around the effectiveness of a biotic index will inhibit the ability for ecologically relevant guidelines to be established (Davies and Jackson 2006) and limit confidence in the tools application.

In order to link a biological index to a pressure-impact relationship, biotic indices are often developed and tested in specific habitats or ecoregions, in an attempt to minimise environmental variation (e.g. Zweig and Rabeni 2001). However, in the case of taxonomic indices, this will likely limit the spatial applicability of any indices that are derived from such data, due to natural variation in species distributions. One approach that has been suggested as a means of developing a biomonitoring tool, which can be

used over large geographic areas and across ecoregions, is the use of biological traits (Townsend et al. 1997b, Statzner et al. 2001b), discussed in Section 2.2.7 of this chapter.

2.2.2 Biomonitoring and ecological theory

The basic principles of biomonitoring are loosely based on ecological theories or concepts (Friberg et al. 2011), such as Niche Theory, the Habitat Templet (Southwood 1977), the Environmental Filtering Hypothesis (Keddy 1992, Poff 1997) and the *r-K* continuum (MacArthur and Wilson 1967). Nevertheless, the development of many biomonitoring tools has neglected to relate to, or utilise ecological knowledge, instead relying only on statistical approaches based on field observations (Relyea et al. 2012, Hubler et al. 2016). Although there is some dispute over the applicability of some of the general ecological theories to freshwater ecology (Death 2010), the general premise of biomonitoring is that organisms have adapted and evolved biological traits (physiological, morphological, behavioural, and life history strategies) to suit a range of optimum environmental conditions in which they can persist (i.e. their fundamental niche), but are also influenced by biotic interactions (i.e. their realized niche). When conditions deviate from these optima, those taxa that are most suited to the new environmental conditions will persist (Menezes et al. 2010). Biomonitoring relies on these organisms to respond in a quantifiable and predictable way to disturbance.

2.2.3 Biotic elements used for biomonitoring tools

Abiotic and biotic conditions have a significant influence on stream communities (Usseglio-Polatera et al. 2000b). When determining which biotic elements to use in a biomonitoring tool, the spatial and temporal variations of the pressure of concern, along with the biological and ecological traits of the organisms, should be considered in order

to select the biota that are likely to be most informative. Furthermore, it is important to consider the inherent variability of biota, which is influenced by both natural and anthropogenically-derived abiotic and biotic conditions. The choice of biotic element is therefore often strongly dependent on the pressure of concern. For example, macrophytes or periphyton may be used to identify nutrient enrichment, as they can respond directly to the pressure (e.g. increased growth) (Willby et al. 2009). More mobile organisms may be required to determine the impacts of other pressures, such as river engineering (e.g. hydropower; Bilotta et al. 2016). Each biotic element will also likely respond to different pressures and to varying degrees over different temporal and spatial scales (Hughes et al. 2000). In Europe, the most commonly utilised biotic elements in biomonitoring tools are macroinvertebrates, fish and phytoplankton (Figure 2.1) (Birk et al. 2012). Under the WFD, member states are required to monitor fish, macroinvertebrates, macrophytes and phytobenthos.

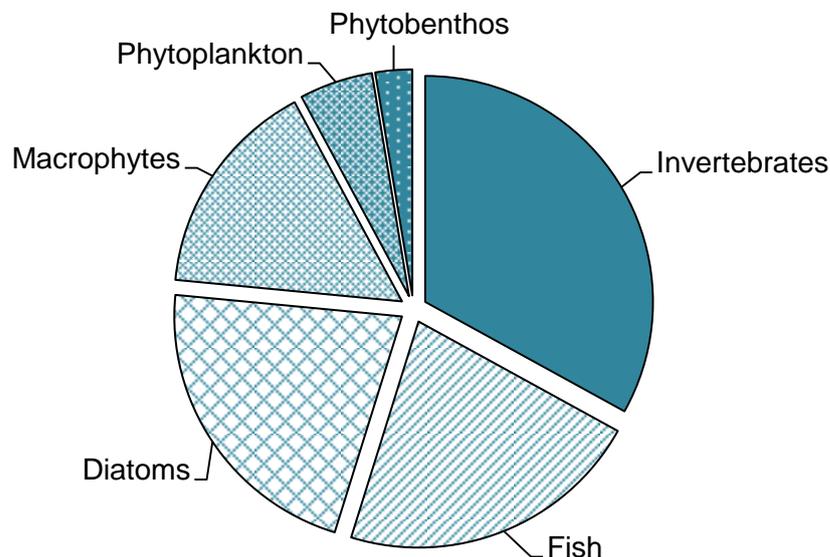


Figure 2.1. Proportion of biotic elements used in riverine biomonitoring schemes throughout Europe. Data extracted from the WISER methods database (Birk et al. 2010).

There are advantages and disadvantages of each of the commonly used biotic elements, including ecological factors, such as the diversity of the assemblage, their distribution and trophic position, as well as logistical considerations, such as the ease of sampling and identification. Resh (2008) reviewed these advantages and disadvantages to discuss the “best” assemblages for biomonitoring programs and concluded that ultimately the “best” assemblage was study- or site-specific. The following sections discuss the three most commonly used biotic elements.

2.2.3.1 Biomonitoring approaches using macroinvertebrates

Macroinvertebrates are key components of freshwater ecosystems, vital for stream functional integrity; processing detritus and nutrient cycling, and are also an important food source for both freshwater and terrestrial vertebrates (e.g. fish, birds, amphibians) (Wallace and Webster 1996). Macroinvertebrates are the most widely used freshwater organisms for biomonitoring approaches, for a number of reasons:

- i) They are the most diverse group of freshwater organisms, with communities that can comprise many potential species, each with their own range of environmental tolerances/sensitivities (Cook 1976, Pratt and Coler 1976, France 1990), having evolved numerous traits and life history strategies in order to subsist and exploit food and habitat resources.
- ii) Taxa occupy a wide range of trophic levels, and therefore changes in community composition can reflect both bottom-up and top-down effects (Wallace and Webster 1996).
- iii) Many taxa are relatively sedentary and are therefore representative of site-specific environmental conditions (Cook 1976).
- iv) The life-span of macroinvertebrates is considered long enough to allow them to integrate the effects of short-term environmental variations (Hutchinson et al.

1998, Clarke et al. 2003).

- v) There is a good range of literature and taxonomic keys available to aid the identification of taxa.

Owing to their suitability, macroinvertebrates have been used in biomonitoring tools developed to identify various pressures in freshwater ecosystems. Nevertheless, despite the availability of taxonomic keys, the identification of macroinvertebrates to species level typically requires highly trained taxonomists, with some groups being particularly difficult to identify (e.g. mites and chironomids). In order to overcome this challenge, certain taxa are often excluded from indices, or incorporated at coarser taxonomic resolutions (Davy-Bowker et al. 2010).

2.2.3.2 Biomonitoring approaches using fish

Fish are the most mobile of the biotic elements used in freshwater biomonitoring. They have a broad range of habitat requirements, which change throughout the various stages of their life cycles, and are therefore often considered useful indicators of ecological integrity (Kemp et al. 2011). Occupying the higher trophic levels of these ecosystems, they can also be used to indicate biotic integrity, as they can reflect impacts in lower trophic levels (Karr 1981, Berkman et al. 1986). Fish are sensitive to most forms of anthropogenic pollution/disturbance to varying degrees, making them useful as indicators (Harris 1995). Furthermore, logistically, they are relatively easy to identify to species level (requiring limited training), with sample processing being conducted on site, and specimens being released once captured (Karr 1981). Another suggested advantage is that given the recreational and cultural importance of fish, the public can relate to any outputs (Resh 2008). Despite these advantages, there are also drawbacks of using fish as indicators. These include their high mobility, which can result in a naturally patchy distribution and relatively large sampling effort (Karr 1981,

Vehanen et al. 2013). Other factors, such as their generation times have been cited as both advantages or disadvantages, and will be dependent on the study/monitoring objectives (Resh 2008). In order to assess for multiple pressures, it is often considered preferential that the assemblage is diverse, as different species are likely to respond differently and to a varying degree to pressures (Friberg 2010). Freshwater fish have relatively low species richness (ca. 66 native and non-native species in the UK; Maitland 2004) in comparison to the other biotic elements (macroinvertebrates: at least ca. 6400 species in the UK; Davies and Edwards 2011), which may limit their use as indicators over subtle gradients of pressures.

2.2.3.3 Biomonitoring using diatoms

Many EU member states use diatoms as a proxy for phytobenthos for WFD reporting (Kelly 2011). This is partly owing to the fact that they have been widely used as biological indicators, due to their ubiquitous nature, ability to infer water quality deterioration, and ease of sampling (Feio et al. 2009). They play a key role in freshwater ecosystems as primary producers at the base of the food web (Tison et al. 2008), their ecology is considered well studied (Potapova and Charles 2007), and crucially, they have been shown to respond to various pressures (Pan et al. 1996). To date, benthic diatoms have been mainly used as indicators of overall ecological status (Kelly et al. 2008), or aspects of water quality, including acidification (Battarbee et al. 1999) and nutrient enrichment (Kelly and Whitton 1995). Despite these examples, there is some debate over the effectiveness of diatoms as biological indicators, and their ability to respond to water quality improvements (Kelly 2011). A further disadvantage is that identification to species level requires highly trained specialists (Feio et al. 2009).

From this point forward the review will focus on the use of macroinvertebrates as they are the most widely used biotic assemblage for biomonitoring, and are both ubiquitous

and speciose. Importantly they are the only biotic assemblage which have been used to identify fine sediment pressure, which may partly be due to the fact that, substrate is thought to be a primary driver of macroinvertebrate community composition (Minshall 1984).

2.2.4 Reference-based approach in the UK – RIVPACS/RICT

Reference condition is defined as:

“no, or only very minor, anthropogenic alterations to the values of the physico-chemical and hydromorphological quality elements for the surface water body type from those normally associated with that type under undisturbed conditions”

Water Framework Directive, Annex 5, Section 2.1

The Centre for Ecology and Hydrology in the UK led the way globally, in developing a predictive multivariate tool for assessing the ecological status of sites, based on data from “reference condition” or minimally impacted sites (Wright et al. 1993, Wright et al. 2000). The River InVertebrate Prediction And Classification System (RIVPACS), now superseded by the River Invertebrate Classification Tool (RICT), is used by regulatory agencies (i.e. Environment Agency, Natural Resources Wales, Scottish Environment Protection Agency and Northern Ireland Environment Agency) to obtain predictions for macroinvertebrate communities if a site was in reference condition. This multivariate tool was developed using macroinvertebrate and environmental data from 835 “reference condition” sites throughout the UK. These sites were carefully selected to represent the best available examples of river types in the UK, and were considered to be minimally impacted by anthropogenic activities (Clarke et al. 2003). From this point forward these sites will be referred to as minimally impacted, rather than reference condition, to reflect that these sites were not necessarily in pristine condition. The sites

comprise a wide range of environments (Table 2.2), varying in their (i) climate, (ii) catchment geology, (iii) topography and (iv) morphometry.

Table 2.2. Characteristics of the River InVertebrate Prediction And Classification System sites (Wright 2000).

Site characteristics	Description
Mean annual precipitation (between 1961 and 1990)	430 mm – 2930 mm
Mean annual temperature (between 1961 and 1990)	7.93°C – 11.45°C
Geology	Various - hard igneous rock to soft sedimentary rock
Altitude at river source	5 m – 1216 m
Average river width	0.4 m – 117 m
Average river depth	0.02 m – 3.00 m
Mean annual discharge	< 0.31 m ³ s ⁻¹ - > 80.00 m ³ s ⁻¹
Slope	0 – 150 m km ⁻¹
Substratum percentage cover of fine sediment (< 2 mm)	0 – 100%
Substratum percentage cover of gravels and pebbles	0 – 98%
Substratum percentage cover of cobbles and boulders	0 – 100%

Macroinvertebrate communities were sampled using a standardised three-minute kick-sample, followed by a one-minute manual search for surface dwelling and attached taxa (EU-STAR 2004). Taxa were identified to species-level where possible, and macroinvertebrate community 'types' were then characterised using Two Way INdicator

SPecies ANalysis (TWINSPAN), a type of cluster analysis, which identified 43 'community types' or 'end groups'. Importantly, these end groups are a technical step and should not be considered as distinct assemblages in the environment (Wright et al. 2000). Following the cluster analysis, environmental 'predictor' variables were linked to the 43 end groups using Multiple Discriminant Analysis (MDA). The result is a tool that can predict the likelihoods of a test site belonging to the various end groups. With this information the model is able to predict the expected macroinvertebrate assemblages and calculate expected scores for any biotic index that has been incorporated into the software (Wright et al. 2000). The RIVPACS/RICT method of predicting the expected fauna at a site, and therefore the expected biotic index scores, is considered ecologically sound as it recognises that macroinvertebrate assemblages form a continuum along environmental gradients, and that an exact match between assemblages is unlikely (Friberg et al. 2011) given this natural variability and also sampling variability (discussed in Section 2.2.5). One important consideration is that any use of this predictive tool (RICT) should be done so using macroinvertebrate data that has been collected using the same methods (three-minute kick-sample plus a one-minute manual search), to avoid any deviations that may result from different sampling efforts. An exception to this is the sampling of deep rivers, for which a variety of methods are utilised, and therefore the use of RICT for deep rivers is not recommended (Clarke 2009).

Other countries in Europe have developed similar reference-based tools, such as SWEPACSRI (Sweden) and PERLA (Czech Republic) but utilise different macroinvertebrate sampling techniques (Clarke et al. 2006, Bennett et al. 2011). Outside of Europe, similar approaches have been developed in Australia (AUStralian River Assessment Scheme; AusRivAS) and Canada (Benthic Assessment of Sediment; BEAST) (Wright et al. 2000).

2.2.5 *Sampling procedures*

The method of macroinvertebrate sampling and the sorting procedure can affect the results obtained from biomonitoring tools (Haase et al. 2004a, Haase et al. 2004b). A variety of different macroinvertebrate sampling techniques are used internationally, including throughout Europe (Bennett et al. 2011). Each EU member state has adopted their own standardised approach, using variations of hand net and kick-sampling, or Surber samplers. Within the UK, the three-minute kick-sample and one-minute manual search method is used. This technique involves sampling all habitats at the site in proportion to their occurrence (EU-STAR 2004). Starting at the downstream end of a site, the substrate is disturbed with the operator's boot, whilst holding the hand net downstream. In areas of apparent homogeneous habitat, the operator should move upstream in a zig-zag pattern. The method is semi-quantitative and has been shown to collect only approximately 50% of the species, and 60% of the families present in six replicate samples (Furse et al. 1981). Despite this, the method is the UK standard, and any biomonitoring tool that is developed for use in the UK should be compatible with this method, to ensure that the tool can be applied to current monitoring programs.

Whilst raw abundance data are likely to provide the most information in terms of community response, the suitability of using such data is dependent on the sampling technique used to collect it. Often when a semi-quantitative technique has been used it is deemed more suitable to transform raw abundance counts to log abundance categories (i.e. 0-9 = 1, 10-99 = 2, 100-999 = 3 etc.) (Monk et al. 2006, Extence et al. 2013). This transformation captures some information on abundance, without placing too much confidence in the sampling technique, but will miss more subtle changes to species abundances. For example, a species may decline from a population of 999 to a population of 100, and will be assigned the same abundance (i.e. an abundance of three). Another alternative is the use of presence/absence data, which provides virtually no information on changes to abundance.

2.2.6 Taxonomy-based indices

The vast majority of biomonitoring tools utilise information on taxonomic community composition to derive index scores and infer ecological condition. This approach is based on the idea that taxonomic units (e.g. species) have different ecological requirements (as discussed in Section 2.2.2), and that certain taxa respond in a predictable way to changes in their environment (Schuwirth et al. 2015).

Many of the biotic assemblages used in biomonitoring, benefit from being taxonomically well described, with tools using information on taxon abundance or richness, often at the family, genus or species level. Despite their widespread use, taxonomy-based indices are often criticised for their lack of spatial applicability, with studies showing that environmental gradients (e.g. geology and altitude) across eco-regions can affect taxonomic community composition (Charvet et al. 2000).

2.2.6.1 Taxonomic resolution of biomonitoring tools

The appropriate taxonomic resolution on which to base biomonitoring tools is dependent on a number of factors, including the biotic assemblage, availability of taxonomic resources, levels of taxonomic expertise, and time and cost constraints (Resh 2008). In the EU, the majority of biotic assemblages are identified to species-level (Figure 2.2). The exception is macroinvertebrates, which are commonly used at family-, or genus-level (Birk et al. 2010), likely due to time and cost constraints. Nonetheless, species-level identification is usually considered preferential (Furse et al. 1984, Resh and McElravy 1993, Schmidt-Kloiber and Nijboer 2004, Monk et al. 2012), as it allows for the consideration of species-specific sensitivity variations, and acknowledges the breadth of ecological niches that have resulted from evolutionary processes (Grinnell 1917, Hirzel and Le Lay 2008, Waringer et al. 2013).

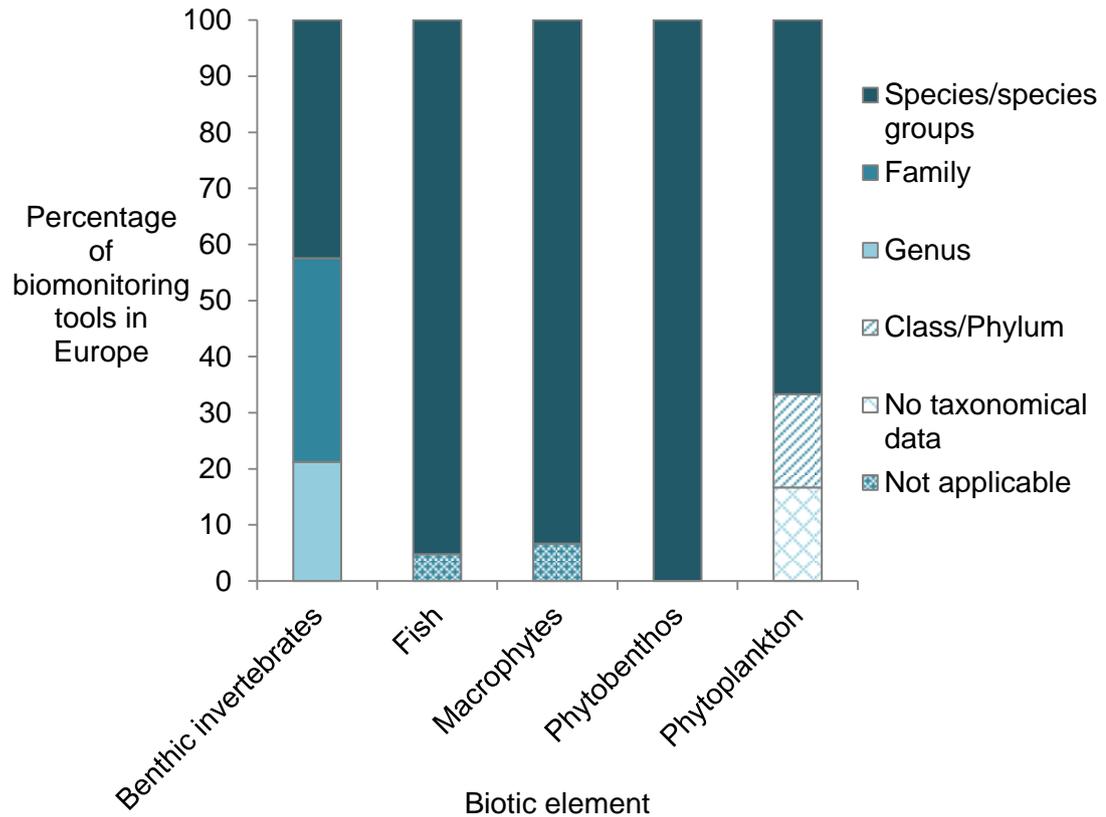


Figure 2.2. Taxonomic resolution utilised by biomonitoring tools in rivers in Europe. Data extracted from WISER database (Birk et al. 2010).

Species-level data ensure that the greatest amount of information regarding the community composition is captured, which may assist with the identification of any deviations in environmental conditions (Schmidt-Kloiber and Nijboer 2004). Indices that use higher taxonomic units (e.g. order, family, genus) follow the assumption that taxa within these units, occupy similar ecological niches, through having similar biological and ecological traits. Commonly, this is not the case; species within the same family, as well as the same genus, can have different ecological requirements, resulting in varying sensitivities to pressures (Resh and McElravy 1993, Schmidt-Kloiber and Nijboer 2004, Monk et al. 2012). For example, many families within the orders of Ephemeroptera, Plecoptera and Trichoptera, are species-rich and have a diverse

range of traits and sensitivities to anthropogenic disturbances (Lenat and Resh 2001, Bonada et al. 2004, Monk et al. 2012). The family Baetidae (Ephemeroptera) includes species with a range of flow preferences; rheobiont species (e.g. *Baetis maurus*) to limnophilic species (e.g. *Cloeon* spp.) (Buffagni et al. 2009, Buffagni et al. 2017). Using these higher taxonomic units can therefore underestimate ecological diversity (Verdonschot 2006, Waringer et al. 2013) and may result in information on the relationship of concern being lost (Schmidt-Kloiber and Nijboer 2004).

A lack of autecological information for some taxon means that the extent of these variations within higher taxonomic units is largely unknown. In some cases family-level resolution data is considered sufficient, for example when assessing the general biological condition. However, when assessing for specific pressures or ecosystem functioning and structure, higher resolution data are preferential (Bennett et al. 2011). For these reasons it is often suggested that species-level identification should be carried out where possible, particularly when data is being used for management or legislative purposes (Vaughan and Ormerod 2010, Monk et al. 2012). Nevertheless, in practice, the selection of an appropriate taxonomic resolution at which to record data is often a trade-off between obtaining the most detailed information, and the associated time and cost constraints. Ultimately, a biomonitoring tool should be developed to align with the protocols of regulatory agencies, to ensure the tool is widely applicable over the intended area.

2.2.7 Trait-based indices

Biological traits and ecological preferences have been studied by ecologists since the 19th century, however only relatively recently have they been explored for their use in biomonitoring tools (Menezes et al. 2010). Traits have a strong influence on the performance and success of an organism (Reyjol et al. 2014), and are often the result

of adaptations that have evolved to deal with ecological characteristics (Menezes et al. 2010). Trait-based studies often include multiple traits that are as distinct as possible to avoid subjectivity and ambiguity (Table 2.3).

Table 2.3. List of biological and ecological traits (Usseglio-Polatera et al. 2000a, Doledec et al. 2006, Archambault et al. 2010).

Traits

Biological traits

Maximal size, life cycle duration, adult life duration, potential number of reproduction cycles per year, aquatic stages, respirations of aquatic stages, reproductive method, dispersal, resistance form, respiratory method, locomotion and substrate relation, food source, feeding habits, body form, body flexibility, method of attachment to substrate, dissemination potential

Ecological traits/preferences

Transversal distribution, longitudinal distribution, altitude, biogeographic regions, substrate (preference), current velocity (preference)

Trait-based approaches to biomonitoring are theoretically underpinned by niche-based theories, such as the Habitat Templet model (Southwood 1977), and also the concept of environmental filtering (Poff 1997). Where ecological characteristics of stream sites are similar, the composition of traits is expected to be similar. When conditions diverge, trait composition is expected to change to reflect the new suite of ecological characteristics (Poff et al. 2006). In order for this divergence to be useful for biomonitoring, traits need to be identified that are sensitive or tolerant of stressors related to the pressure of concern (Statzner et al. 2001a). Importantly, traits may provide a mechanistic linkage between species' responses and pressures, allowing for

a priori predictions of effects (Statzner and Beche 2010, Culp et al. 2011). While it is still necessary to taxonomically identify an organism (to determine what its associated traits are), research suggests that at least in the case of large biomonitoring programs, trait data based on coarser taxonomic resolution (family or genus) may be sufficient (Gayraud et al. 2003, Statzner et al. 2005, Archambault et al. 2010).

Some of the advantages of using biological traits are that they are considered to be relatively stable across biogeographic regions, thus increasing the spatial applicability of trait-based indices (Usseglio-Polatera et al. 2000a, Statzner et al. 2001a). A study of reference condition streams found that traits were relatively unaffected by environmental gradients across ecoregions (Charvet et al. 2000). Traits are also thought to be relatively stable both seasonally and annually, reducing some of the variation seen in taxonomic approaches (Culp et al. 2011). Certain traits are also likely to be sensitive to specific pressures whilst others vary independently of pressures (Statzner and Beche 2010).

Whilst this approach appears to have a number of significant benefits over taxonomy-based approaches, there are also a number of disadvantages or limitations (Culp et al. 2011) that warrant consideration: (i) The natural variation in trait community patterns under minimally impacted conditions requires further investigation, (ii) the lack of trait databases in many countries, and limited understanding of trait-stressor relationships, represents a significant barrier to widespread use of traits for biomonitoring, (iii) various traits may be correlated, something that should be considered when attempting to link a trait to a stressor/pressure, (iv) trait-based tools often consider traits as static, when in fact they may vary over the life history of the taxa, which could potentially lead to a misinterpretation of outputs, (v) whilst the sampling, sorting and identification efforts for the most part are similar to taxonomy-based tools (if trait databases are linked to taxonomic recording programs), certain traits such as body size, will require further efforts, (vi) traits are often considered in isolation, instead of as a combination of

characteristics that determine the ecological preference of a taxon. For example, utilising knowledge of feeding mechanisms, species with very different ecological requirements can be grouped together (e.g. limnophilic and rheophilic filter feeders; *Pisidium obtusale* and *Hydropsyche* spp., respectively) (Tachet et al. 2010). The recent development of trait databases and continued work on these throughout Europe (Schmidt-Kloiber and Hering 2015) and North America (Vieira et al. 2006), represent a significant resource for trait-based biomonitoring approaches.

Trait correlations and combinations have the potential to result in misleading conclusions being drawn from the outputs of trait-based indices (Schuwirth et al. 2015). Correlations between traits can occur for a number of reasons. Firstly, the traits may be mechanistically linked, for example, a long adult lifespan is often accompanied by semivoltinism (Poff et al. 2006). They can also be the result of phylogenetic relationships - with closely related taxa being physiologically similar (Buchwalter et al. 2008), or the result of convergent or parallel evolution – with unrelated taxa evolving similar traits (Culp et al. 2011).

2.2.8 Pressure-specific biomonitoring tools

In order to develop or test a pressure-specific tool, it is often necessary to limit any confounding pressures, whether natural environmental variations or anthropogenic derived perturbations (Pont et al. 2006). To minimise these confounding pressures, authors have focused development and validation efforts on areas of naturally restricted environmental conditions, such as geographically restricted areas (e.g. ecoregions or single river basins) (Pont et al. 2006). A number of pressure-specific biomonitoring tools have been proposed in the literature, to identify environmental pressures such as acidification (Sandin and Johnson 2000, Braukmann 2001, Sandin et al. 2004, Davy-Bowker et al. 2005) organic pollution (Zelinka and Marvan 1961,

Armitage et al. 1983, Rae 1989, Zamora-Munoz and Alba-Tercedor 1996), nutrient enrichment (Hynes 1960, Hellawell 1978, 1986, Mason 1996, Paisley et al. 2011), heavy metal pollution (Winner et al. 1980, Poulton et al. 1995, Smolders et al. 2003), hydromorphological degradation (Extence et al. 1999, Buffagni et al. 2004, Lorenz et al. 2004, Friberg et al. 2009), and fine sediment pollution (Zweig and Rabeni 2001, Relyea 2007, Bryce et al. 2010, Extence et al. 2013, Hubler et al. 2016).

Combinations of pressure-specific indices can also be used to improve bio-diagnostic capabilities (Clews and Ormerod 2009). Using these pressure-specific tools as part of a suite of biomonitoring tools can prove useful for regulatory agencies attempting to identify the most likely causes of a deviation in ecological condition. The WFD requirements to identify specific pressures and the effects of these pressures, has placed an importance on bio-diagnostic capabilities (Clews and Ormerod 2009).

2.2.9 Confounding pressures

In Europe, the majority of rivers and streams are impacted by numerous anthropogenic activities. A recent study of 9330 sites throughout 14 European countries, found 47% of rivers (90% of lowland rivers) to be subject to multiple pressures relating to hydrology, morphology, water quality and connectivity (Schinegger et al. 2012). It is therefore likely that the biotic communities in many rivers and streams are not responding to a single pressure, rather, multiple interacting pressures (that can be related and unrelated) are influencing them. Relatively few studies have considered the interactions and co-occurrence of in-stream pressures, and their influence on stream communities, something that is likely to affect the performance of biomonitoring tools. Friberg (2010) hypothesise that the presence and influence of multiple pressures is the reason that a “wedge shape” relationship is often observed between a single metric and the pressure of concern (Figure 2.3); when the pressure of concern is low, other pressures play a

more influential role in shaping the biotic community; as the pressure of concern increases, it becomes the dominant force in terms of shaping the community.

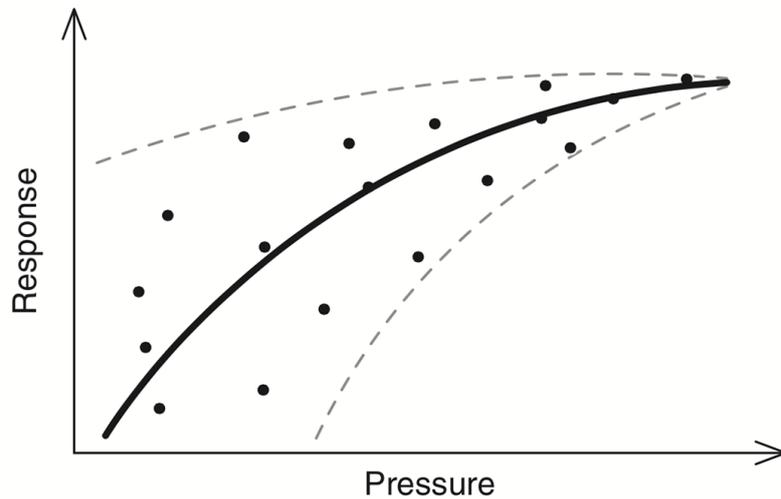


Figure 2.3. “Wedge-shaped” relationship often observed between a single pressure and response (Friberg 2010).

As fine sediment is also a natural component of rivers and streams, its interactions and associations have been widely studied. Globally, a number of studies have analysed the relationships between fine sediment and other physical, chemical and biological pressures (Table 2.4), research that will be an important consideration when determining the performance and independent testing of the biomonitoring tool.

Table 2.4. Confounding pressures (physical, chemical and biological) commonly associated with fine sediment in rivers and streams globally, and their effect on macroinvertebrates.

Associated pressure	Details	Country of study	Authors	Effect on macroinvertebrates
Physical				
Flow	Discharge, velocity	Austria, France, New Zealand, UK	(Slattery and Burt 1997, Tockner et al. 1999, Wood and Armitage 1999, Lefrançois et al. 2007, Petticrew et al. 2007, Matthaei et al. 2010)	Decrease/increase in abundance, dependent on the ecological requirements of macroinvertebrates (Extence et al. 1999).
Chemical				
Nutrients	Nitrogen, phosphorus	China, Korea, New Zealand, UK, USA	(Carpenter et al. 1998, Owens and Walling 2002, Kim et al. 2003, Jarvie et al. 2006, Brazier et al. 2007, Owens et al. 2007, Sun et al. 2009, Piggott et al. 2015)	Hypoxic conditions following eutropication can reduce the abundance of species with high oxygen requirements. Inputs of nitrogenous compounds, can also lead to ammonia, nitrite and nitrate toxicity (reviewed in Camargo and Alonso 2006).
Pesticides	Polychlorinated biphenyls,	Australia, Brazil, China, India, Italy,	(McKenzie-Smith et al. 1994, Camusso et al. 2002, Torres et al.	Various pesticides have been linked to declines in both abundance and richness,

Associated pressure	Details	Country of study	Authors	Effect on macroinvertebrates
	pyrethroid, organochlorines, organophosphate	Vietnam, UK, USA	2002, Warren et al. 2003, Zhang et al. 2003, Weston et al. 2004, Minh et al. 2007)	(reviewed in Wijngaarden et al. 2005), which is also likely to influence predator/prey interactions.
Metals	Aluminium, cadmium, chromium, copper, iron, lead, manganese, mercury, zinc	Italy, Pakistan, Turkey, UK, USA	(Abernathy et al. 1984, Tariq et al. 1996, Dawson and Macklin 1998, Owens et al. 2001, Camusso et al. 2002, Akcay et al. 2003, Walling et al. 2003)	Responses to metal pollution are varied, but for intolerant groups (e.g. Ephemeroptera), abundance and richness are typically reduced in impacted streams (reviewed in Clements 1991).
Organic matter		Austria, Luxemburg, Taiwan, UK	(Tockner et al. 1999, Yu et al. 2001, Von Bertrab et al. 2013, Glendell and Brazier 2014)	Excessive inputs of organic matter and the subsequent biological decomposition, can lead to reduced oxygen levels, impacting those species with high oxygen requirements (e.g. <i>Leuctra</i> spp.), whilst favouring those with low oxygen requirements (e.g. <i>Chironomus</i> spp.) (Friberg et al. 2010).

Associated pressure	Details	Country of study	Authors	Effect on macroinvertebrates
Other	Polycyclic aromatic hydrocarbons (PAHs)	Canada, Malaysia, UK, USA	China, Taiwan, (Ashley and Baker 1999, Yunker et al. 2002, Zakaria et al. 2002, Stevens et al. 2003, Doong and Lin 2004, Zhang et al. 2004)	Polycyclic aromatic hydrocarbons are toxic to many macroinvertebrates, and can lead to reduced richness and abundance (Ankley et al. 1994).
Biological				
Invasive species	<i>Pacifastacus leniusculus</i>	UK	(Johnson et al. 2010, Harvey et al. 2011, Johnson et al. 2011, Harvey et al. 2014, Albertson and Daniels 2016)	The signal crayfish (<i>Pacifastacus leniusculus</i>) has been shown to result in changes to invertebrate community composition, and fine sediment dynamics (Harvey et al. 2014, Mathers et al. 2016a).
Bacteria/ pathogens	<i>Escherichia coli</i> , <i>Salmonella sp.</i> , <i>Cryptosporidium sp.</i>	Austria, Canada, New Zealand, USA	(Mallin 2000, Muirhead et al. 2004, Jamieson et al. 2005, Droppo et al. 2009, Kernegger et al. 2009)	The effects of the many sediment-associated pathogens on macroinvertebrates is unclear. <i>Cryptosporidium</i> and <i>Giardia</i> accumulate within some macroinvertebrates, but with uncertain consequences (Reboredo-Fernández et al. 2014).

2.2.10 Sediment-specific biomonitoring

The benefits of pressure-specific biomonitoring tools have been discussed. Fine sediment pollution is one of the most widespread forms of water quality impairment (Richter et al. 1997), and as such, water managers are increasingly looking for ways to identify this pressure. A significant challenge to developing a sediment-specific index lies with the nature of the pressure; being a highly variable (both spatially and temporally), natural component of freshwater ecosystems. Furthermore, identifying when and where sediment is the primary cause of ecological degradation is a challenging task. Biological communities undergo natural cycles and variation over time, and respond to a range of physical, chemical and biological characteristics. Fine sediments are also commonly associated with numerous other pressures that are likely to influence aquatic biota (Table 2.4). The use of conventional, non-biological monitoring to assign cause and effect would necessitate measurement of multiple parameters, at sufficient temporal resolution, and for a significant period of time. Biomonitoring tools may provide a valuable alternative means to detecting the effects of this pressure. Macroinvertebrates are thought to be a suitable group of organisms for indicating fine sediment pressure, as substrate is considered to be a primary factor affecting macroinvertebrate community composition (Rabeni and Minshall 1977, Minshall 1984).

Monitoring of physical stream attributes, in this case substrate composition, could be considered more challenging than for chemical parameters, which can be instrumented and monitored at high resolution (Sandin et al. 2004). Biomonitoring tools designed to identify changes to physical attributes such as sedimentation, are hard to evaluate for a number of reasons: (i) It is not clear which sediment metric is the most biologically relevant (Sutherland et al. 2012), (ii) the accuracy of the methods used for monitoring deposited fine sediment are often uncertain (Wohl et al. 1996, Bunte and Abt 2001, Faustini and Kaufmann 2007, Whitacre et al. 2007) and (iii) many of these methods

offer only a snapshot of sediment conditions and so may not capture the true range of the pressure. These and other uncertainties inherent in the development of a sediment-specific biomonitoring tool should ideally be considered and where possible incorporated into the development and testing stages (Demars et al. 2012).

2.2.10.1 Sediment-specific indices developed globally

In order to develop a sediment-specific index, biotic responses should ideally be linked to quantitative estimates of sediment conditions (Bryce et al. 2010). Therefore, the sediment metric to which an index is calibrated should be biologically-relevant. Despite these challenges, a small number of macroinvertebrate based, sediment-specific indices and non-specific indices have been suggested as potential indicators of fine sediment pressure (Zweig and Rabeni 2001, Relyea 2007, Sutherland et al. 2012, Extence et al. 2013, Murphy et al. 2015, Hubler et al. 2016). A number of these have shown general changes in macroinvertebrate community composition as a result of sedimentation, but they have not provided a mechanistic linkage (with the exception of Extence et al. 2013) for these changes. Furthermore, many of those that have developed sediment-specific tools have used relatively small development/test datasets (e.g. Zweig and Rabeni 2001) or used flow as a proxy for fine sediment (e.g. Extence et al. 2013).

The Fine Sediment Biotic Index (FSBI) (Relyea 2007) benefits from being developed using data from 1025 streams from nine 'ecoregions' in the Pacific North-West (USA) and a validation process that used data from a further 255 streams. The sediment tolerances of the 93 common macroinvertebrate taxa (those that occurred in more than 2% of sites, and excluding taxa that were only able to be identified to a coarse taxonomic level, i.e. Chironomidae) were assigned using empirical data from this large dataset. The main drawback of this approach is that it neglects previous literature or

expert knowledge regarding macroinvertebrate sensitivities/tolerances, being based solely on empirical data, and therefore lacks a biological basis and mechanistic linkage between the index and fine sediment pressure. Additionally, other variables/pressures do not appear to have been considered, and so the presence/absence or abundance of the tolerance rated macroinvertebrates could be due to other factors, particularly as there is no explicit biological basis behind the sediment tolerance values.

A study of four streams in Missouri, USA, observed moderate–strong correlations ($r_s = -0.534$ to -0.907 , $p < 0.001$) for deposited sediment ranging from 0 to 100% fines (visual estimates) with various biological indices including the Deposited Sediment Biotic Index (DSBI) (Zweig and Rabeni 2001). The DSBI is based on 30 taxa that occurred in at least two of the four streams and comprised >10% abundance in any one sample. These taxa were assigned to one of three groups: intolerant, moderately tolerant and tolerant based on the cumulative frequency curves and the value of % deposited fine sediment at which 50% of the taxon's cumulative abundance occurred (Zweig and Rabeni 2001). Despite this sediment-specific index having moderate-strong correlations with fine sediment metrics, the local scale, lack of a biological basis or consideration of confounding pressures, limits the index being conclusively linked to fine sediment pressure. Another criticism is that the relatively few taxa included in the index, may limit its spatial applicability.

A number of indices have been developed using statistical approaches, which are designed to detect general environmental degradation, including impacts arising from fine sediment pollution (Bennett et al. 2004, Weiss and Reice 2005). Others have incorporated metrics related to macroinvertebrate sediment tolerance in multimetric approaches (Fore et al. 1996, Weigel et al. 2002, Herlihy et al. 2005). Whilst not developed as a sediment-specific index, the Hilsenhoff (1988) Modified Family Biotic Index (MFBI) was shown to strongly correlate with fine sediment ($r^2 = 0.78$, $p < 0.001$) across 15 agricultural streams in Canada (Sutherland et al. 2012). Although this is a

strong correlation, the index is based on the tolerance of macroinvertebrates to organic pollution and therefore lacks a mechanistic linkage. Furthermore, the small scale of the study limits the spatial applicability of the index as well as confidence in its ability to identify sediment pressure.

The Proportion of Sediment-sensitive Invertebrates (PSI) index is a biomonitoring tool that is designed to identify the degree of sedimentation in streams throughout the UK (Extence et al. 2013). It was developed using a similar approach to the Lotic invertebrate Index for Flow Evaluation (LIFE) (Extence et al. 1999); through assessment of biological and ecological traits and previous literature, macroinvertebrate taxa are assigned to one of four Fine Sediment Sensitivity Ratings (FSSR). The weighted relative abundance of taxa is used to calculate a PSI score on a linear scale; 0 being heavily sedimented, 100 being unsedimented. The PSI index is linked to ecological niche theory and may therefore also relate to ecosystem functioning (Friberg et al. 2011). However, despite this sound biological basis of the tool, until now, the PSI index has only been tested against observed fine sediment data in two catchments in the UK (Glendell et al. 2014). Other published applications of the PSI index have relied on inferred sediment values when evaluating the index in the UK and also in Guinea (Africa), based on assumed relationships between flow regime or land-use/habitat modification and fine sediment levels (Extence et al. 2013, Poole et al. 2013).

Internationally, Ephemeroptera, Plecoptera and Trichoptera (EPT) metrics are often used to infer fine sediment pressure and habitat degradation (Wagenhoff et al. 2012). These orders of insects include many taxa that are considered sensitive of fine sediment, for example, due to periphyton being smothered (Larsen and Ormerod 2010), filter nets becoming clogged (Strand and Merritt 1997), and interstices being filled (Pollard and Yuan 2010). Whilst some studies have shown strong relationships between various EPT indices and fine sediment metrics (Angradi 1999, Kaller and

Hartman 2004, Sutherland et al. 2012, Burdon et al. 2013), the three orders of invertebrates also contain families, genera, and species that are not considered to be sensitive to fine sediment (e.g. many Caenidae and Limnephilidae species), which may lead to misinterpretations of outputs from these indices. The metrics have not been extensively evaluated for their empirical relationships with fine sediment.

The stringent criteria suggested by Bonada et al. (2006), for the 'ideal' macroinvertebrate biomonitoring tool, provides a benchmark for these types of approach, however it should be noted that it is unlikely in practice that any one tool will meet all 11 criteria (Table 2.5).

Table 2.5. Modified from Bonada et al. (2006) to include criteria relevant to pressure-specific biomonitoring tools and with additional criteria added.

Criteria	PSI	EPT	MFBI	DSBI	FSBI
Rationale					
i) Derived from sound theoretical concepts in ecology (relating to fine sediment)	✓	✗	✗	✗	✗
ii) <i>A priori</i> predictive	✓	✗	✗	✗	✗
iii) Potential to assess ecological functioning	?	?	?	?	?
iv) Potential to discriminate between pressures	?	?	?	?	?
Implementation					
v) Low costs for sampling and sorting	✓	✓	✓	✓	✓
vi) Simple sampling protocol	✓	✓	✓	✓	✓
vii) Low cost for taxa identification (specialists not required)	✓	✓	✓	✓	✓
viii) Easily integrated into current monitoring schemes	✓	✓	✓	✓	✓
Performance					
ix) Large-scale applicability (across ecoregions)	?	?	?	?	?
x) Reliable indication of changes to fine sediment conditions	?	?	?	?	?
xi) Human impact indication on linear scale	?	?	?	?	?
Proportion of Sediment-sensitive Invertebrates (PSI); Ephemeroptera, Plecoptera, Trichoptera (EPT) metrics; Modified Family Biotic Index (MFBI); Deposited Sediment Biotic Index (DSBI); Fine Sediment Biotic Index (FSBI). Unclear (?), Yes (✓), No (✗)					

2.2.11 Uncertainties

Understanding sources of error and variability is crucial if biomonitoring tools are to be used appropriately (Ostermiller and Hawkins 2004). Despite this being widely acknowledged, the majority of biomonitoring tools currently in use in the EU fail to consider the uncertainties associated with each tool (Figure 2.4). Where the uncertainties have been considered, in most cases this does not include the accuracy of indices predictions as to the level of pressure (Demars 2013).

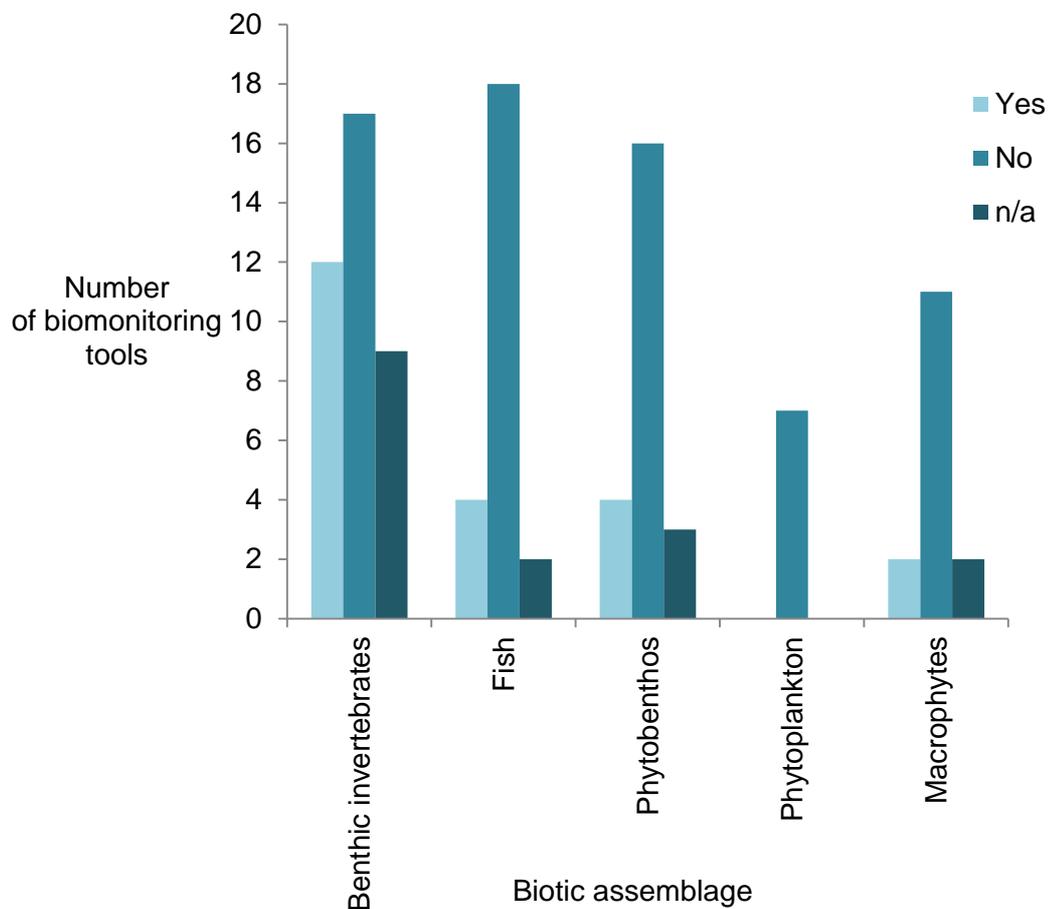


Figure 2.4. Consideration of uncertainties in 107 riverine biomonitoring tools used throughout the EU (n/a: data not available). Data extracted from the WISER database (Birk et al. 2010).

The uncertainties that are most often considered are those concerned with the precision of a biomonitoring tool such as the error and variability arising from sampling effort or method (Ostermiller and Hawkins 2004, Clarke et al. 2006). Other uncertainties can arise from the sorting procedure (taxa may be missed or under-represented in a subsample) and the level of taxonomic expertise (leading to misidentification) (Haase et al. 2004a). As previously discussed, this is one reason that a standardised sampling procedure should be followed if comparisons are to be made between sites. It is also important to test a biomonitoring tool using objective, quantitative data to determine its relationship with the pressure of concern, and where possible, determine levels of uncertainty regarding both the accuracy and precision of predictions (Friberg et al. 2011).

2.3 Conclusion

The monitoring and accurate assessment of freshwater ecosystems is essential to protect freshwater ecosystems and their biodiversity, as well as legislate effectively. Given the consequences of incorrect assignment of ecological status, it is important that biomonitoring tools are well designed and based on sound science. Despite the widespread use of biomonitoring tools to meet monitoring requirements, a comprehensive best practice approach to their development has not been established. Furthermore, although fine sediment is widely acknowledged as being one of the most widespread causes of ecological degradation in rivers and streams, a sediment-specific biomonitoring tool has not been developed and tested over the full range of river and stream sites to which it will be required to apply.

This review highlights the potential considerations for a best practice approach to developing biomonitoring tools, including the theoretical and technical aspects of tools, with a particular focus on pressure-specific tools. Based on this review of the literature,

there are five key characteristics that an “ideal” biomonitoring tool should possess, these include:

- i) Wide spatial applicability – allowing for methods to be standardised across regions, and comparisons to be made between outputs, without the need for intercalibration.
- ii) Low cost – ideally utilising monitoring methods currently in place, to maximise the potential for application of the tool by regulatory agencies.
- iii) Biological basis – to provide a mechanistic linkage for the pressure-response relationship and reduce the likelihood of a correlation being simply a statistical artefact.
- iv) Established empirical relationship with the pressure of concern – to ensure that the theory behind the development of a biomonitoring tool is validated, and that the output is accurate.
- v) Tested across the full range of sites to which it will be applied. Not only should this include minimally impacted sites, but also those with multiple anthropogenically-derived pressures, reflecting realistic evaluations of the tools performance.

Regulatory agencies require a sediment-specific biomonitoring tool with these attributes, which is able to quantify fine sediment pressure. At present there is no biomonitoring tool available, which meets these important criteria. Given the costs and limitations associated with conventional methods of monitoring fine sediment, regulatory agencies require a biomonitoring tool that has been subject to robust development and testing, and allows them to monitor this widespread form of pollution.

The most common biotic assemblage used in biomonitoring is the macroinvertebrate community. Given their diversity, ubiquity, ease of sampling, and the importance of substrate for macroinvertebrate community composition, they are likely to represent a suitable assemblage on which to base a sediment-specific biomonitoring tool. A

macroinvertebrate-based biological index has been highlighted in this review, for having the potential to be applicable as a sediment-specific biomonitoring tool across temperate rivers and streams. The PSI index has a sound biological basis, but neither its spatial applicability, nor its relationship to fine sediment, have been evaluated. Using tools with these significant uncertainties could risk environmental damage, or the imposing of unjustified burdens on water users.

An objective evaluation of the current performance of the potential sediment-specific indices is necessary, across the full range of temperate river and stream ecosystems. This will provide a foundation and an understanding of the predictive capabilities and shortcomings of the tools. Crucially, the evaluation will inform the design and development of an enhanced tool, able to identify fine sediment impacts, being based on sound science and best practice, whilst addressing the uncertainties commonly ignored during and after the development of biomonitoring tools.

Chapter 3 Evaluating the performance of sediment-specific biomonitoring tools across a wide range of temperate river and stream ecosystems

Part of the content of this chapter is reproduced from the following:

Turley, M. D., G. S. Bilotta, C. A. Extence, and R. E. Brazier. 2014. Evaluation of a fine sediment biomonitoring tool across a wide range of temperate rivers and streams. *Freshwater Biology* 59:2268-2277.

Contributions: MT led the analysis and writing of this chapter (and publication), supported and supervised by GB (primary supervisor), CE & RB (external supervisors).

3.1 Abstract

An important step that is often missed in the development of biomonitoring tools is the testing of the tool over the full range of rivers and stream “types” to which it will be applied. This chapter reports the results of a comprehensive analysis of both sediment-specific and generic biomonitoring tools across a wide range of minimally impacted temperate stream and river ecosystems, including 835 sites with data on deposited fine sediment (ranging from 0% to 100% sand, silt and clay) and 451 sites with data on suspended solids (mean annual concentrations ranging from 1 mg L⁻¹ to 46 mg L⁻¹). Chapter 2 identified the Proportion of Sediment-sensitive Invertebrates (PSI) index as having a biological basis and being developed in the UK, but with testing using fine sediment data limited to two catchments. The previous chapter also identified the Ephemeroptera, Plecoptera and Trichoptera (EPT) metrics as being commonly used internationally as indicators of habitat degradation and fine sediment pressure. These indices, along with the Average Score Per Taxon (ASPT), and the Lotic-invertebrate Index for Flow Evaluation (LIFE), both commonly used indices in UK surface waters,

were included in the analysis in this chapter. The results showed that the PSI index was more correlated with fine sediment metrics than the other biological indices tested, for both deposited fine sediment ($r_S = -0.64$, $p < 0.01$; $n = 2502$) and suspended solids ($r_S = -0.50$, $p < 0.01$; $n = 1353$). The effect of taxonomic resolution on the performance of the PSI index was also examined, as was the correlation between the PSI index and the non-sediment-specific indices. The study highlights the optimal conditions for applying the PSI index in its current form. Given the variability in the relationship between PSI and fine sediment metrics, the tool may benefit from further development, to enhance the performance of the model for future applications and advance understanding of fine sediment dynamics and the pressure–response relationship.

3.2 Introduction

The previous chapter reviewed a number of biomonitoring tools that have been used as indicators of fine sediment. Of these, the Proportion of Sediment-sensitive Invertebrates (PSI) index (Extence et al. 2013) was identified as having a biological basis and as being developed for temperate rivers and streams, but as lacking suitable testing. In addition, the Ephemeroptera, Plecoptera and Trichoptera (EPT) metrics, which are commonly used globally as indicators of habitat degradation and fine sediment pressure, were highlighted as being applicable across biogeographic regions. In this chapter, the current ability of these tools to identify fine sediment conditions will be evaluated across a wide range of temperate rivers and streams. In addition, the Lotic-invertebrate Index for Flow Evaluation (LIFE) (Extence et al. 1999) will also be evaluated due to the relationship between river flow and fine sediment dynamics, and since previous studies have shown strong correlations between LIFE and PSI (Glendell et al. 2014). Other tools that were reviewed in Chapter 2 are not evaluated either due to their lack of a biological basis, local scale development (e.g. few streams, single

regions) or the small number of taxa included in the index (limiting the applicability across ecoregions).

The importance of testing biomonitoring tools with empirical data has been previously emphasised (Bonada et al. 2006). Whilst a number of biomonitoring tools have been suggested as indicators of sediment pressure, none have been tested across the full range of pressure gradients or across a wide range of environments. Instead, where testing has been carried out, it has been done on a local scale (Zweig and Rabeni 2001, Sutherland et al. 2012), or using proxies for fine sediment (Extence et al. 2013). The use of large datasets taken from national monitoring programmes provides an opportunity to assess the spatial applicability of these biomonitoring tools (Friberg et al. 2011). This “opportunistic” data analysis will often comprise relatively low-resolution temporal data, but usually benefits from the use of standardised methods and long-term data. In the UK, data from the Environment Agency (EA), the Scottish Environment Protection Agency (SEPA), Natural Resources Wales (NRW) and the Northern Ireland Environment Agency (NIEA) monitoring programmes are freely available and encompass thousands of rivers and streams. One limitation of these data is that the sites are likely to be impacted by numerous anthropogenic disturbances, which are likely to result in multiple pressures on the macroinvertebrate community. An exception to this is the River InVertebrate Prediction And Classification System (RIVPACS) database comprised of 835 minimally impacted sites, sampled between 1978 and 2004. The sites were selected for being minimally impacted by anthropogenic disturbances and were thought to represent the best examples of their ‘river types’ (Clarke et al. 2003). Given that substratum and suspended solids concentrations vary naturally, these minimally impacted sites provide an opportunity to assess the ability of these biomonitoring tools to discriminate between fine sediment conditions, in an environment with few confounding pressures.

3.2.1 *Aims and objectives*

The aim of this chapter was to evaluate the performance of a range of biomonitoring tools in terms of their ability to indicate fine sediment conditions throughout minimally impacted, temperate river and stream ecosystems. This comprehensive analysis will allow for a strategic approach to any further development of the indices. To further develop an index, it is useful to know where it performs well and where further work is needed. In addition, any correlations obtained from this initial evaluation can be used as benchmarks to determine the effectiveness of the further development stages.

The hypotheses were that:

- (i) The biomonitoring tools will be correlated to the percentage of the substratum consisting of fine sediment.
- (ii) The biomonitoring tools will be correlated to mean suspended solids concentration.

Further aims of this chapter were to:

- (i) Determine whether the taxonomic resolution (family-level, mixed-level, species-level), and sampling season, used to calculate the biomonitoring scores, influenced the strength of the correlation with fine sediment metrics.
- (ii) Determine whether there was a correlation between potential sediment-specific tools and other non-specific, commonly used biological indices; Average Score Per Taxon (ASPT) (Murray-Bligh 1999), Lotic-invertebrate Index for Flow Evaluation (LIFE) (Extence et al. 1999).

3.2.2 *Ephemeroptera, Plecoptera and Trichoptera metrics*

Ephemeroptera, Plecoptera and Trichoptera are some of the most widely studied freshwater macroinvertebrates. Perhaps partly for this reason, EPT metrics are commonly used as biomonitoring tools, often broadly considered to be sensitive to poor water quality (Lenat 1988). These orders of insects comprise numerous families, genera and species, some of which have been shown to respond differently to pressures (Descloux et al. 2013). Nevertheless, they have been used in studies investigating water quality impairment (Lenat 1988), the impacts of water abstraction (Dewson et al. 2007), sediment pressure (Angradi 1999, Zweig and Rabeni 2001) and multiple agricultural pressures (Wagenhoff et al. 2012).

3.2.3 *The Proportion of Sediment-sensitive Invertebrates (PSI) index*

As previously discussed, the PSI index was developed using expert judgement following an extensive literature review, and an assessment of macroinvertebrate biological and ecological traits, giving the tool a biological basis (rather than a statistical basis). The traits used to determine macroinvertebrate sensitivities included locomotion, functional feeding group, mode of respiration, and substrate preference. Taxa were assigned to one of four Fine Sediment Sensitivity Ratings (FSSRs) (Table 3.1) with the group assignment influencing the taxon's position in the PSI equation (Equation 3.1), as well as the abundance related score.

Table 3.1. The Fine Sediment Sensitivity Ratings (FSSRs) and relevant abundance related weights that form the basis of the PSI index (Extence et al. 2013).

	Fine Sediment Sensitivity Rating (FSSR)	Log abundance categories			
		1-9	10-99	100-999	>1000
A	Highly sensitive	2	3	4	5
B	Moderately sensitive	1	2	3	4
C	Moderately insensitive	1	2	3	4
D	Highly insensitive	2	3	4	5

$$PSI = \frac{\sum \text{Scores for Fine Sediment Sensitivity Groups A \& B}}{\sum \text{Scores for all Fine Sediment Sensitivity Groups A, B, C \& D}} \times 100$$

Equation 3.1. Formula used to calculate PSI scores using abundance-weighted scores (Extence et al. 2013).

In the numerator of the PSI equation (Equation 3.1), abundance related weights for those taxa deemed to be highly sensitive and moderately sensitive of fine sediment, are summed. In the denominator of the equation, the abundance related weights of both sensitive and insensitive taxa are summed. Finally, the value is multiplied by 100, to provide a PSI score of between 0 and 100, with a suggested interpretation of: 0 – 20 = heavily sedimented, 21 – 40 = sedimented, 41 – 60 = moderately sedimented, 61 – 80 = slightly sedimented, and 81 – 100 = minimally sedimented.

3.2.3.1 *Evaluating the theoretical basis and development of the PSI index*

When comparing the PSI index to the 11 criteria suggested by Bonada et al. (2006) alongside other potential sediment-specific indices (Table 2.5 – Chapter 2) it is clear

that although the index has a number of benefits, there is significant scope for further development. Being based on biological and ecological traits that were considered either sensitive or tolerant to fine sediment, the PSI index recognises that organisms are adapted to a specific range of environmental conditions (the niche concept), and as such is based on sound theoretical principles, which may increase the potential for the tool to discriminate between pressures, providing a mechanistic linkage. Although the index has this biological basis, it is not clear whether the index is able to indicate fine sediment conditions or discriminate between multiple pressures, as the index's performance has not been evaluated across the necessary range of fine sediment conditions or river and stream types. This is a crucial part of the development of biomonitoring tools, as identified in Chapter 2.

The PSI index has both family and species resolution versions, and therefore it can be applied to historic datasets (which were often recorded to family-level) as well as to mixed-level (family, genus and species) data currently being collected by the UK regulatory agencies. As discussed in Chapter 2, using species-level resolution data recognises that a significant amount of variation can exist, in terms of ecological preferences between species within the same family and genera.

The index uses abundance data transformed into log abundance categories (0 – 9 = 1; 10 – 99 = 2; 100 – 999 = 3; > 1000 = 4), to allow consideration of the semi-quantitative nature of the kick-sampling method of sampling macroinvertebrates, whilst maintaining some indication of changes to abundances. The costs associated with application of the tool itself are minimised as it can be incorporated into existing monitoring protocols, by applying the index to routinely collected data. This is an important logistical consideration, which is likely to determine the adoption of any biomonitoring tool by regulatory agencies.

3.2.3.2 Uncertainties associated with the PSI index

The uncertainties associated with applying the PSI index using the UK's predictive modelling approach, the River Invertebrate Classification Tool (RICT) model, are considered in Figure 3.1. Whilst many of these uncertainties are beyond the scope of this thesis, as they relate to the use of the reference-based model or sampling protocols that are UK standard techniques, it is important to consider the range of these uncertainties, which will influence the application of the PSI index, as well as other biological indices that are applied using this reference-based approach.

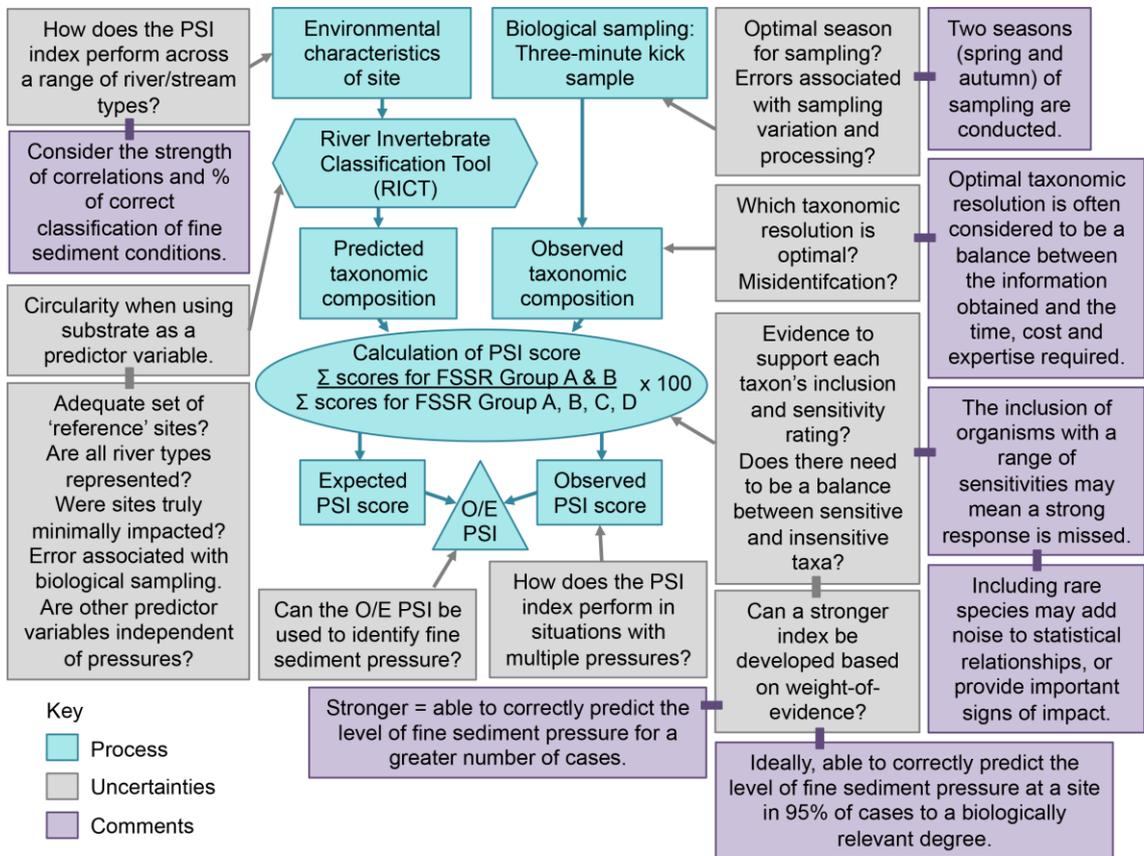


Figure 3.1. The process of calculating the observed (O) and expected (E) Proportion of Sediment-sensitive Invertebrates (PSI) index scores for a site using the “River Invertebrate Classification Tool” approach, highlighting areas of uncertainty.

3.3 Methods

3.3.1 Data

The main dataset used in this study is the RIVPACS IV (May 2011 version) dataset (River Invertebrate Prediction and Classification System - NERC [CEH] 2006. Database rights NERC [CEH] 2006 all rights reserved) described in detail by Wright (2000) and Clarke et al. (2003), and is summarised here. The database contains macroinvertebrate, water quality and catchment characteristics data, recorded at each site over at least one year, between 1978 and 2004. The 835 reference condition sites, on streams and rivers across the UK (Figure 3.2), encompass a wide range of environments, varying in their: (i) Climate – mean annual precipitation totals between 1961-1990 of 430 to 2930 mm and mean annual air temperatures between 1961-1990 ranging from 7.93 to 11.45 °C, (ii) Geology - varying from catchments dominated by hard igneous rocks to catchments dominated by soft sedimentary rocks, and (iii) Topography - altitudes at river source varying from 5 to 1216 m above sea level. The stream and river sites also vary in their morphometry with widths ranging from 0.4 to 117 m, and average depths ranging from 0.02 to 3.00 m (widths and depths are a mean of three seasonal measurements). All of the sites were considered to be minimally impacted, and as close to reference condition as it is possible in the UK, with no, or only very minor, anthropogenic alterations to the values of water chemistry and hydromorphology, supporting biota usually associated with such undisturbed or minimally disturbed conditions.

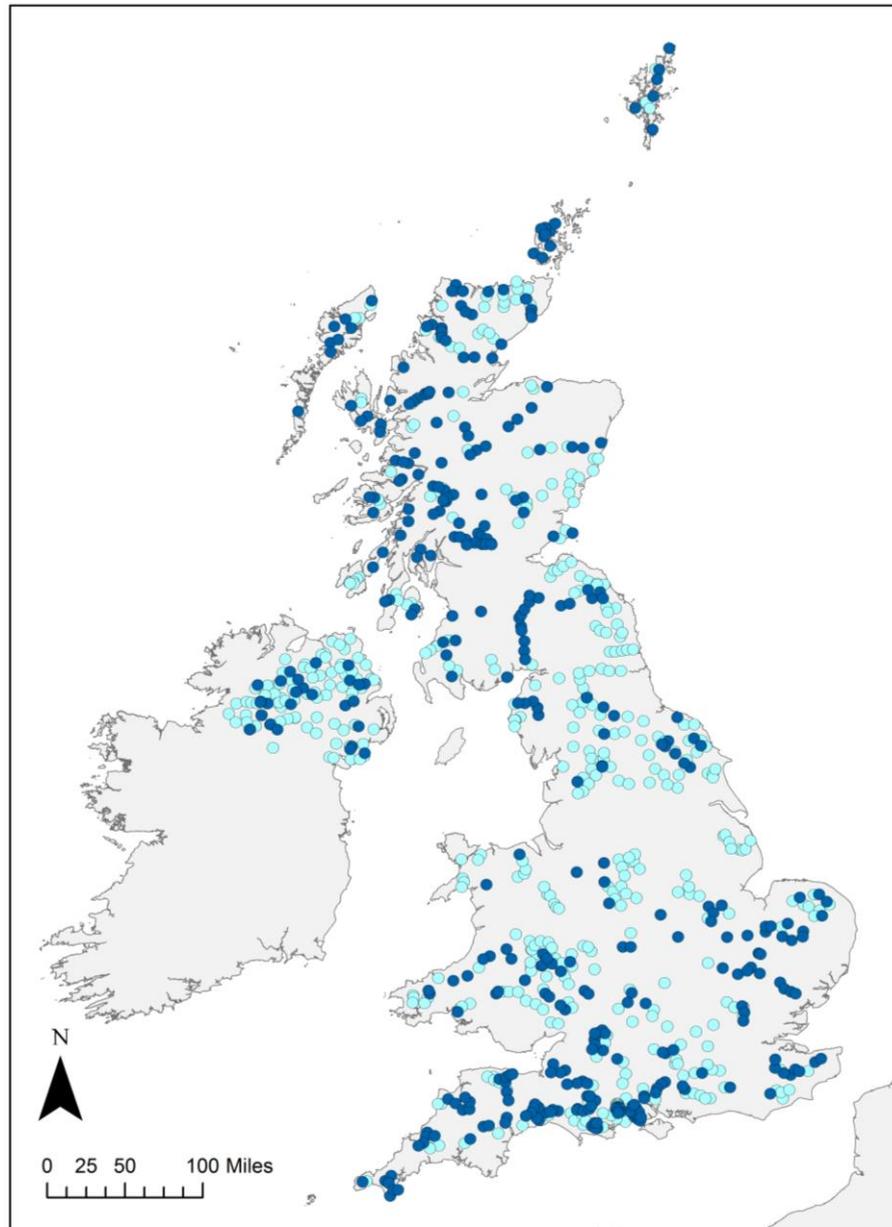


Figure 3.2. Distribution of RIVPACS 'reference condition' sites. Light blue dots are those sites with both substratum composition data and ≥ 12 suspended solids measurements, dark blue dots are those sites with only substratum composition data.

3.3.1.1 Macroinvertebrate data

The macroinvertebrate data within the RIVPACS IV database were collected from the 835 sites, using the UK standard sampling protocol, described in the Standardisation of River Classifications project proposal (EU-STAR 2004). Briefly, this involved

conducting a three-minute active kick-sample technique with a 900 µm mesh pond net, where all in-stream habitats within the site were sampled in proportion to their occurrence, followed by a one-minute manual search (EU-STAR 2004). The abundance of different macroinvertebrates identified to species level or to the lowest possible taxonomic unit, were recorded numerically (Wright 2000). There are season-specific records of community composition: Spring being the samples collected during March – May, summer being the samples collected during June – August, and autumn being the samples collected during September – November. There are no records for winter (December – February). The three taxonomic resolutions recorded within the database and used in this study were (i) family-level (103 taxa), (ii) mixed-level (415 taxa) and (iii) species-level (652 taxa).

3.3.1.2 Fine sediment data

The fine sediment data available within the RIVPACS IV database, includes measurements of suspended solids (SS), and observations describing the percentage of the substratum consisting of: (i) Silt and clay (< 0.06 mm), (ii) sand (< 2 mm, ≥ 0.06 mm), and (iii) sand, silt and clay combined. All of the 835 RIVPACS IV sites have data describing the substratum composition. These data were collected using the visual estimate method described in the Standardisation of River Classifications project protocol (EU-STAR 2004). Briefly, this involves the operator, estimating the percentage cover of substratum classes over a given reach, based on a visual inspection. The values used represent a mean of three seasonal measurements. Whilst this technique does not quantify the volume of deposited fine sediment, which PSI is designed to relate to, it does provide a measure of the percentage cover, which theoretically should be related to the PSI index (Glendell et al. 2014).

Four hundred and fifty one of these sites had 12 or more SS measurements taken over

at least one year of sampling (between 1978 and 2004), and these were selected for use in this study. Concentrations of SS were determined using the standard gravimetric method which involves filtration of a known volume of sample through a dried and pre-weighed 0.7 μm pore-size glass fibre filter paper, followed by drying at 105 °C and reweighing (Anon 1980, Gray et al. 2000). The sites exhibited a range of mean annual SS concentrations (1 mg L^{-1} to 46 mg L^{-1}) measured between 1978 and 2002 at the 451 sites used in this paper. Although the concentrations based on this frequency of sampling may not capture the full range of SS values that occur at each site, they do provide a good indication of mean annual background SS concentrations.

3.3.2 *Statistical analyses*

The relevant data were extracted from the RIVPACS IV database and compiled in Microsoft Excel prior to analysis. The PSI index, EPT metrics (EPT abundance, % EPT abundance, EPT richness and % EPT richness), LIFE, and ASPT were calculated using the data of compatible taxonomic resolutions. All indices were assessed for any correlation to fine sediment metrics. The different taxonomic resolutions are identified from this point forward by the subscript following the index name. For example, the PSI index was calculated using data of 652 taxa (mostly to species-level), 415 taxa (family, genus or species-level), and 103 taxa (family-level), and these different taxonomic resolutions are identified as PSI_{sp} , $\text{PSI}_{\text{mixed}}$ and PSI_{fam} , respectively. Given the semi-quantitative methods used for macroinvertebrate sampling, log abundance categories (0 – 9 = 1; 10 – 99 = 2; 100 – 999 = 3; > 1000 = 4) were used to calculate PSI, EPT abundance, % EPT abundance and LIFE scores. Using SPSS statistical software (IBM® SPSS® Statistics 20, NY, USA) the data were found to be non-normally distributed and show heteroscedasticity, and could not be transformed successfully.

The non-parametric Spearman's rank correlation was used to analyse the relationship

for spring, summer, and autumn, between the biological indices of different taxonomic resolution and fine sediment metrics (see Supplementary Material A). The seasonal data were then combined to provide a single Spearman's rank correlation for each biological index and sediment metric. All correlations were interpreted using the Dancey and Reidy (2007) classifications of correlations; 0.1 – 0.39 = weak, 0.4 – 0.69 = moderate, 0.7 – 0.99 = strong. The index and fine sediment metric, which exhibited the strongest correlation was analysed using the Kruskal-Wallis test, as it was expected that this relationship would have the greatest predictive capabilities. This was done for both deposited fine sediment and suspended solids metrics by grouping the scores into independent groups (e.g. 0 – 20, 21 – 40, 41 – 60, 61 – 80, 81 – 100); the Kruskal-Wallis test returns a p -value which is used to determine if any of the groups are significantly different. Pairwise comparisons were then performed using Dunn's (1964) procedure with a Bonferroni correction for multiple comparisons, to determine which groups were significantly different.

3.4 Results

3.4.1 Biological indices and fine sediment metrics

The PSI index was negatively correlated to the percentage of the substratum consisting of fine sediment (Figure. 3.3a). The strongest relationship, for all seasons and all taxonomic levels, was between PSI and the percentage of the substratum consisting of sand, silt and clay; the highest of which was $r_s = - 0.65$, $p < 0.01$ (observed in summer). The strongest relationship for the percentage of substratum consisting of silt and clay was $r_s = - 0.63$, $p < 0.01$ (observed in spring). For the percentage of the substratum consisting of sand, the strongest relationship with PSI was $r_s = - 0.42$, $p < 0.01$ (observed in summer). The PSI score was also negatively correlated to mean SS concentration (Figure. 3.3b), the strongest relationship, for all seasons and all taxonomic levels being $r_s = - 0.55$, $p < 0.01$. The EPT metrics were

also negatively correlated to the percentage of the substratum consisting of fine sediment, but to a lesser extent, with the strongest correlation ($r_s = -0.55$, $p < 0.01$) being between % EPT abundance and % sand, silt and clay.

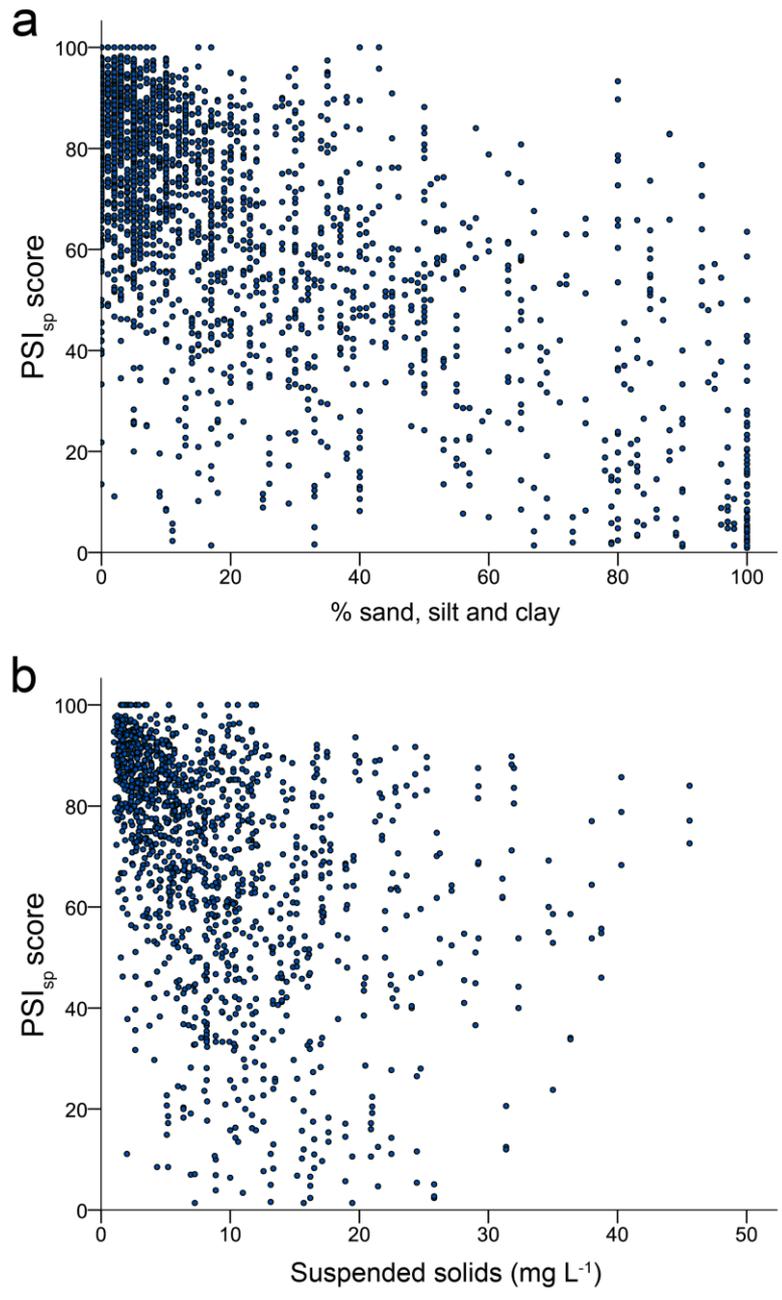


Figure 3.3. Relationship between PSI_{sp} score (combined seasons) and (a) % sand, silt and clay substratum (based on visual estimates) (b) suspended solids (mean; $n \geq 12$), derived from RIVPACS data.

Due to the similar correlations between index scores and fine sediment metrics (both deposited and suspended) across all sampling seasons; for example PSI_{sp} versus % sand, silt and clay varied by 0.01 (spring $r_s = -0.64$, summer $r_s = -0.65$, autumn $r_s = -0.64$, $p < 0.01$), the three seasons were combined, and from this point forward will be considered as one set of data (Table 3.2).

Table 3.2. Spearman's correlation coefficients between combined seasons of biological indices and fine sediment metrics at minimally impacted RIVPACS sites.

Biological index	Silt & clay	Sand, silt & clay	Sand	Suspended solids mean (mg L ⁻¹)
$ASPT_{fam}$	-0.44	-0.50	-0.29	-0.34
$LIFE_{fam}$	-0.53	-0.57	-0.33	-0.28
$LIFE_{mixed}$	-0.51	-0.55	-0.34	-0.28
$LIFE_{sp}$	-0.54	-0.58	-0.36	-0.31
PSI_{fam}	-0.58	-0.61	-0.37	-0.41
PSI_{mixed}	-0.60	-0.63	-0.40	-0.47
PSI_{sp}	-0.62	-0.64	-0.40	-0.50
EPT abundance	-0.20	-0.21	N/S	-0.11
% EPT abundance	-0.55	-0.59	-0.35	-0.46
EPT_{fam} richness	-0.24	-0.26	-0.06	-0.22
% EPT_{fam} richness	-0.52	-0.55	-0.31	-0.47

Unless otherwise stated, correlations are significant at the 0.01 level (2-tailed). Subscript text indicates the taxonomic resolution (family-, mixed- or species-level) used for index calculation. N/S indicates that the correlation was not significant.

3.4.2 Taxonomic resolution

The PSI scores based on family-level data had consistently weaker correlations with all fine sediment metrics compared to PSI scores calculated using mixed-level and species-level data. The correlations between mean SS and PSI_{sp} , and mean SS and PSI_{fam} , had the largest differences ($r_s = -0.50$, $p < 0.01$ compared to $r_s = -0.41$, $p < 0.01$, respectively). Spearman's rank correlations (Table 3.2) between PSI and fine sediment show that PSI_{sp} and PSI_{mixed} had similar moderate correlations to fine sediment metrics, despite the greater number of taxa and species-level data used to calculate PSI_{sp} (e.g. sand, silt and clay: $r_s = -0.64$, $p < 0.01$ compared to $r_s = -0.63$, $p < 0.01$, respectively).

3.4.3 Discrimination ability of the best performing index

In order to evaluate the ability of PSI to discriminate between different levels of sedimentation, PSI_{sp} scores were grouped and analysed using the Kruskal-Wallis test. The process of selecting group sizes for PSI scores, was a balance between selecting groups which were very small (i.e. PSI scores of 0 – 5, 5 - 10, 10 – 15, etc.) which would need to be based on highly accurate and precise measurements of fine sediment, and selecting groups that were very large, which would limit the potential for discrimination between different levels of sedimentation. Grouped PSI scores of 20 % were tested (Figure. 3.4) and found to have significant differences between groups ($p < 0.05$). Pairwise comparisons identified all groups as being significantly different from each other ($p < 0.05$). Similarly, this analysis was carried out on the relationship between PSI and mean SS concentration. A statistically significant difference ($p < 0.05$) was observed between mean SS concentrations grouped by PSI, with only the two upper groups (PSI 60.1 – 80 and 80.1- 100), being statistically different from each other and the three lower groups (PSI 0 – 20, 20.1 – 40 and 40.1 – 60.0).

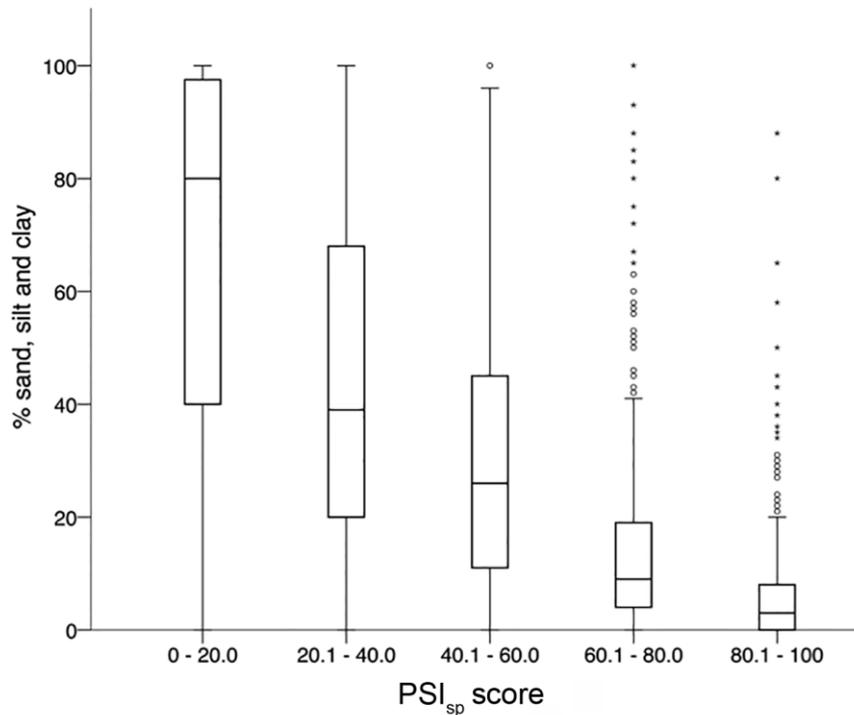


Figure 3.4. Boxplots showing the relationship between grouped PSI_{sp} scores and % sand, silt and clay substratum (based on visual estimates) derived from minimally impacted RIVPACS sites, using combined seasons data. SPSS identifies potential outliers as: > 1.5 times (°) or > 3 times (*) the interquartile range above the 75th percentile.

3.4.4 Comparisons between the biological indices

The PSI index had slightly to moderately, stronger correlations with all fine sediment metrics than all other biological indices tested. The % EPT abundance, % EPT_{fam} richness and LIFE_{sp}, had slightly weaker, moderate correlations with % sand, silt and clay ($r_s = -0.59$, $r_s = -0.55$, and $r_s = -0.58$, $p < 0.01$, respectively, compared to $r_s = -0.64$, $p < 0.01$ for PSI_{sp}) as well as for % silt and clay ($r_s = -0.55$, $r_s = -0.52$ and $r_s = -0.54$, $p < 0.01$, respectively, compared to $r_s = -0.62$, $p < 0.01$ for PSI_{sp}). Two indices (EPT abundance and EPT_{fam} richness) had much weaker correlations with all sediment metrics, their strongest correlations being with % sand, silt and clay ($r_s = -0.21$ and $r_s =$

-0.26, $p < 0.01$, respectively).

The correlations between the biological indices were also analysed using Spearman's rank correlation. Strong, statistically significant, positive relationships ($r_s = > 0.74$, $p < 0.01$) were observed between PSI, and a number of the non-sediment-specific indices; % EPT abundance, % EPT_{fam} richness, LIFE_{fam}, LIFE_{mixed}, LIFE_{sp} and ASPT_{fam} (Table 3.3).

Table 3.3. Spearman's correlation coefficients for relationships between biological indices from combined seasons, at minimally impacted streams in RIVPACS.

Biological index	PSI _{fam}	PSI _{sp}	PSI _{mixed}
ASPT _{fam}	0.79	0.74	0.75
LIFE _{fam}	0.89	0.85	0.86
LIFE _{sp}	0.85	0.89	0.89
LIFE _{mixed}	0.83	0.85	0.87
EPT abundance	0.33	0.27	0.29
% EPT abundance	0.83	0.80	0.80
EPT _{fam} richness	0.41	0.36	0.37
% EPT _{fam} richness	0.82	0.78	0.79

All correlations are significant at the 0.01 level (2-tailed). Subscript text indicates the taxonomic resolution (family, mixed or species) used for index calculation.

3.5 Discussion

3.5.1 *The ability of the best-performing index to indicate fine sediment conditions*

The results of this study show that the PSI index was moderately, negatively correlated to: (i) The percentage of the substratum consisting of sand, silt and clay, (ii) percentage of the substratum consisting of % silt and clay, (iii) mean SS concentration, and therefore both hypotheses are accepted. The strongest correlation observed when seasons were combined, was between PSI_{sp} and the percentage of the substratum consisting of sand, silt and clay ($r_s = -0.64$, $p < 0.01$). To put this into context, a study of 297 bio-assessment methods (comprising invertebrate, macrophyte, phytoplankton and diatom indices), which are used for the implementation of the Water Framework Directive (WFD) across Europe, found the median correlation coefficient of invertebrate-based indices to be 0.64 in relation to their respective stressor (Birk et al. 2012). Based on that analysis, the correlation observed in this study (between PSI and the percentage of the substratum consisting of sand, silt and clay), is comparable to other indices used in the implementation of the EU WFD. Nevertheless, given the implications of incorrect assignment of ecological status of streams for both water and land managers, greater effort is needed to evaluate and improve the performance of biological indices to achieve robust models.

Statzner et al. (2005) concluded that a robust model should be able to predict group assignments correctly in c.70% of cases. The results from the Kruskal-Wallis and post-hoc test show that when PSI_{sp} was grouped (0 – 20, 21 – 40, 41 – 60, 61 – 80, 81 – 100), the % of sand, silt and clay in the groups were statistically significantly different between all groups. However, the large overlaps between groups (Figure. 3.4) prevent the development of an effective predictive model and highlight the need for detailed validation and further development of the index.

The variability and 'wedge shaped' response observed for PSI to fine sediment metrics, is at least partly due to the natural variability in biological communities (Resh and

Jackson 1993) and environmental characteristics (Zweig and Rabeni 2001). It may also be attributed to the invertebrates responding to multiple pressures (Ormerod et al. 2010), although, as these are minimally impacted sites it is more likely to be due to the quality of the underlying biological or fine sediment data (Friberg 2010). For example, there is likely to be error introduced by utilising the sediment metrics which are annual averages, and (i) are not necessarily intended to quantify the rate or degree of sedimentation, (ii) do not necessarily provide information on the sediment conditions preceding the biological sampling, and (iii) rely on the visual estimate method (deposited sediment) which is subjective and will have been collected by different observers potentially adding to the variance (Wang et al. 1996). Despite these limitations, the visual estimate method is one of the most widely used methods of quantifying deposited fine sediment, and the data used in this study benefits from its large spatial coverage and high number of minimally impacted sites.

Furthermore, Sutherland et al. (2012) found the visual estimate method to provide the strongest correlation of eight sediment metrics, ($r^2 = 0.78$, $p < 0.001$) to their Modified Family Biotic Index, in 15 agricultural streams in Canada. Similarly, Zweig and Rabeni (2001) observed moderate-strong correlations ($r_s = -0.534$ to -0.907 , $p < 0.001$) for deposited sediment ranging from 0 - 100% fines (visual estimates) with various biological metrics across 4 streams in Missouri, USA. The lower correlations seen in this present study may be partly due to the wide range of ecosystems and regions from which the data are collected. Nonetheless, it is important from a policy perspective that indices are applicable and standardised over larger scales (Statzner and Beche 2010), therefore, the use of large datasets, which derive from a wide range of environments are essential if we are to develop improved biomonitoring tools.

There is likely to be further error related to the inability of macroinvertebrate sampling to collect information on the full diversity at sampling sites. A UK study found that one standardised three-minute kick-sample typically contained 50% of the species and 60%

of the families found amongst six replicate samples at the same site (Wright 2000). However, the biological sampling method of kick-sampling is the UK standard protocol for sampling macroinvertebrates under the EU WFD, and the biological data gained from this technique are the basis for calculating the biological indices utilised by regulatory agencies.

To refine sediment-specific indices, it may be necessary to collect more objective and quantitative measures of deposited fine sediment, particularly as increased sedimentation is often accompanied by other factors such as flow variation, removal of riparian vegetation, and nutrient enrichment, all of which can alter macroinvertebrate communities (Zweig and Rabeni 2001). The empirical testing of the pressure-response relationship of a biological index is therefore an essential step in the development and validation process (Borja et al. 2011, Friberg et al. 2011), yet for approximately 30 % of biological assessment methods used in Europe for the purpose of assessing WFD ecological status, this has not occurred (Birk et al. 2012).

3.5.2 Effect of taxonomic resolution on the performance of the index

The results highlight that increasing the taxonomic resolution (family to species level data), increased the strength of the relationship between PSI and fine sediment metrics. The effect of taxonomic resolution on biological indices has been an extensively reviewed topic, with consensus being that species identification is preferred due to the variability of sensitivity within families and genera (Furse et al. 1984, Resh and McElravy 1993, Schmidt-Kloiber and Nijboer 2004, Monk et al. 2012). The collection and use of species data is often considered to be appropriate as ecological niche concept recognises that each species is adapted to thrive in a particular “ecological space” with specific abiotic and biotic characteristics (Grinnell 1917, Hirzel and Le Lay 2008). However, identification to a coarser taxonomic resolution (i.e. family)

may be time- and cost- efficient, likely to result in fewer identification errors, and does not require taxonomic specialists (Furse et al. 1984, Bailey et al. 2001). The calculation of PSI scores using species data in this study shows some benefits to that of PSI scores calculated with family data, the greatest of which being between SS and PSI_{sp} , $r_{s=}$ -0.50 compared to SS and PSI_{fam} , $r_{s=}$ -0.41. This may be an important consideration for regulatory agencies with time and budget constraints. However, with more quantitative measures of deposited fine sediment, the importance of species responses may become more apparent.

3.5.3 Effect of substratum particle size on the performance of the index

The different strength correlation coefficients between PSI and the four, fine sediment metrics suggest that the correlation between PSI score and substratum varies with substratum particle size. The correlations between PSI and % silt and clay, and % sand, silt and clay, were stronger than with mean SS concentration. This may be expected as the PSI index is designed to identify sedimentation i.e. deposited fine sediment, and not necessarily suspended solids (Extence et al. 2013). The moderate correlation between PSI and SS, is likely to be due to the link between SS and deposited sediment as well as the impacts caused by the SS to aquatic invertebrates (reviewed in Bilotta and Brazier 2008).

3.5.4 Independence of the biological indices

The results of this study show that the PSI index had a much stronger correlation with the fine sediment metrics, compared to EPT abundance and EPT richness, and was slightly more strongly correlated than LIFE, ASPT, % EPT abundance and % EPT richness, which also showed moderate relationships to fine sediment metrics; the PSI index was able to explain slightly more of the variation (% sand silt and clay: PSI_{sp} , $r_{s=}$

-0.64, $p < 0.01$ compared to % EPT abundance, $r_s = -0.59$, $p < 0.01$). Whilst this is a relatively small difference, the benefits of the PSI are that it also provides a mechanistic linkage for the macroinvertebrate responses to fine sediment, being based on biological and ecological traits that cause the organism to be sensitive or tolerant of fine sediment (Extence et al. 2013). In contrast, the EPT indices are more generic, but nevertheless are often used as indicators of fine sediment impacts or overall habitat degradation (Wagenhoff et al. 2012). Treating EPT indices as sediment-specific indices may potentially provide misleading results in some situations, given that some EPT are relatively tolerant of fine sediment (e.g. many Caenidae and Limnephilidae species). Despite this, other studies have shown weak to strong correlations between fine sediment metrics and EPT indices. Angradi (1999) observed relatively subtle changes to EPT taxa richness, at sites with a narrow range of sediment characteristics (5-30% fine sediment, < 2 mm). Whereas, Zweig and Rabeni (2001) found Spearman's rank correlations between visual estimates of fine deposited sediment and EPT density and EPT richness which ranged from $r_s = -0.498$ to $r_s = -0.868$, $p < 0.01$. These strong correlations may be due in part to the experimental design, which included sampling only four streams, with similar habitat characteristics (except substrate composition). Another study of 18 streams (32 reaches) found that at the reach-scale, fine sediment could not be related to EPT indices, but at the patch-scale, in eight streams (12 reaches), found significant declines in EPT richness (25% less EPT taxa) at sediment-rich sites (Larsen et al. 2009). EPT relative abundance has also previously been shown to be moderately correlated to visual estimates of fine sediment (Sutherland et al. 2012, Burdon et al. 2013). Contrary to their expectations, Descloux et al. (2013) found that EPT abundance was highest in heavily sedimented reaches due to increases in some species of Ephemeroptera (*Baetis* sp. and *Rhithrogena* sp.). Other studies have also found Baetidae, particularly *Baetis* spp. to be relatively tolerant of fine sediment, being present at sites with high fine sediment (Culp et al. 1983, Wallace and Gurtz 1986, Relyea et al. 2000). Whilst in some instances EPT metrics may be useful as

indicators of fine sediment conditions, in the present study, which includes over 835 sites (three seasons, $n = 2502$) from a wide range of different temperate river and stream ecosystems, these metrics do not appear to be useful as indicators of fine sediment.

All indices showed at least some moderate relationships with fine sediment metrics, but all were strongly correlated with the PSI index; demonstrating the need for further development of the PSI index if it is to be considered a fine sediment-specific index. The positive correlation between PSI_{fam} and $ASPT_{fam}$ ($r_s = 0.79$, $p < 0.01$) and PSI_{fam} and $LIFE_{fam}$ ($r_s = 0.89$, $p < 0.01$), can be expected as higher scoring ASPT and LIFE taxa are likely to be more prevalent at sites with higher PSI scores (sites with better water quality and faster flows). Additionally, the strong statistically significant correlation between PSI_{fam} and $LIFE_{fam}$ ($r_s = 0.89$, $p < 0.01$), may be due to the relationship between flow and fine sediment transport-deposition (Matthaei et al. 2010). Similar correlations between PSI, LIFE and ASPT were also observed in two contrasting catchments in the south west of the UK (Glendell et al. 2014). In contrast, a study of conservation priority habitats (woodland, agri-environment schemes and organic farming) in the Upper Thames catchment reported a link between PSI and these land uses, with no such link for the LIFE index. This suggests a certain independence between PSI and LIFE, although the lack of fine sediment metrics in the study prevents the PSI index being conclusively linked to sedimentation (Poole et al. 2013).

3.6 Conclusion

This chapter represents the first evaluation of the PSI index and EPT metrics, across a wide range of temperate rivers and streams. Given the importance of empirically testing the ability of biological indices to identify particular stressors, further work is

needed in order to test the PSI approach. Whilst this work highlights the relationship between the PSI index and visual estimates of the percentage of substratum consisting of sand, silt and clay, it also shows the large variances observed, even over minimally impacted sites. With the documented methods for measuring deposited fine sediment consisting largely of destructive, semi-quantitative and subjective techniques, the understanding of deposited fine sediment dynamics and the pressure-response relationship would benefit from a more objective, quantitative method at the reach-scale. In addition, information on the size and geochemical composition of the sediment may help disentangle the pressure-response relationship. Data from a more objective, quantitative method of measuring deposited fine sediment could also benefit the further development of the PSI index, with the aim of enabling accurate predictions of the levels of fine sediment. Further work should also evaluate the theoretical and technical aspects of the PSI index (as the best performing tool), including the sensitivity scores on which the tool is based.

Chapter 4 Developing an improved biomonitoring tool for fine sediment: Utilising expert knowledge and empirical data

Part of the content of this chapter is reproduced from the following:

Turley, M. D., G. S. Bilotta, T. Krueger, R. E. Brazier, and C. A. Extence. 2015. Developing an improved biomonitoring tool for fine sediment: Combining expert knowledge and empirical data. *Ecological Indicators* 54:82-86.

Turley, M. D., G. S. Bilotta, R. P. Chadd, C. A. Extence, R. E. Brazier, N. G. Burnside, and A. G. Pickwell. 2016. A sediment-specific family-level biomonitoring tool to identify the impacts of fine sediment in temperate rivers and streams. *Ecological Indicators* 70:151-165.

Contributions: MT led the analysis and writing of this chapter (and publications), supported and supervised by GB (primary supervisor), CE & RB (external supervisors). TK provided training on modeling techniques, and assisted with model troubleshooting. RC helped with the identification of macroinvertebrates and gave advice on invertebrate ecology. NB assisted with obtaining data and matching sampling locations using GIS. AP provided assistance obtaining data and advice on macroinvertebrate identification.

4.1 Abstract

The previous chapter highlighted the Proportion of Sediment-sensitive Invertebrates (PSI) index as the best performing sediment-specific biomonitoring tool, and the most viable option for application within the UK. The PSI index has a sound biological basis, with taxon sensitivity ratings that were assigned using existing literature and knowledge of biological and ecological traits. The family-level and mixed-level versions of PSI allow it to be easily integrated into standardised biological sampling and recording

methods. However, the evaluation of these versions of the index across a wide range of temperate river and stream ecosystems, showed it to have moderate correlations with fine sediment ($r_s = -0.61$, $p < 0.01$ and $r_s = -0.64$, $p < 0.01$, respectively), which although comparable to other biomonitoring tools used throughout Europe, may somewhat limit confidence in its application. In this chapter, the PSI index is used as a basis for the development of an improved macroinvertebrate family-level and mixed-level biomonitoring tool for fine sediment. The biologically-based classifications of sediment sensitivity used in the PSI index, are supplemented by using empirical data of macroinvertebrate abundance and percentage fine sediment, collected across a wide range of minimally impacted temperate river and stream ecosystems (model training dataset $n = 2252$) to assign individual indicator weights to taxa. Indicator weights were restrained based on the taxon's original classification as sensitive or insensitive. An optimum set of weights were identified, using a method of non-linear optimisation, as those that resulted in the highest Spearman's rank correlation coefficient between the new index (called the Empirically-weighted Proportion of Sediment-sensitive Invertebrates index; E-PSI) scores and deposited fine sediment in the model training dataset. A new version of the family-level E-PSI index is also developed to account for the likelihood of significant intra-family variation regarding sediment sensitivity. The optimum taxon weights were used to calculate E-PSI scores for sites in an independent test dataset ($n = 84$) across a range of temperate river and stream sites. A strong correlation was observed between both the family-level and mixed-level versions of the index, and fine sediment, indicating an improved performance and suggesting that the tool may be a useful addition to the suite of tools currently used by regulatory agencies and other water managers.

4.2 Introduction

Biomonitoring tools are used globally to monitor and inform the management of freshwater ecosystems. Despite an increasing reliance on these tools to determine the ecological condition of surface waters, many of them have been developed and documented with little explicit consideration of the theoretical underpinning and the various decisions that are taken during the process of model development. Furthermore, a recent review of biomonitoring tools used throughout Europe, found that 31% of lotic indices had no documented empirical testing with their respective pressure (Birk et al. 2012). Ideally, for reasons discussed in more detail in the following sections, the future development of biomonitoring tools should fully document the steps taken and the theory behind the models development, and ensure that where possible they: (i) have a biological basis, (ii) can be easily integrated into standardised biological sampling and recording methods, (iii) are developed and tested over sites from the full range of river and stream ecosystems to which they are intended to be applied, and (iv) have a strong relationship with the pressure of concern.

As the best-performing tool in Chapter 3, the PSI index is, in this chapter, the focus of further development and evaluation. Prior to this, the index is considered in terms of the above criteria, highlighting its strengths, weaknesses, and areas of opportunity for further development and evaluation.

4.2.1 *Biological basis*

There is an emerging consensus among those involved in developing biomonitoring tools, that more reliable and useful tools have a biological basis, i.e. they use biological and ecological traits that influence the tolerance of organisms to a given pressure (and the resulting stressors), and are linked to ecological theory (Bonada et al. 2006, Friberg et al. 2011). One of the reasons for this is that these types of tools are not vulnerable to

statistical artifacts that may affect purely statistical models. The biological traits (e.g. respiration, locomotion, dispersal, feeding) of taxa in a community reflect the spatial and temporal variations in the environmental factors of a habitat, that act as “filters”, with successful combinations of traits enabling survival and reproduction (Poff 1997, Townsend et al. 1997a, Statzner et al. 2001b). Certain traits or combinations of traits can result in sensitivities/tolerances to particular environmental pressures and therefore these traits have the potential to be used in biomonitoring to discriminate between types of human disturbance (Statzner et al. 2001a). If taxa are selected for inclusion and their sensitivities determined based on their biological and ecological traits, which influence their sensitivity to a given pressure, the resultant biomonitoring tool will have a mechanistic linkage (rather than a purely correlative linkage) between the pressure of concern and the biotic response (Friberg 2014).

As previously discussed, the PSI index is a pressure-specific biomonitoring tool, designed to identify the degree of sedimentation in river and streams, using standardised kick-samples of the benthic invertebrate community (Extence et al. 2013). The tool was developed using previous literature and expert knowledge of invertebrate traits that are associated with either a sensitivity or tolerance to fine sediment, in order to select and assign taxa to one of four Fine Sediment Sensitivity Ratings (FSSRs) (Table 3.1 – Chapter 3). The tool thus has a sound biological basis and is linked to ecological niche theory (Hirzel and Le Lay 2008). Extence et al. (2013) considered the presence of traits that may result in sensitivity to fine sediment stressors, including feeding, locomotion and respiratory attributes. For example, scrapers/grazers and passive filter feeders may experience decreased food availability or damage to feeding appendages as a result of deposited fine sediment (Nerbonne and Vondracek 2001, Larsen and Ormerod 2010).

The use of tolerance values or ratings is common practice for biomonitoring tools. Often a range of values are assigned, which are thought to represent the range of

sensitivities to the pressure of concern (Chang et al. 2014). However, it has been suggested that the development of tolerance values should be more transparent and rigorous (Chang et al. 2014). With only four sensitivity ratings, the basis of the PSI index is unlikely to be representing the true breadth of ecological niches that invertebrates occupy (Bonada et al. 2004). Furthermore, within the original FSSRs certain taxa are likely to be more useful than others as indicators of fine sediment pressure. Whilst a taxon may be sensitive to fine sediment, it may not be useful as an indicator taxon if it does not respond in a predictable manner, or if it's typically associated with a sediment-free microhabitat that occurs alongside a moderately or heavily sedimented microhabitat. For example, the larvae of the caseless caddis, *Wormaldia* sp. typically inhabit areas of boulders with vertical flows (Edington and Hildrew 1995), which are often associated with scour pool formation and sediment storage on the leeward side (Beschta and Platts 1986). Similarly, black fly (Simuliidae) larvae are considered sensitive to fine sediment (Extence et al. 2013), as they require relatively clean and stable substrate to attach to (Bass 1998), but such microhabitats can occur alongside fine sediment. Despite the benefits of having a biological basis, the use of empirical data alongside the biologically based classifications, may provide a range of taxon weightings that more accurately reflect the combination of taxon sensitivity, and their usefulness as an indicator.

4.2.2 Easily integrated into standardised biological sampling and recording methods

One of the many benefits of biomonitoring, as opposed to conventional monitoring of multiple environmental parameters, is that the biological data collected from the same biological sample can be interpreted by an array of different biomonitoring tools to identify potential pressures. In order for this efficiency and cost-effectiveness to be realised, the biomonitoring tools must be able to make use of data collected using standardised biological sampling methods and recorded to standardised taxonomic

resolutions. Regulatory agencies in different countries commonly carry out macroinvertebrate identification and recording at different taxonomic resolutions. For example, within Europe, where the level of invertebrate taxonomic knowledge and available resources vary between countries, a variety of different taxonomic resolutions are recorded, resulting in a lack of comparability between data (Hering et al. 2010, Schmidt-Kloiber and Hering 2015). Within the UK, England and Wales recently moved from family-level resolution macroinvertebrate recording to a mixed resolution¹, consisting of family-, genus- and species-level identifications, with many taxa being identified to species (Davy-Bowker et al. 2010). However, for routine sampling, Scotland and Northern Ireland currently record at family-level². A family-level tool is not only essential for those countries that record at this taxonomic resolution, but is also crucial for those countries which have recently switched to a higher taxonomic resolution, as it will allow for retrospective analysis of historic family-level data. This ability to assess the historic conditions at sites is particularly important when attempting to distinguish between natural temporal variations (Resh et al. 2005) and those caused by anthropogenic activities, as this can require numerous years of baseline data (e.g. Bilotta et al. 2017). Furthermore, a lower-cost family-level biomonitoring tool is likely to be highly desirable for non-regulatory work (e.g. river restoration projects, aquatic research and citizen science programmes) where budgets and taxonomic expertise may be limited.

The PSI index has both family-level and mixed-level versions, allowing it to be easily integrated into standardised biological sampling and recording methods. Despite the importance of a family level version, intra-family variation in terms of sediment sensitivity will likely have necessitated some generalisations in the original family level FSSRs, which may be hindering the performance of this version of the tool.

¹ This mixed resolution identification is referred to as TL5 by the Environment Agency

² This family resolution identification is referred to as TL2 by the Environment Agency

4.2.3 Developed and tested over the full range of different rivers and streams

There are a number of reasons why biomonitoring tools should be developed and tested using data from sites across the full spectrum of rivers and streams to which they will be applied. Firstly, biological communities and species distributions vary naturally, partly as a result of environmental gradients, biotic interactions (McGill et al. 2006) and their ecological requirements (Schmidt-Kloiber and Hering 2015). As such, a biomonitoring tool must incorporate a range of taxa to ensure that any sampled site will have the potential to include a sufficient number of taxa with sensitivity/indicator weightings. Secondly, it cannot be assumed that biological communities will respond uniformly to the same pressure in different rivers and streams. Not only may the pressure of concern occur alongside other pressures (potentially having synergistic or antagonistic effects) (Folt et al. 1999), but differences in environmental characteristics (e.g. habitat complexity) may also affect a biological community's resistance and resilience to a specific pressure (Lake 2000, Dunbar et al. 2010a, Dunbar et al. 2010b).

The recent evaluation of the PSI index (Chapter 3; Turley et al. 2014) across a wide range of temperate river and stream ecosystems was the first evaluation of the index on this scale. The 835 sites throughout the UK were considered to be minimally impacted at the time of sampling, and therefore the analysis does not consider the tools performance alongside confounding pressures that are present in many rivers and streams (Schinegger et al. 2012).

4.2.4 Strong relationship with pressure of interest

Given the implications of incorrect assignment of ecological status of streams, for both water and land managers (from unjustified burdens being placed on the users of water resources, to environmental damage going undetected), it is important that biomonitoring tools have a strong relationship with their pressure, in addition to the

previous three criteria. Nevertheless, a strong correlation does not rule out the possibility of the tool indicating other aquatic pressures or variables that may occur in parallel with the pressure of concern. A statistical approach may yield strong correlations to the pressure of concern, but these can be the result of statistical artifacts e.g. an inadvertent relationship with an associated pressure (e.g. Table 2.4 – Chapter 2). Although a biological basis provides a mechanistic linkage for a correlation, biological traits are not always unique to a particular pressure, and as such the influence of confounding pressures also cannot be ruled out (Schuwirth et al. 2015).

The previous chapter showed versions of the PSI index to have moderate correlations with fine sediment ($r_s = -0.61$, $p < 0.01$ and $r_s = -0.64$, $p < 0.01$, respectively). Based on an analysis of 297 biomonitoring tools used throughout Europe (Birk et al. 2012), which found the median correlation coefficient of invertebrate-based indices to be 0.64 in relation to their respective pressure, the correlation between PSI score and percentage cover of fine sediment is comparable to other indices used in the implementation of the EU WFD. However, further development is needed to improve the performance of the PSI index and other similar indices, if regulatory agencies are able to rely on them to identify the causes of ecological degradation.

4.2.5 Aims

To investigate if the performance of the family-level and mixed-level versions of the PSI index could be enhanced, through empirical-weighting techniques, whilst retaining their biological basis, and ensuring that the tools are compatible with historic and contemporary datasets recorded to family-level and/or mixed-level, and have a strong relationship with fine sediment.

4.3 Methods

4.3.1 Data

It was important that empirical data from minimally impacted sites were used in this study, in order to reduce the influence of confounding pressures in the process of assigning indicator weights. In this instance, a freely available dataset from the UK was utilised. However, this study can be seen as an exemplar to be used in other countries where such datasets exist. The RIVPACS IV (May 2011 version) dataset (River Invertebrate Prediction and Classification System – NERC [CEH] 2006. Database rights NERC [CEH] 2006 all rights reserved) is described in detail by Wright (2000) and Clarke et al. (2003), and is summarised in Chapter 3 (Section 3.3.1). The database benefits from including macroinvertebrate, water quality, and catchment characteristic data from 835 minimally impacted sites that encompass a wide range of environments (Table 4.1), varying in their (i) climate, (ii) catchment geology, (iii) topography and (iv) morphometry.

The macroinvertebrate data within the RIVPACS IV dataset were collected from the 835 sites, using the UK standard protocol (three-minute kick-sample and one-minute manual search) described in Chapter 3 (Section 3.3.1.1) and in the Standardisation of River Classifications project protocol (EU-STAR 2004). The database has abundance records of different macroinvertebrates identified to (i) family level, (ii) mixed level, and (iii) species level (Wright et al., 2000). Each site has a season-specific record of community composition³; spring (March to May), summer (June to August) and autumn (September to November). As the technique used for macroinvertebrate sampling was semi-quantitative, model development and testing utilised log abundance data rather than raw abundance or presence/absence data. This recognises the potential importance of changes in abundance when attempting to identify pressure gradients,

³ 834 sites have three seasons of data, one site has only two seasons of data.

without placing spurious confidence in the semi-quantitative sampling technique (Furse et al. 1981).

Table 4.1. Characteristics of the 835 RIVPACS sites.

Site characteristics	
Mean annual precipitation (1961 – 1990)	430 mm – 2930 mm
Mean annual temperature (1961 – 1990)	7.93°C – 11.45°C
Geology	Various - hard igneous to soft sedimentary rock
Average river width	0.4 m – 117 m
Average river depth	0.02 m – 3.00 m
Mean annual discharge	<0.31 m ³ s ⁻¹ - >80.00 m ³ s ⁻¹
Slope	0 – 150 m km ⁻¹
Percentage cover of fine sediment (< 2 mm)	0 – 100%
Percentage cover of gravels and pebbles	0 – 98%
Percentage cover of cobbles and boulders	0 – 100%

The fine sediment data within the RIVPACS IV database consisted of visual estimates of the percentage of the substrate cover composed of silt and clay (<0.06 mm) and sand (≥0.06 and <2.00 mm), as an annual average based on three seasonal measurements. The visual estimate method used to collect these data is briefly described in Chapter 3 (Section 3.3.1.2) of this thesis and in the Standardisation of River Classifications project protocol (EU-STAR 2004).

Family and mixed level macroinvertebrate data, and site substrate cover were extracted from the RIVPACS database and compiled in Microsoft Excel. The percentage of the substratum cover consisting of sand, silt and clay, were combined to provide an overall estimate of fine sediment (< 2mm) for each of the 835 minimally impacted sites. The reasons for this were (i) that the previous chapters evaluation of the PSI index found this sediment metric to be the most correlated to PSI scores (Turley et al. 2014) and (ii) to acknowledge the difficulties in differentiating between the various fractions using the visual estimate method.

To ensure that the new index could be tested using an independent dataset, with similarly, minimally impacted sites, the dataset of 835 sites was split into training (751 sites with three seasons of data; $n = 2252$; Figure 4.1) and testing datasets (84 sites, autumn season only, $n = 84$; Figure 4.2) using random allocation, to ensure testing of the indices could be considered independent of the development stages (see Supplementary Material B). Similar to Kelly et al. (2012), this 90:10 split was chosen to ensure that the indices were developed using a large number of sites, whilst leaving a suitable number for independent testing. Only the autumn invertebrate data were used in the testing stages to avoid any potential autocorrelation between seasons at the same site. The annual averages of fine sediment (collected in spring, summer and autumn) therefore represent the antecedent conditions.

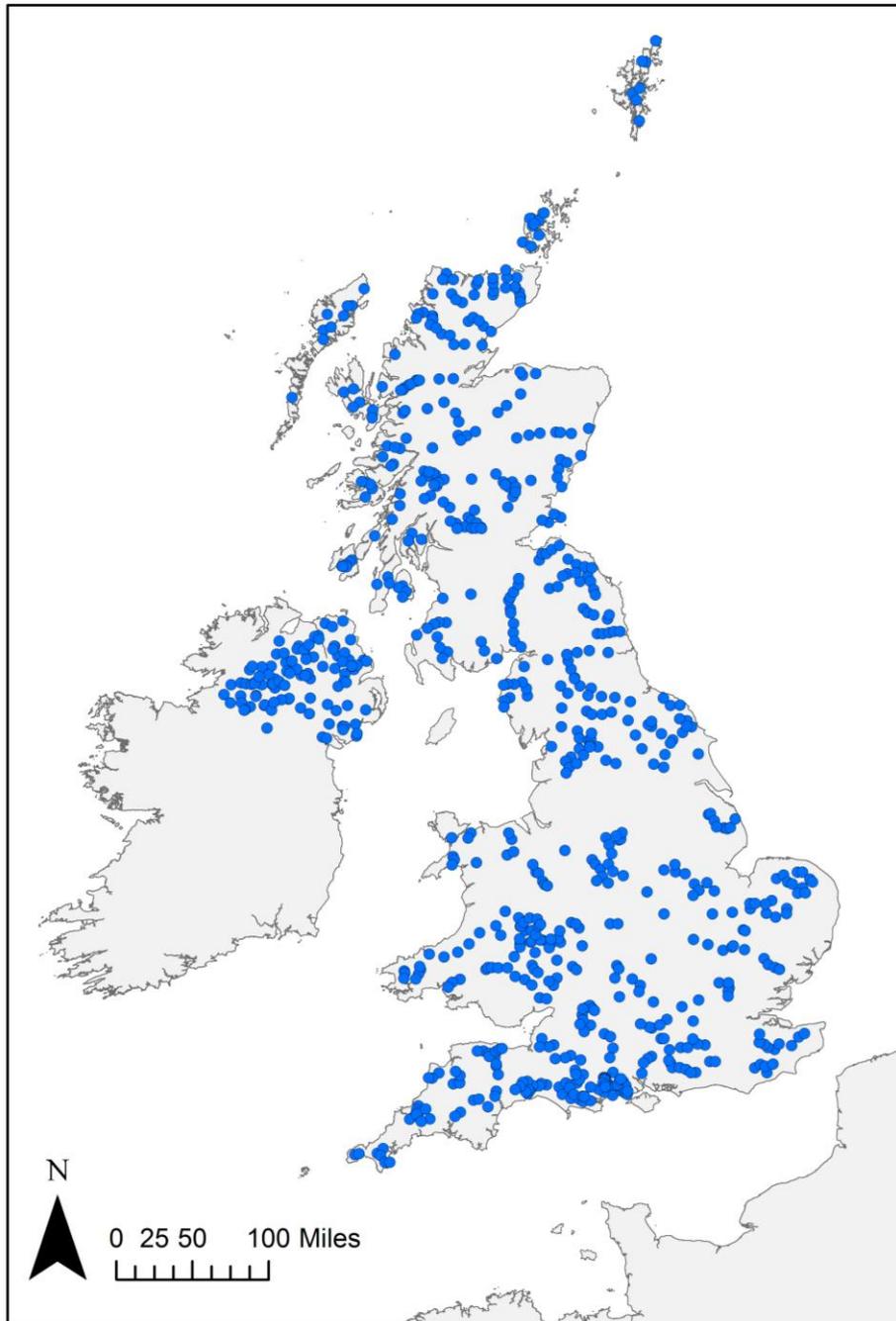


Figure 4.1. Distribution of minimally impacted sites throughout the UK that formed the training dataset ($n = 751$) for development of the E-PSI index.

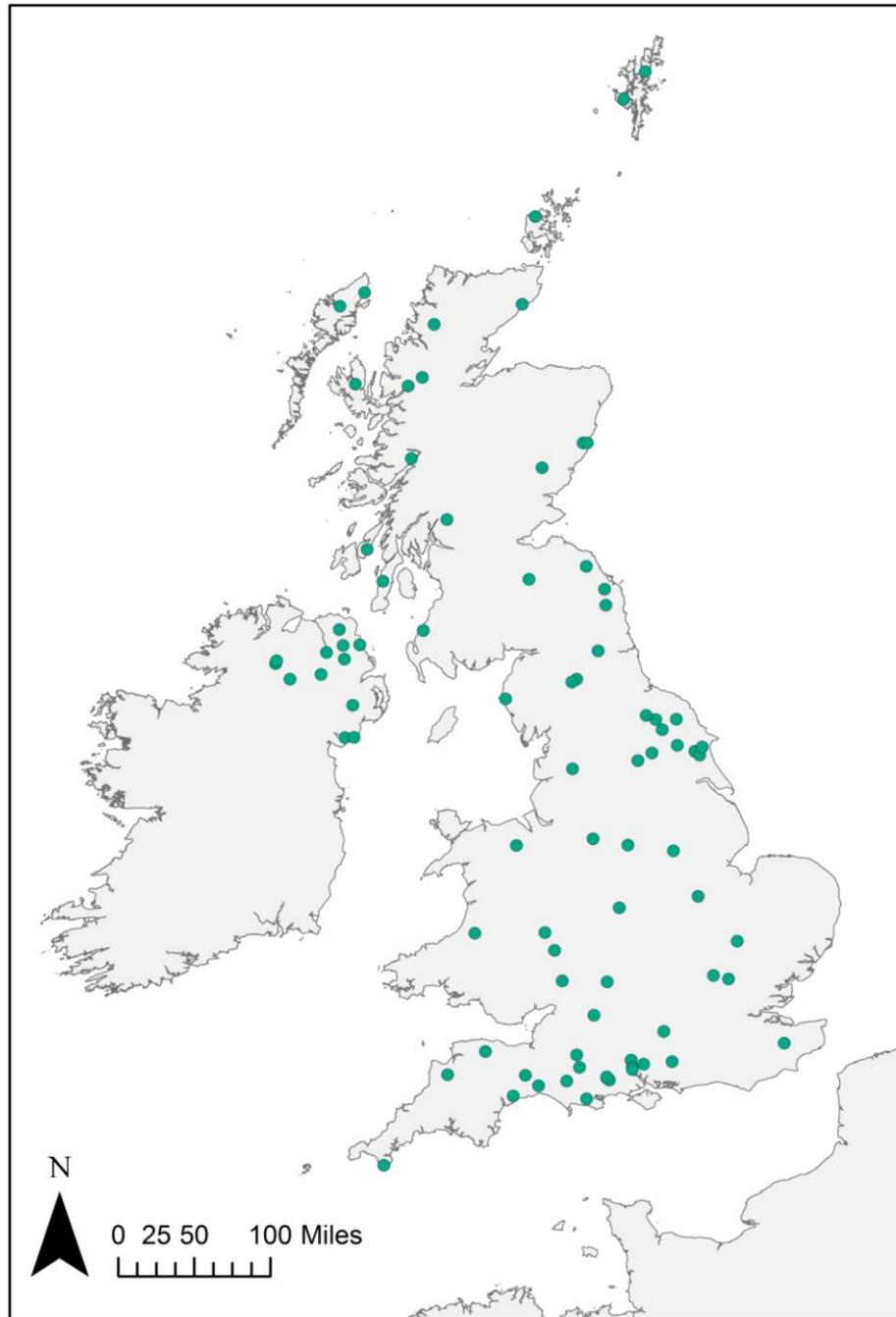


Figure 4.2. Distribution of minimally impacted sites throughout the UK that formed the independent dataset ($n = 84$) for testing of the E-PSI index.

4.3.2 Developing the Empirically-weighted PSI (E-PSI) index

For both the family-level and mixed-level versions of the E-PSI index, the *fmincon* function (active-set algorithm), a nonlinear optimisation method of MATLAB (Mathworks, version R2014a), was used to test up to 100,000 iterations of taxon indicator weights, to find an optimum set of weights that resulted in the strongest Spearman's rank correlation coefficient between index scores and fine sediment in the training dataset. The Spearman's rank correlation was used, as the data were found to be non-normally distributed and show heteroscedasticity, and could not be successfully transformed. The PSI formula was re-cast to allow for the use of taxon-specific indicator weights (Equation 4.1).

$$\text{E-PSI} = \frac{\sum(\log A_{sens} * W)}{\sum(\log A_{all} * W)} * 100$$

Equation 4.1. Formula used to calculate E-PSI scores. The sum of each sensitive taxon's log abundance ($\log A_{sens}$) multiplied by the corresponding taxon-specific indicator weight (W), is divided by the sum of all taxon log abundances ($\log A_{all}$) multiplied by the corresponding taxon-specific indicator weight (W). This value is then multiplied by 100 to provide the E-PSI score. Note: Log abundance categories in E-PSI were simplified (compared to PSI) to: 1 – 9 individuals present = 1; 10 – 99 = 2; 100 – 999 = 3; >1000 = 4. E-PSI scores range from 0 to 100; 0 representing a site dominated by fine sediment, 100 representing a site with minimal amounts of fine sediment.

In the original PSI index, all taxa within the same FSSR receive the same log-abundance weights, which were developed through an extensive literature review and expert judgements (Extence et al. 2013), and were based on macroinvertebrate traits such as physiological and/or morphological adaptations that are associated with either a sensitivity or tolerance to fine sediment. In this study, taxon-specific weightings were

constrained within a range around their original estimates of sensitivity (i.e. sensitive or insensitive) so as to deviate only slightly, from the expert judgements and biological basis. Those taxa originally identified (FSSRs) as moderately to highly sensitive to fine sediment were assigned a weight between 0.5 and 1.0, whilst taxa identified as moderately to highly insensitive were weighted between 0.0 and < 0.5. As such, whether the taxa were originally identified as being sensitive or insensitive, remains unchanged. Sensitive taxa were assigned this larger weighting as they were deemed to be the most significant in terms of identifying sediment pressure, whereas those taxa identified as insensitive, are tolerant of fine sediment but not necessarily reliant on it. Using a range of weights acknowledges the breadth of ecological niches that invertebrates occupy and also their differing potential as indicators.

4.3.2.1 Developing the family level E-PSI index

In total, 79 invertebrate families with PSI sensitivity weightings were present in the family level training dataset. Following the nonlinear optimisation procedure described in Section 4.3.2, an optimum set of family indicator weights formed the E-PSI_{fam} index.

4.3.2.2 Adjustments for family-level sensitivity variations and indicator suitability

In the process of developing the original, family-level PSI index, inevitably some generalisations were made regarding the sensitivities of macroinvertebrate families to fine sediment. Families can comprise numerous genera and species, each with a range of different environmental requirements and sensitivities (Lenat and Resh 2001), making assignment of sensitivities at family-level a difficult process. To determine whether all families identified as sensitive by the original PSI index were correctly identified as useful indicators at this taxonomic level, the empirical data were investigated. Using data from all 835 sites within the RIVPACS dataset, the percentage

of fine sediment that corresponded with the 75th percentile of the family's total abundance was calculated for each taxon. This involved using raw abundance invertebrate data, sorting all 2504 samples in order of increasing fine sediment, and calculating the cumulative abundance for each taxon. The 75th percentile of abundance was then calculated for each family, and this value or the closest (greater) value was cross-referenced to obtain the corresponding sediment value for that abundance.

The family-specific sediment values, represented the maximum fine sediment percentage at which at least 75% of the family's abundance occurred. Similarly to Relyea et al. (2012), this 75% value was used as it recognises the fact that taxa can occur in sub-optimal conditions, often at reduced densities. These family-specific fine sediment values were used to inform the creation of a modified version of the family-level E-PSI index, by excluding taxa whose sediment value was $\geq 33\%$. The families that exceeded this threshold were removed from the index altogether (instead of being reassigned to the insensitive category) to maintain the biological basis. The removal of these families is not an admission of their tolerance to fine sediment, rather it acknowledges that when considering fine sediment at the reach scale, they are not useful as indicator taxa. The 33% threshold was used as previous research showed that at the patch scale, sediment cover of approximately one third resulted in significant declines in invertebrate richness (Larsen et al. 2009). Once these taxa had been removed from the index, the nonlinear optimisation procedure was performed to return a new set of optimum indicator weights based on the new altered community composition/taxon list, forming the E-PSI_{fam69} index

The exclusion process resulted in 10 families being removed entirely from the calculation of index scores (Table 4.2). These sensitive families had corresponding sediment values of between 36% and 83% fine sediment. Three of these families had taxa rated as insensitive in the mixed level tool: Limnephilidae, Scirtidae and Gammaridae.

Table 4.2. Sensitive macroinvertebrate families removed from $E\text{-PSI}_{\text{fam69}}$ calculations, based on a 33% threshold being applied to their corresponding sediment values.

Removed sensitive families	Number of sensitive taxa in $E\text{-PSI}_{\text{mixed}}$	Number of insensitive taxa in $E\text{-PSI}_{\text{mixed}}$	Corresponding sediment value (%)
Sisyridae	0	0	83
Piscicolidae	1	0	73
Limnephilidae	5	15	46
Beraeidae	3	0	45
Scirtidae	1	1	45
Dixidae	N/A	N/A	42
Goeridae	3	0	38
Gammaridae	2	3	37
Polycentropodidae	7	0	37
Psychomyiidae	5	0	36

N/A identifies taxa that are included in the mixed-level tools at family-level.

4.3.3 *Developing the mixed level E-PSI index*

In addition to the family-level index, a mixed-level version was developed using the same procedures as the $E\text{-PSI}_{\text{fam}}$ index. In total, 355 invertebrate taxa with PSI sensitivity weightings were present in the mixed level training dataset. Following the nonlinear optimisation procedure outlined in Section 4.3.2, an optimum set of indicator weights formed the $E\text{-PSI}_{\text{mixed}}$ index.

4.3.4 *Testing the E-PSI index*

The developed versions of the E-PSI index were tested using the minimally impacted, independent dataset ($n = 84$). The performance of these indices were evaluated and compared using their Spearman's rank correlations with fine sediment, due to the non-normally distributed and heteroscedastic residuals, and the inability to successfully transform. All correlations were interpreted using the Dancey and Reidy (2007) classifications of correlations; 0.1 – 0.39 = weak, 0.4 – 0.69 = moderate, 0.7 – 0.99 = strong.

4.3.5 *Ability of the E-PSI index to discriminate between fine sediment conditions*

In order to evaluate the ability of the E-PSI index to discriminate between different fine sediment conditions, Kruskal-Wallis tests were carried out on both PSI and E-PSI, by grouping the scores into independent groups (0 – 10, 11 – 20, 21 – 30, 31 – 40, 41 – 50, 51 – 60, 61 – 70, 71 – 80, 81 – 90 and 91 – 100). The Kruskal-Wallis test returns a p -value, which is used to determine whether any of the groups are significantly different. Groups of this size were selected due to the importance of discriminating between different levels of sedimentation, but also to account for the uncertainties in both the sediment and invertebrate data (smaller groups would need to be based on highly accurate and precise data). Pairwise comparisons were then performed using Dunn's (1964) procedure with a Bonferroni correction for multiple comparisons, to determine which groups were significantly different.

4.3.6 *Comparisons with other biomonitoring tools*

The Lotic-invertebrate Index for Flow Evaluation (LIFE) (Extence et al. 1999) and the recently published Combined Fine Sediment Index (CoFSI) (Murphy et al. 2015) were calculated and their relationship to both fine sediment, PSI and E-PSI, were assessed

in terms of their Spearman's rank correlation coefficients. LIFE was chosen to determine the influence of known interactions between flow and fine sediment deposition and re-suspension (Dewson et al. 2007).

The predominantly species- and genus-level CoFSI was included in this analysis, as it is a purely statistically based index designed to indicate fine sediment pressure (Murphy et al. 2015). The authors used partial canonical correspondence analysis to rank taxa in terms of their sensitivity to fine sediment, based on empirical data from 179 stream sites.

All mixed-level indices were calculated using data of the same taxonomic detail, at the level used for Water Framework Directive reporting.

4.4 Results

4.4.1 Biological indices and fine sediment

The best performing family-level and mixed-level versions of the E-PSI index were strongly, negatively correlated to fine sediment metrics in the minimally impacted dataset (Table 4.3; Figure 4.3).

Table 4.3. Spearman's rank correlation coefficients (2 d.p.) between versions of PSI, E-PSI, LIFE, CoFSI and fine sediment, in the minimally impacted test dataset.

Index	No. of sensitive taxa	Minimally impacted sites ($n = 84$)
Family level		
PSI _{fam}	36	-0.59
E-PSI _{fam}	36	-0.66
E-PSI _{fam69}	26	-0.72
LIFE _{fam}	n/a	-0.57
Mixed level		
PSI _{mixed}	139	-0.60
E-PSI _{mixed}	139	-0.70
CoFSI _{mixed}	n/a	-0.72
LIFE _{mixed}	n/a	-0.51

Subscripted text identifies the taxonomic resolution/version of the tool (family or mixed). Correlations significant at the 0.01 level (2-tailed).

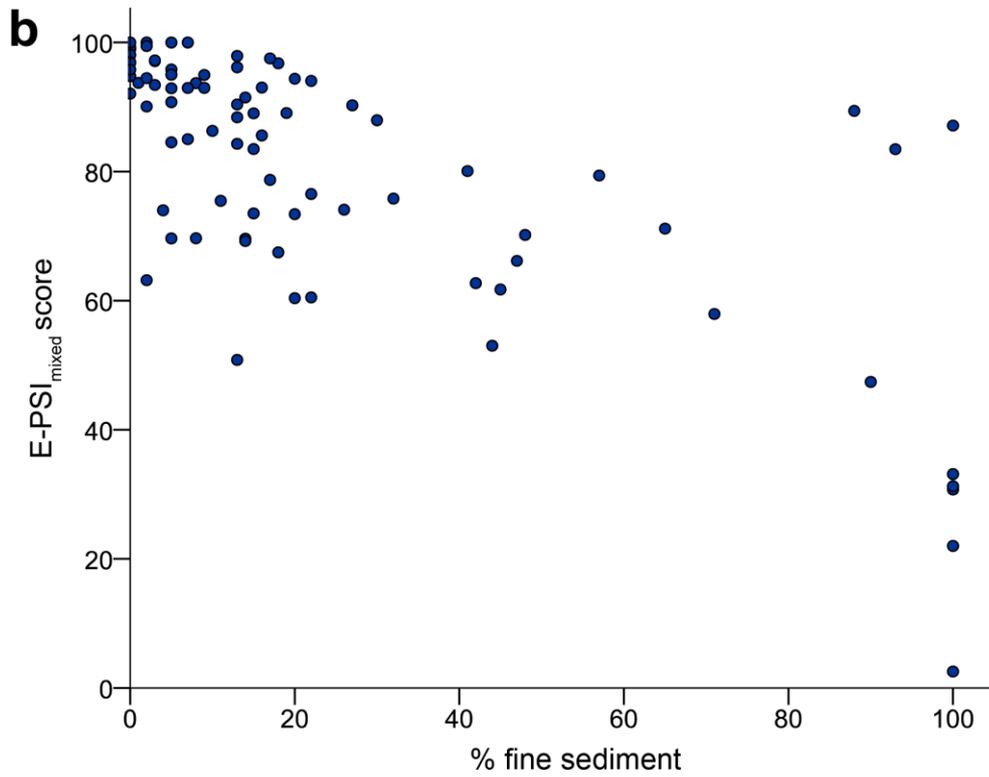
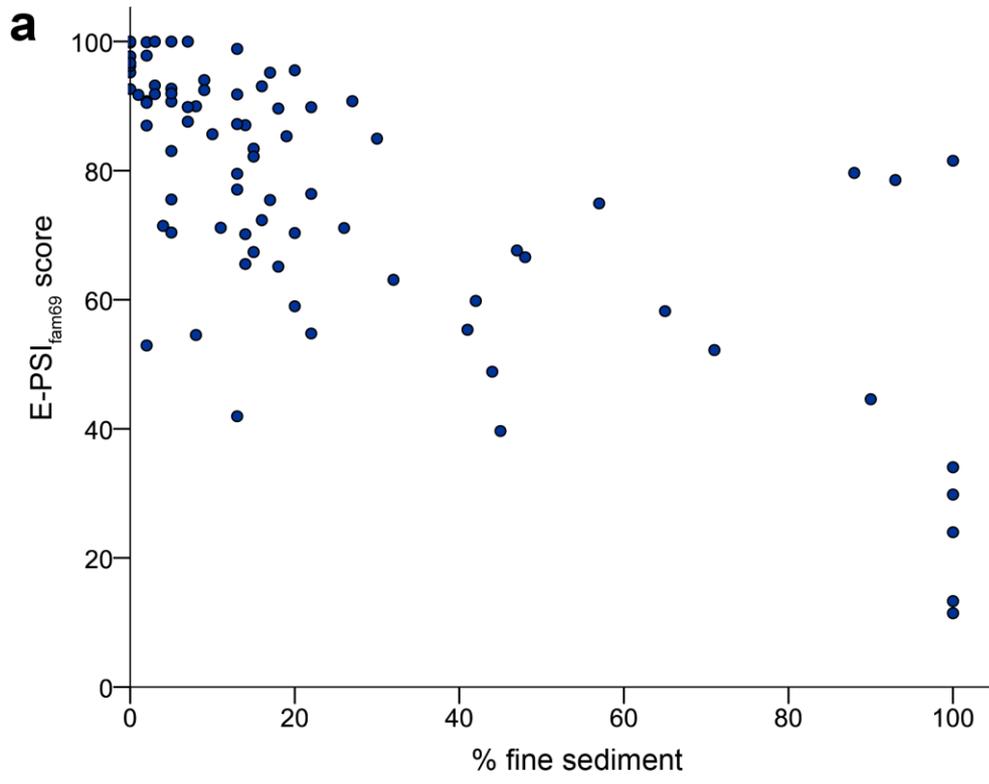


Figure 4.3. Relationship between (a) E-PSI_{fam69} and fine sediment, and (b) E-PSI_{mixed} and fine sediment, across the independent minimally impacted test dataset ($n = 84$).

The versions of the E-PSI index with the strongest correlations with fine sediment in the minimally impacted test dataset were E-PSI_{fam69} and E-PSI_{mixed} ($r_s = -0.72$ and $r_s = -0.70$, $p < 0.01$, respectively). The optimisation process was responsible for improving the correlations between fine sediment and the E-PSI_{fam} index compared to the PSI_{fam} index ($r_s = -0.66$, $p < 0.01$ compared to $r_s = -0.59$, $p < 0.01$). The largest improvement following the optimisation process was for the E-PSI_{mixed} index, which represented an improvement of 10 percentage points compared to PSI_{mixed} ($r_s = -0.70$, $p < 0.01$ compared to $r_s = -0.60$, $p < 0.01$). LIFE_{fam}, and LIFE_{mixed} had moderate correlations with fine sediment ($r_s = -0.57$ and $r_s = -0.51$, $p < 0.01$, respectively), and CoFSI had a strong correlation with fine sediment ($r_s = -0.72$, $p < 0.01$).

4.4.2 *Assigning sediment indicator weightings*

The indicator weightings that formed the best performing versions of the E-PSI index (E-PSI_{fam69} and E-PSI_{mixed}) in the minimally impacted test dataset are detailed in Appendix A and B.

4.4.3 *Ability of the E-PSI to discriminate between fine sediment conditions*

Grouped PSI_{fam} scores and the best performing family-level E-PSI (E-PSI_{fam69}) scores are shown in the boxplots in Figure 4.4. The Kruskal–Wallis test returns a p -value, which is used to determine whether any of the groups are significantly different. Pairwise comparisons identified which groups were statistically significantly different from each other. Three groups of PSI_{fam} had statistically significant different distributions of fine sediment values compared to five statistically significant different distributions of fine sediment values for grouped E-PSI_{fam69} scores.

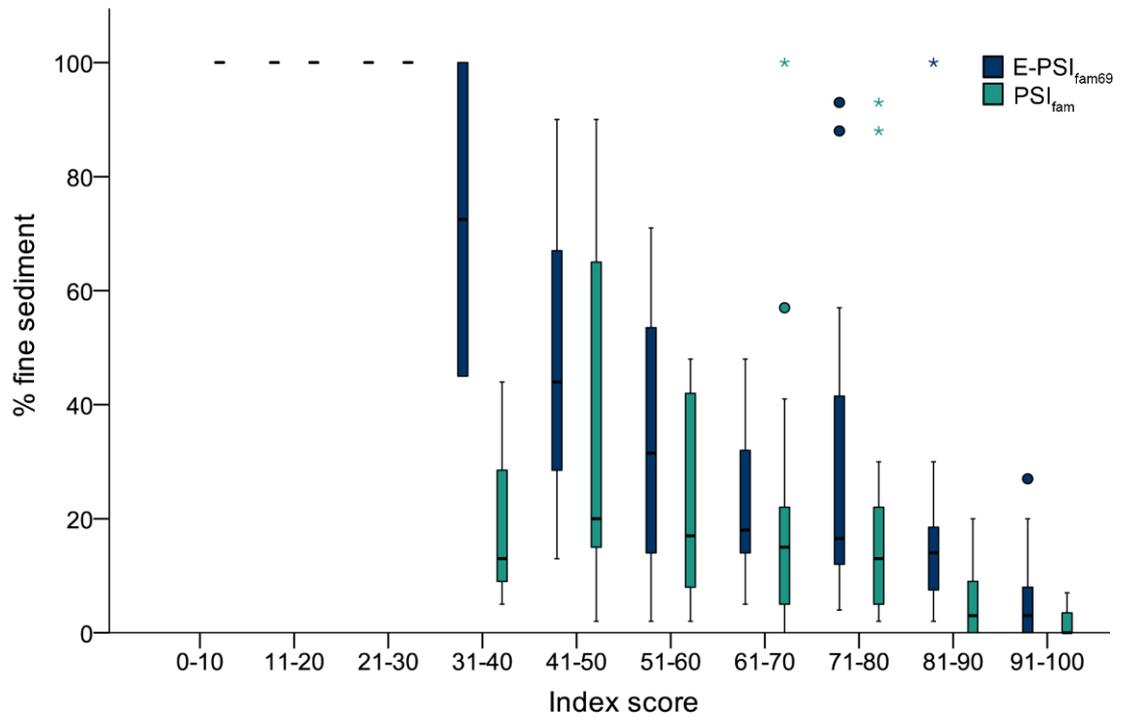


Figure 4.4. Boxplot showing the relationship between percentage fine sediment (based on visual estimates) and grouped E-PSI_{fam69} and PSI_{fam} scores for the minimally impacted test dataset ($n = 84$). Note: Minimum, Maximum (shown in the lower and upper whiskers respectively), Median (-), Interquartile range (boxes). SPSS identifies potential outliers as >1.5 times (\circ) or >3 times (*) the interquartile range above the 75th percentile.

4.4.4 Comparisons between biomonitoring tools

In addition to comparing the E-PSI index to fine sediment, the relationships between indices were considered. The various indices had strong correlations with each other, ranging from $r_s = 0.79$ to $r_s = 0.98$, $p < 0.01$ (Table 4.4). All versions of the E-PSI index had weaker correlations with LIFE_{fam} and LIFE_{mixed}, compared to the original versions of the PSI index.

Table 4.4. Spearman's rank correlation coefficients (2 d.p.) between biological indices, in the minimally impacted test dataset ($n = 84$).

Index	PSI _{fam}	E-PSI _{fam}	E-PSI _{fam69}	LIFE _{fam}	PSI _{mixed}	E-PSI _{mixed}	CoFSI _{mixed}
Family level							
PSI _{fam}		0.95	0.93	0.92	0.96	0.93	0.87
E-PSI _{fam}	0.95		0.98	0.85	0.94	0.96	0.93
E-PSI _{fam69}	0.93	0.98		0.85	0.91	0.96	0.94
LIFE _{fam}	0.92	0.85	0.85		0.89	0.86	0.79
Mixed level							
PSI _{mixed}	0.96	0.94	0.91	0.89		0.95	0.88
E-PSI _{mixed}	0.93	0.96	0.96	0.86	0.95		0.92
CoFSI _{mixed}	0.87	0.93	0.94	0.79	0.88	0.92	
LIFE _{mixed}	0.88	0.83	0.79	0.85	0.91	0.85	0.80

Subscripted text identifies the taxonomic resolution/version of the tool (family- or mixed-level). Correlations significant at the 0.01 level (2-tailed).

4.5 Discussion

4.5.1 Identifying fine sediment pressure

The results of this chapter show that the use of empirical data to assign indicator weights within the PSI index's original biologically-based sensitivity ratings, has improved the performance of the various versions of the E-PSI index, in terms of their correlation with fine sediment. As a result, both the family-level and mixed-level versions of the E-PSI index have correlation coefficients with fine sediment, that are stronger than the average invertebrate-based, pressure-specific biomonitoring tool

used throughout Europe in the implementation of the WFD (Birk et al. 2012). Furthermore, by including only those taxa whose 75th percentile of abundance corresponded with a fine sediment value of < 33%, the E-PSI_{fam69} index has a strong correlation with fine sediment in the minimally impacted test dataset, similar to that of E-PSI_{mixed} (E-PSI_{fam69} versus fine sediment: $r_s = -0.72$, $p < 0.01$). Applying this threshold to the family-level index was intended to acknowledge the likelihood of significant intra-family variation in terms of their sensitivity to fine sediment, as well as identify those taxa that occur alongside fine sediment, and are therefore not useful as indicators at the reach scale. This exercise appears to have had the desired effect, reducing some of the variation in the relationship that may have been caused by varying sensitivities within families.

Despite the improved correlations between the E-PSI index and fine sediment, in comparison to the PSI index, few improvements were visible in terms of discriminatory power between sites grouped by E-PSI scores. The reason for this may be due to the grouped scores being arbitrarily assigned, and not being biologically relevant, or due to the fact that the relationship is not one of simple cause and effect, and the likelihood of invertebrates responding to fine sediment conditions differently in streams varying in their physical, chemical and biological characteristics.

The strong and moderate correlations do not prove causality and they should be interpreted with some caution, partly because many in-stream influences are associated with each other in riverine environments (Allan 2004). However, the benefit of biologically-based indices such as the PSI and E-PSI indices is that the biological basis provides a mechanistic linkage between index scores and fine sediment conditions. The PSI index utilises existing knowledge and expert judgement of biological and ecological traits and sensitivities to fine sediment, to assign sensitivity ratings, which the E-PSI index combines with empirical data to assign indicator weightings (0 – 1).

Based on the available data on the ecological preferences of macroinvertebrates from Schmidt-Kloiber and Hering (2015), those families, which have been assigned the highest indicator weightings in E-PSI_{fam69} (Heptageniidae, Perlidae, Aphelocheiridae, Chloroperlidae, Lepidostomatidae, Leuctridae, and Perlodidae), are dominated by species with a strong affinity to coarse substrate microhabitats, with the exception of Lepidostomatidae that have some affinity to coarse substrates, but are more often associated with woody debris. Habitat preferences are determined by an invertebrate's biological traits or combination of traits. These preferences relate not only to the direct physical properties of the habitat (e.g. substrate), but also the flow velocities, hydrological and thermal regimes, resource availability and biotic interactions associated with them. These taxa with the greatest E-PSI indicator weightings are mainly clingers, with tarsal claws allowing them to "grip" the surface of pebbles, cobbles and boulders in shearing flows (Pollard and Yuan 2010). Nevertheless, they depend on the heterogeneity of coarse substrates to provide refugia from these shearing flows, as well as from predators. Previous research has shown reduced densities and richness of clingers, with increasing fine sediment cover (Rabeni et al. 2005, Pollard and Yuan 2010). Additionally, the turbulence caused by coarse substrate and the resulting oxygenation is likely to be particularly important for many of these sensitive families.

4.5.2 Statistically based sediment-specific tools

As discussed in Chapter 2, other studies have attempted to develop fine sediment-specific indices, using empirical data and statistical methods to select taxa for inclusion and to assign indicator weights to these taxa, to achieve a correlation between tool outputs and the pressure of concern. These approaches have achieved moderate-strong correlations with fine sediment but lack a biological basis and mechanistic linkage, which are important for disentangling the multiple pressures in rivers and

streams (Friberg 2014). Murphy et al. (2015) developed CoFSI, using sediment data collected using the re-suspension method of quantifying fine sediment, averaging samples from two erosional and two depositional patches to achieve a contemporaneous reach-scale average. Despite this technique necessitating some subjective assessment of “erosional” and “depositional” patches, and the fact that macroinvertebrates are not likely to be responding only to sediment conditions at the time of sampling, but also the preceding days, weeks and months, moderate to strong correlations ($r_s = -0.54$ to -0.70 , $p < 0.05$) were observed between the resultant sediment data and CoFSI in their test dataset (Murphy et al. 2015). Due to the difficulties of selecting sites with minimal confounding pressures that at the same time represent a gradient of sediment pressure, their resulting test dataset ($n = 83$) is somewhat geographically restricted and is focused on agricultural streams.

Murphy et al. (2015) made attempts to isolate catchments suffering from *just* agricultural fine sediment pressure using models to predict sediment delivery, however, the reality is that many of these sites will also be simultaneously affected by other pressures, including nutrients, pesticides, and heavy metals, but also potentially ecosystem alterations caused by the presence of invasive species such as signal crayfish (*Pacifastacus leniusculus*) (Allan 2004, Weston et al. 2004, Matthaei et al. 2010). Their selection of these sites also relied on highly uncertain estimates of modelled fine sediment pressure using PSYCHIC, a sediment delivery model that has been shown to exhibit root mean squared error of $> 200 \text{ kg ha}^{-1} \text{ yr}^{-1}$ (Strömquist et al. 2008), and as such the sites may not be ideal as the basis for tool development. Nevertheless, in the present study, CoFSI is shown to have a similar correlation with fine sediment compared to the best performing family- and mixed-level versions of E-PSI. CoFSI is a mixed-level (predominantly genus- and species-level) biomonitoring tool, and as such cannot be applied to present and historic datasets recorded at family-level.

Most recently, in North America, Hubler et al. (2016) developed a statistically based index for Oregon streams (the Biological Sediment Tolerance Index; BSTI) using weighted averaging to assign tolerance values, and found a moderate correlation ($r^2 = 0.58$) between the index and fine sediment in their independent test dataset ($n = 50$). Similarly, Zweig and Rabeni (2001) found a moderate correlation ($r_s = -0.59$, $p < 0.01$) between their Deposited Sediment Biotic Index (DSBI) and visual estimates of fine sediment, using data from four streams in Missouri. The authors used the sediment values that corresponded with each taxon's median abundance, to assign tolerance ratings, and used the same data for testing, therefore lacking an independent test dataset. In addition, the DSBI is currently limited by the small development dataset, which may restrict its spatial applicability. A further example from North America is the Fine Sediment Biotic Index (FSBI), which again used empirical data to assign sensitivity weights to taxa (Relyea et al. 2012). The FSBI showed a large amount of variation, with the data forming a wedge-shaped response that has been suggested as a typical response to multiple pressures (Friberg 2010); when sediment pressure is low, it has minimal impact on the macroinvertebrate community and other pressures dominate. This wedge-shaped response is also observed for PSI, E-PSI and CoFSI (Appendix C), supporting this generalisation.

4.5.3 Sources of uncertainty and natural variation

Despite the strong correlations between versions of the E-PSI index and fine sediment, the scatterplots of the data highlight the amount of variation in the relationship. There are a number of likely explanations as to why this is the case. Firstly, as previously mentioned, the standardised sampling technique used to collect macroinvertebrates is semi-quantitative and has been shown to collect approximately 50% of the species and 60% of the families present at a site, based on six replicate samples (Furse et al. 1981). This sampling technique also ignores that certain microhabitats and their

associated taxa may be more influenced/impacted by fine sediments, and due to variations in microhabitats between sites, may be represented to varying degrees in different samples. This uncertainty is likely to lead to variability between samples and between sites, particularly as the likelihood of a taxon being collected may be taxon-specific (certain taxa may be more likely to be missed by kick-sampling), as well as site-specific (depending on the microhabitats and heterogeneity of the site). Secondly, macroinvertebrate assemblages can exhibit pronounced seasonal fluctuations in community composition, both in terms of a species' abundance and diversity, or the presence/absence of a species. These seasonal fluctuations are often the result of macroinvertebrate life cycles (e.g. egg stages, adult flight stages), but can also be the result of seasonal disturbance (e.g. floods, droughts), invertebrate drift and colonization (Reece et al. 2001, Malmqvist 2002).

A further source of uncertainty in the relationships between pressure-specific biomonitoring tools and the pressure of concern, is the presence of multiple pressures (Friberg et al. 2011). Although the data used in this chapter were collected from minimally impacted sites (between 1978 and 2004) that were considered to have had minimal disturbance to their physical, chemical and biological characteristics at the time of sampling, it is likely that certain pressures were underestimated or not foreseen, and were influencing the invertebrate communities to some degree. Furthermore, in addition to anthropogenically-derived pressures, the presence of naturally occurring confounding factors and collinearity are likely to contribute to the variation in the relationship between riverine biomonitoring tools and their pressure of concern. Lastly, an area of uncertainty that is a probable source of significant variation in the relationship, is the fine sediment data. Quantifying fine sediment in a way that is biologically relevant and accurately represents spatial variation is a further limitation to the development and/or testing of sediment-specific biomonitoring tools. Visual estimates of substrate composition were used in this study due to the data being from

an existing database, which had the benefits of a wide geographic coverage. Although the technique is subjective (Clapcott et al. 2011) it provides a measure of fine sediment surface drape, which is likely to directly influence macroinvertebrate communities and as such is likely to be a biologically relevant metric (Conroy et al. 2016), relating to niche theory and habitat suitability (Hirzel and Le Lay 2008) over the entire reach.

4.5.4 Taxonomic resolution

In contrast to previous findings (Relyea et al. 2012, Turley et al. 2014), the taxonomic resolution had little effect on the indices correlations with fine sediment; $E\text{-PSI}_{\text{fam69}}$ and $E\text{-PSI}_{\text{mixed}}$ had similar strong correlations with fine sediment. Relyea et al. (2012) found that sensitivities varied within invertebrate families and concluded that family level was insufficient for pressure-specific tools. This intra-family variation of sensitivities to fine sediment was expected to result in large differences in terms of the correlation between family and mixed level indices and fine sediment. Despite the similar correlations observed, it is likely that the mixed level tool will provide more diagnostic information, particularly at sites subjected to multiple pressures.

The taxonomic sufficiency is a key consideration when developing an invertebrate-based biomonitoring tool, as the taxonomic resolution can affect the accuracy of predictions (Schmidt-Kloiber and Nijboer 2004) as well as the costs associated with processing biotic samples (Marshall et al. 2006, Jones 2008). The higher the taxonomic resolution, the more resource intensive biomonitoring becomes (identification requires more time and expertise/training). For macroinvertebrates, some studies have shown that family level is often sufficiently able to detect environmental change (Marshall et al. 2006, Mueller et al. 2013), whilst others conclude that a higher resolution is preferential, particularly for genera-rich families or when attempting to identify subtle environmental changes (Waite et al. 2004, Monk et al. 2012).

Furthermore, although our knowledge of species level biology is incomplete, intra-family and intra-generic environmental requirements have been shown to vary significantly for various families of macroinvertebrates (reviewed in Lenat and Resh 2001). In terms of a pressure-specific biomonitoring tool, the optimum taxonomic resolution in terms of model predictions is likely to be dependent on the scale or gradient of environmental pressure, but will inevitably be a compromise between the costs associated with high taxonomic resolution and the ecological information lost at lower resolutions. Nevertheless, the development of new pressure-specific tools should ideally align with national/international regulatory agencies, to ensure that they can be applied to existing protocols and historical data.

As the E-PSI index is not a purely statistically based tool, it is able to include relatively rare taxa (low abundances or small distribution range), which are often removed in the development stages of statistically based indices (Relyea et al. 2012, Murphy et al. 2015). These rare taxa, specifically those with small distribution ranges, may be important indicators of subtle environmental change as they are likely to be more specialized, having a narrower range of optimal conditions, and as such may be the first taxa to indicate the effects of a pressure (Cao et al. 1998, Schmidt-Kloiber and Nijboer 2004). Whilst these assumptions are intuitive, there is some debate as to the suitability of rare taxa in multivariate analyses particularly relating to their redundancy and their influence on observed versus expected scores (Cao et al. 2001, Marchant 2002, Van Sickle et al. 2007). Furthermore, when conservation issues become a concern for these rare species, their collection should be carefully considered. The E-PSI index only uses taxa that are collected in routine samples by regulatory agencies and as such makes the most use of existing data. The River Invertebrate Classification Tool (RICT) used in the UK to derive expected index scores for sites, includes rare species, calculating scores based on the probability of their occurrence (Clarke et al. 2003).

4.5.5 *Application of the E-PSI index*

In application, most indices should be used within an Ecological Quality Index (EQI) framework, based on observed versus expected invertebrate community composition (Glendell et al. 2014), particularly when being used for regulatory purposes. This requires a predictive model that is independent of the pressure of concern. At present, in the UK, the predictive model RICT is not independent of fine sediment, as it uses substrate composition as a predictor variable (Clarke et al. 2011). Whilst it is possible to exclude substrate composition from the model, it is an important predictor variable (Davy-Bowker et al. 2006, Clarke et al. 2011), and the resulting predictions (excluding substrate composition) have not been suitably tested. Furthermore, it is over-simplistic to assume that a deviation of observed from expected community composition (and therefore index score) can be explained by a single measurement at one point in time. Aside from the fact that the structure of macroinvertebrate communities is determined not simply by the contemporaneous fine sediment, but by local sediment dynamics spanning the preceding days, weeks and months, there are multiple reasons for this not being a simple cause-effect relationship. Firstly, fine sediment has numerous direct and indirect effects on invertebrates (reviewed in Bilotta and Brazier 2008), many of which are poorly understood. Secondly, not only are there often dissociated pressures present, but studies have also shown a number of common sediment-associated physical, chemical and biological pressures (Table 2.4 – Chapter 2) that depend on catchment land use, in-stream influences, geomorphology, hydrology and so on. For example, fine sediment pressure is commonly associated with stream flow, due to the influence of stream velocity on the transport, deposition and re-suspension of fine sediment (Wood and Armitage 1997). Additionally, depending on the sorptive properties of the sediment (i.e. organic carbon content, particle size, clay content and cation exchange capacity) hydrophobic contaminants often become associated via adsorption or absorption, potentially acting as confounding pressures (Warren et al. 2003). Lastly, in disturbed sites, the biological communities have often been subjected

to this range of pressures over an extended period of time and therefore the observed macroinvertebrate community composition at any point in time is partly reflective of the legacy of these pressures (Allan 2004).

4.6 Conclusion

The $E\text{-PSI}_{\text{fam69}}$ and $E\text{-PSI}_{\text{mixed}}$ indices that have been developed in this chapter utilise both expert knowledge, and empirical data from minimally impacted sites. By assigning indicator weights to taxa using empirical data, but restricting the weights to a range based on whether the taxon was deemed sensitive or insensitive of fine sediment (based on existing literature and knowledge of biological traits and ecological preferences), the indices maintain their mechanistic linkage, something that is important for pressure-specific tools. The two versions of the E-PSI index developed in this chapter are compatible with the taxonomic resolution of data currently collected by the four UK regulatory agencies, as well as the taxonomic resolution of historic protocols. This will ensure that the tools can be implemented into existing monitoring programs at minimal cost, and maximise the information provided by the various taxonomic resolutions. The family-level version of the tool is likely to be an extremely useful tool for researchers and water managers, allowing it to be used to determine long term patterns in deposited sediment conditions, something that is likely to be necessary when considering the effects of abstraction, river restoration programs, and development.

Despite the strengths of developing a tool in this way, it remains crucial to test the tool over the range of river and stream types, at which the tool will be utilised. The testing in this chapter is therefore an important step in the evaluation of the E-PSI index, highlighting its applicability throughout the UK. Nevertheless, in practice, the vast majority of sites to which the index will be applied will be subject to multiple pressures

arising from anthropogenic activities, and it is therefore essential that indices be tested over such sites. The confounding pressures that have been shown to occur alongside fine sediment pressures were discussed in Chapter 2 (see Table 2.4) and include physical, chemical and biological pressures. The following chapter is focused on testing of the indices with consideration of naturally occurring confounding pressures, in catchments that are largely dominated by agricultural activities, and as such are impacted by an array of different pressures.

Chapter 5 Testing the Empirically-weighted Proportion of Sediment-sensitive Invertebrates (E-PSI) index with consideration of longitudinal gradients, and in the presence of agricultural pressures.

Part of the content of this chapter is reproduced from the following:

Turley, M. D., G. S. Bilotta, R. P. Chadd, C. A. Extence, R. E. Brazier, N. G. Burnside, and A. G. Pickwell. 2016. A sediment-specific family-level biomonitoring tool to identify the impacts of fine sediment in temperate rivers and streams. *Ecological Indicators* 70:151-165.

Contributions: MT led the analysis and writing of this chapter (and publications), supported and supervised by GB (primary supervisor), CE & RB (external supervisors). RC helped with the identification of macroinvertebrates and gave advice on invertebrate ecology. NB assisted with obtaining data and matching sampling locations using GIS. AP provided assistance obtaining data and advice on macroinvertebrate identification.

5.1 Abstract

Biomonitoring tools are commonly used by water managers to provide information on the ecological condition of freshwater habitats. Given the implications of misinterpretation, and/or misclassification of ecological status, these tools should be subjected to thorough testing across the full range of environments to which they are intended to be applied, as well as across the full pressure gradient and alongside other potentially confounding pressures. In this chapter, the family-level Empirically-weighted Proportion of Sediment-sensitive Invertebrates (E-PSI_{fam69}) index, developed in the previous chapter, is tested using historical (family-level) macroinvertebrate data from

routine monitoring of rivers and streams in agriculturally impacted catchments. The independent dataset includes sites ($n = 754$) with upstream catchment land-use ranging from 0-90% arable fields. The family level tool had a moderate negative correlation to fine sediment ($r_s = - 0.68$ $p < 0.01$) comparable to those in the previous chapter using data from minimally impacted sites. The performance of E-PSI_{fam69} and the mixed-level version of E-PSI (E-PSI_{mixed}) are also evaluated using data from minimally impacted sites, grouped based on their specific stream power, in an attempt to reduce collinearity with variables associated with longitudinal gradients, particularly those relating to flow (e.g. discharge, velocity and slope). A similar evaluation is carried out for the E-PSI_{fam69} index using the agricultural dataset. The results highlight a trend of decreasing correlation between E-PSI and the Lotic-invertebrates Index for Flow Evaluation (LIFE), with increasing specific stream power group. The chapter demonstrates the performance of E-PSI_{fam69} over impacted sites, and highlights the ease with which it can be integrated into contemporary monitoring agency protocols and can be applied retrospectively to historical datasets. Given the challenges of non-biological conventional monitoring of fine sediments and determining the biological relevance of the resulting data, a sediment-specific biomonitoring approach is highly desirable and will be a useful addition to the suite of pressure-specific biomonitoring tools currently used to infer the causes of ecological degradation.

5.2 Introduction

Biomonitoring plays an important role in the environmental assessments carried out by water managers. Biomonitoring tools are used to determine the ecological condition of a habitat, often by identifying a deviation from the predicted community composition and providing information on the potential causes of any deviation. Given the implications of drawing incorrect conclusions concerning the ecological condition of a river or stream, it is important that biomonitoring tools are tested using data from sites

across the full spectrum of rivers and streams to which they are intended to be applied. The reasons for this are that biological communities vary naturally, partly as a result of environmental gradients, biotic interactions (McGill et al. 2006) and their ecological requirements (Schmidt-Kloiber and Hering 2015). Furthermore, biological communities in different rivers and streams may not respond to the pressure of concern to the same degree, for a number of reasons; (i) different natural environmental characteristics (physical, chemical and biological) may result in a greater resilience or vulnerability to a given pressure; (ii) the widespread occurrence of multiple anthropogenically derived pressures in rivers and streams may result in antagonistic or synergistic effects. In particular, there are a multitude of pressures that are associated with fine sediment that may exhibit collinearity, and are therefore likely to confound the relationship between a sediment-specific index and fine sediment. These confounding pressures were highlighted in Chapter 2, and include physical (e.g. discharge, velocity), chemical (e.g. pesticides, nitrogen, phosphorus, metals) and biological (e.g. invasive species) pressures. Disentangling multiple pressures in river ecosystems is a complex challenge (Friberg 2014), but is an area of important investigation for biomonitoring tools. Many of these pressures can be the result of agricultural activities, and therefore this chapter evaluates the performance of the family-level Empirically-weighted Proportion of Sediment-sensitive Invertebrates ($E\text{-PSI}_{\text{fam69}}$) index using a dataset of sites situated in agricultural catchments. In addition, the potential for covariates associated with longitudinal gradients to confound the relationships between $E\text{-PSI}_{\text{fam69}}$, and the mixed-level E-PSI ($E\text{-PSI}_{\text{mixed}}$), and fine sediment is investigated.

Given that rivers vary in their natural sediment conditions/dynamics (Bilotta et al. 2012a, Grove et al. 2015), in order to determine if a site is impacted by fine sediment (rather than the observed community composition and E-PSI score, reflecting its “unimpacted” condition) the index is designed to be used alongside a reference-based model (e.g. River Invertebrate Classification Tool; Davy-Bowker et al. 2008), where

observed E-PSI scores can be compared to the expected minimally impacted E-PSI scores. At the time of writing this thesis, the current version of the River Invertebrate Classification Tool (RICT) used to determine predicted index scores, uses substrate composition for these predictions. Whilst it is possible to bypass the entry of this predictor variable, substratum is a strong predictor of macroinvertebrate community composition in the model (Davy-Bowker et al. 2006, Clarke et al. 2011), and RICT has not been tested without it. Therefore, a modified version of RICT is required to overcome this circularity, and to test sediment-specific indices. Nevertheless, in the absence of a sediment-independent model for determining predicted biotic index scores, the correlation between E-PSI scores and fine sediment serves as a useful indication of the performance of the tool.

The aim of this chapter was to test the performance of the E-PSI index using historical regulatory agency data, from catchments with varying degrees of agricultural influence. Index performance is evaluated in terms of the correlation between index scores and fine sediment, over all sites, and within groups of sites with similar specific stream powers.

5.3 Methods

5.3.1 Data

5.3.1.1 Agriculturally impacted sites

The agriculturally impacted dataset used in this chapter comprises data described by Pearson et al. (2016) and additional data obtained from the Environment Agency. The data were derived from routine monitoring, and included stream sites in England and Wales (Figure 5.1) that represented a continuum of agricultural impact. In brief, this dataset included sites ($n = 754$) with upstream catchment land-use ranging from 0-90% arable fields, average widths between 0.80 m and 50 m, average depths between 0.03

m and 3.00 m, seasonal macroinvertebrate data, and seasonal fine sediment data (between 0% and 100%) that were collected during routine monitoring by the Environment Agency in 2006. To allow the calculation of the E-PSI index, the raw macroinvertebrate abundance data were obtained from the Environment Agency, for samples collected in the autumn, along with fine sediment data based on the mean of spring and autumn visual estimates (% sand, silt and clay; EU STAR, 2004), and Environmental Quality Ratios (based on the observed and expected scores - calculated using RICT) for the Walley Hawkes Paisley Trigg (WHPT) index, designed to indicate the effects of organic pollution (Paisley et al. 2014). All 754 sites used in the present study were located on independent water bodies, as detailed by the water body names and grid references provided by the Environment Agency.

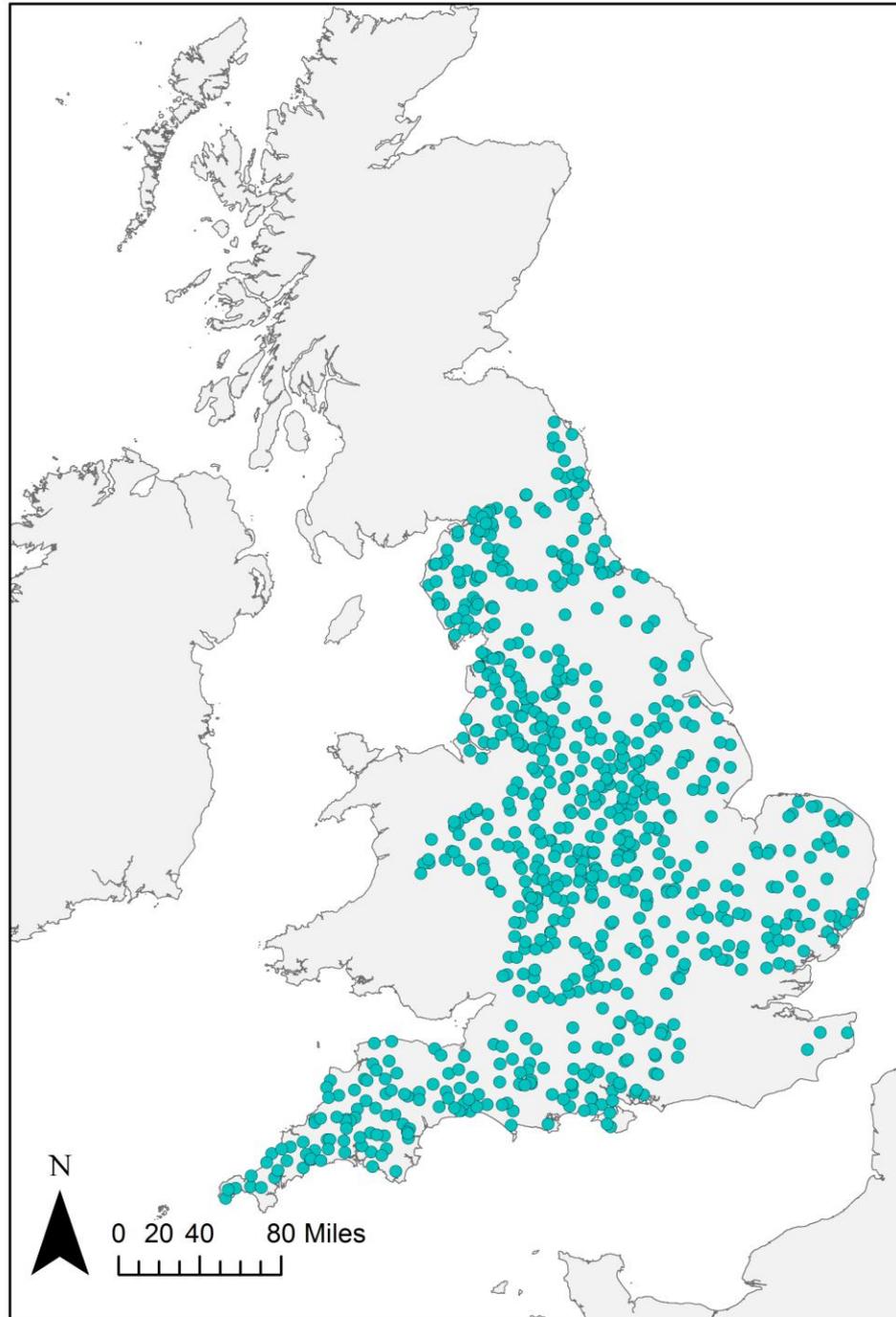


Figure 5.1. Location of sites ($n = 754$) from the agriculturally impacted test dataset.

5.3.1.2 *Minimally impacted sites*

Data from the minimally impacted sites in the RIVPACS database described in Chapter 3 (Section 3.3.1) and 4 (Section 4.3.1), were also utilised in this chapter. Of the 835 minimally impacted sites, 443 sites were selected due to them having annual averages of stream discharge based on at least 12 monthly samples, and macroinvertebrate samples collected in the autumn.

5.3.2 *Testing of the E-PSI index over agriculturally impacted sites*

The $E\text{-PSI}_{\text{fam69}}$ index was tested using the agriculturally impacted dataset ($n = 754$), described in the previous section of this chapter. The performance of the index was evaluated using its Spearman's rank correlation with fine sediment. This non-parametric measure of correlation was used due to the non-normally distributed and heteroscedastic residuals, and the inability to successfully transform. All correlations were interpreted using the Dancey and Reidy (2007) classifications of correlations; $0.1 - 0.39 = \text{weak}$, $0.4 - 0.69 = \text{moderate}$, $0.7 - 0.99 = \text{strong}$.

5.3.3 *Fine sediment covariates*

To examine the relationship between E-PSI and fine sediment further, a separate test was conducted using the minimally impacted sites and the agricultural dataset. The aim of this exercise was to restrict the potential collinearities between fine sediment and covariates relating to longitudinal gradients, particularly those relating to flow (e.g. discharge, velocity and slope), and to assess the independence of E-PSI from flow and the Lotic-invertebrate Index for Flow Evaluation (LIFE) (Extence et al. 1999). To achieve this, the minimally impacted sites were grouped by specific stream power ($W\ m^{-2}$) into five approximately equal size groups. The Spearman's rank correlations within each group between $E\text{-PSI}_{\text{fam69}}$, $E\text{-PSI}_{\text{mixed}}$ and fine sediment were then calculated.

In the absence of actual discharge data for the agriculturally impacted sites, the specific stream powers were calculated using the discharge category for each stream. The discharge categories ranged from 1 to 9 (Table 5.1), and therefore in order to calculate the specific stream power, the middle value for each category was used as a coarse value of average stream discharge. Based on these specific stream power values, the agricultural dataset ($n = 754$) was then split into five approximately equal size groups (1 – 5; low – high). The Spearman's rank correlations within each group between $E\text{-PSI}_{\text{fam69}}$ and fine sediment were then calculated.

Table 5.1. Discharge categories used by the Environment Agency (EU-STAR 2004).

Discharge category	Mean annual discharge ($\text{m}^3 \text{s}^{-1}$)		
1	0.01	-	<0.31
2	0.31	-	0.62
3	0.62	-	1.25
4	1.25	-	2.50
5	2.50	-	5.00
6	5.00	-	10.00
7	10.00	-	20.00
8	20.00	-	40.00
9	40.00	-	80.00
10		>80.00	

Specific stream power (Ω) was used as it is one of the most frequently utilised measures of flow energy and is often used when considering channel processes (Haschenburger and Church 1998, Knighton 1999, Petit et al. 2005). It represents a measure of energy expenditure per unit area of streambed, and is calculated using the following equation:

$$\Omega = (\gamma Qs)/w$$

Equation 5.1. Specific stream power equation, where γ is the specific weight of water (9810 N m^{-3}), Q is the average water discharge ($\text{m}^3 \text{ s}^{-1}$), w is the average stream width, and s is the energy slope (m m^{-1} , which can be approximated by the river slope), (Knighton 1999, Naden et al. 2016).

5.3.4 Comparisons with other biomonitoring tools

The Lotic-invertebrate Index for Flow Evaluation was calculated and its relationship to both fine sediment and E-PSI were assessed in terms of Spearman's rank correlation coefficients across all sites, and within stream power groups. LIFE was chosen to determine the influence of known interactions between flow and fine sediment deposition and re-suspension (Dewson et al. 2007).

5.4 Results

5.4.1 Biological indices and fine sediment

The E-PSI_{fam69} index was moderately correlated to fine sediment ($r_s = -0.68$, $p < 0.01$) in the agriculturally impacted test dataset (Figure 5.2). The agriculturally impacted test dataset had WHPT scores of 2.5 – 8.0 and Environmental Quality Ratios of between 0.4 and 1.3 (see Supplementary Material C) indicating that the sites were, at the very least, impacted to varying degrees by organic pollution.

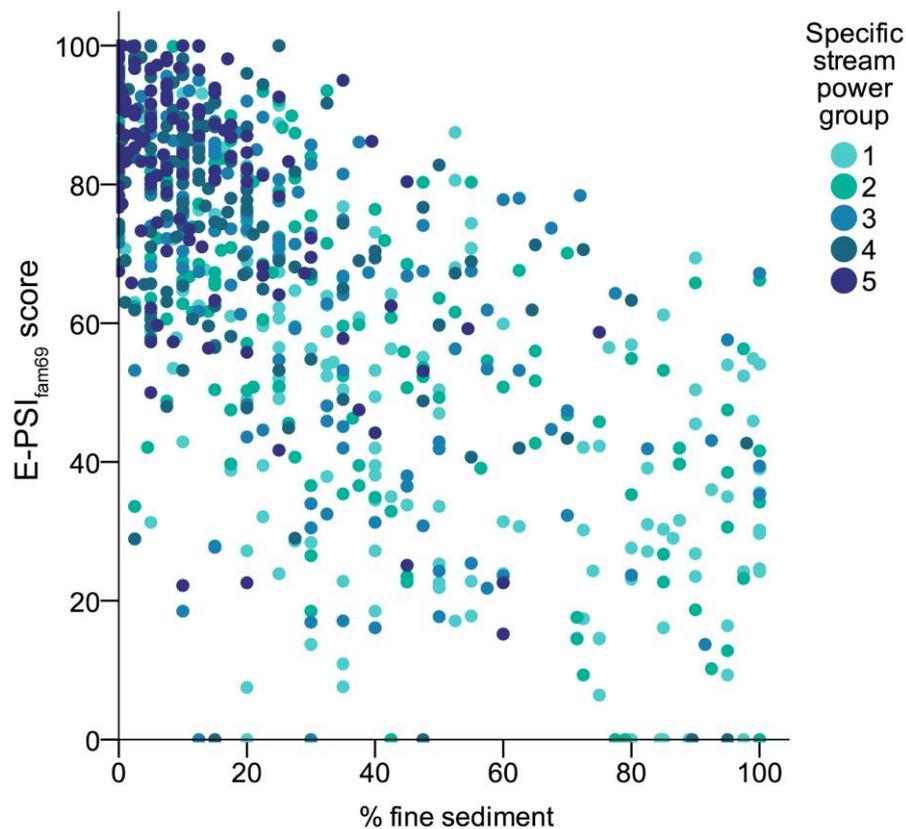


Figure 5.2. Relationship between E-PSI_{fam69} and fine sediment across agriculturally impacted river and stream sites ($n = 754$). The colour of the dots illustrates the specific stream power group for each site (1 – 5; low – high).

5.4.2 Restricting the collinearities between fine sediment and longitudinal gradients

The $E\text{-PSI}_{\text{fam69}}$ index was strongly correlated to fine sediment in the minimally impacted dataset (all sites: $r_s = -0.73$, $p < 0.01$) (Figure 5.3).

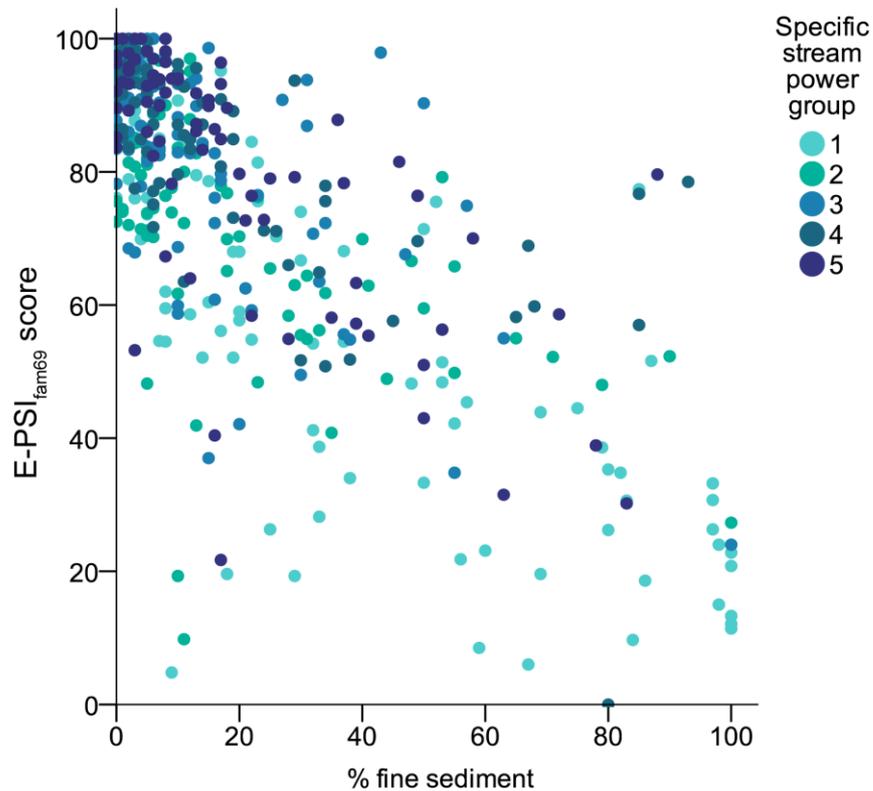


Figure 5.3. Relationship between $E\text{-PSI}_{\text{fam69}}$ and fine sediment across minimally impacted river and stream sites ($n = 443$). The colour of the dots illustrates the specific stream power group for each site (1 – 5; low – high).

Within the stream power groups in the minimally impacted test dataset, the strongest correlation between $E\text{-PSI}_{\text{fam69}}$ and fine sediment was $r_s = -0.77$, $p < 0.01$ in the group of sites with low stream power. All other groups had moderate or strong correlations between $E\text{-PSI}_{\text{fam69}}$ and fine sediment (ranging from $r_s = -0.57$ to $r_s = -0.76$, $p < 0.01$) (Table 5.2).

Table 5.2. Spearman's rank correlation coefficients between the best performing versions of the E-PSI index, LIFE and fine sediment, in the minimally impacted dataset, using all sites with discharge data ($n = 443$). Sites are grouped by specific stream power ($W m^{-2}$).

Stream power group	E-PSI _{fam69}	E-PSI _{mixed}	LIFE _{fam}	LIFE _{mixed}	E-PSI _{fam69}	E-PSI _{mixed}
	versus fine sediment				versus LIFE _{mixed}	
All sites ($n = 443$)	-0.73	-0.72	-0.58	-0.56	0.80	0.84
Group 1 (0.02 – 1.04 $W m^{-2}$)	-0.77	-0.79	-0.75	-0.76	0.92	0.96
Group 2 (1.04 – 2.52 $W m^{-2}$)	-0.69	-0.68	-0.47	-0.59	0.89	0.91
Group 3 (2.54 – 5.66 $W m^{-2}$)	-0.57	-0.58	-0.39	-0.31	0.73	0.78
Group 4 (5.75 – 20.23 $W m^{-2}$)	-0.73	-0.73	-0.51	-0.50	0.67	0.68
Group 5 (20.30 – 6697.69 $W m^{-2}$)	-0.76	-0.70	-0.53	-0.36	0.57	0.68

Stream power groups 1 and 5 ($n = 88$), stream power groups 2, 3 and 4 ($n = 89$).

All correlations are significant at the 0.01 level (2-tailed).

The correlation between E-PSI_{mixed} and fine sediment maintained moderate to strong correlations ($r_s = -0.58$ to $r_s = -0.79$) throughout the stream power groups. In contrast, although the correlations between E-PSI and LIFE_{mixed} were strong in the lower stream power groups, these correlations decreased in successive stream power groups, from $r_s = 0.92$ to $r_s = 0.57$, $p < 0.01$ for E-PSI_{fam69} versus LIFE_{mixed}. The correlation between

LIFE_{mixed} and fine sediment was strongest ($r_s = 0.76$, $p < 0.01$) in the group of sites with low stream power, and was weakest at sites with high stream power.

The Spearman's rank correlations between E-PSI_{fam69} and fine sediment in the agriculturally impacted dataset within stream power groups were moderate ($r_s = -0.40$ to $r_s = -0.63$). Similarly to the minimally impacted dataset, the correlations between E-PSI_{fam69} and LIFE_{fam} in the agricultural dataset decreased, with increasing stream power group (Table 5.3).

Table 5.3. Spearman's rank correlation coefficients between the E-PSI index, LIFE and fine sediment, in the agriculturally impacted dataset ($n = 754$). Sites are grouped by specific stream power ($W m^{-2}$).

Stream power group	E-PSI _{fam69}	LIFE _{fam}	E-PSI _{fam69}
	versus fine sediment		versus LIFE _{fam}
All sites ($n = 754$)	-0.68	-0.62	0.89
Group 1 (0.01 – 1.05 $W m^{-2}$)	-0.54	-0.51	0.91
Group 2 (1.05 – 2.42 $W m^{-2}$)	-0.59	-0.50	0.87
Group 3 (2.43 – 5.09 $W m^{-2}$)	-0.63	-0.54	0.88
Group 4 (5.09 – 11.11 $W m^{-2}$)	-0.40	-0.30	0.82
Group 5 (11.40 – 296.67 $W m^{-2}$)	-0.51	-0.42	0.68

Stream power groups 1, 2, 3 and 4 ($n = 151$), stream power group 5 ($n = 150$). All correlations are significant at the 0.01 level (2-tailed).

5.5 Discussion

5.5.1 Identifying fine sediment pressure

The E-PSI_{fam69} index showed moderate correlations ($r_s = -0.68$, $p < 0.01$) over the agriculturally impacted sites, suggesting that the index performs similarly over sites that are impacted by the multitude of pressures associated with agricultural land-use as it does over minimally impacted sites (see Chapter 4).

5.5.2 Collinearity between longitudinal gradients and fine sediment

A considerable challenge for research carried out over large spatial extents is the occurrence of collinearity between the variable of interest and other environmental variables (Pearson et al. 2016). To investigate the influence of collinearities within the dataset, sites were grouped using specific stream power to restrict the collinearities between fine sediment and variables associated with longitudinal gradients, particularly those relating to flow. Stream power incorporates stream discharge and channel gradient, and is widely used to assess the ability of the stream to transport, erode or deposit sediment (Church 2002, Bizzi and Lerner 2015).

The results of the analysis showed that flow (indicated by LIFE) was moderately to strongly correlated to fine sediment cover, and as such LIFE and E-PSI are moderately to strongly correlated. However, this relationship between fine sediment cover and flow weakens as stream power increases, likely because after the critical thresholds for particle entrainment and transport have been exceeded, further increases in stream power cease to result in much further entrainment, transport and removal of fine sediment from the river bed (though the increases in stream power may trigger entrainment and transport of coarser particles as bed load). This is likely to be the reason why the relationship between LIFE and E-PSI, and LIFE and fine sediment weaken in successively increasing stream power groups. These results add confidence

to the interpretation that although fine sediment cover is related to stream power (for physical reasons), and there will therefore be a moderate to strong relationship between scores derived from sediment-specific and flow-specific biomonitoring tools; these relationships are not fixed, and thus where one pressure (e.g. flow) is relatively stable, whilst the other is variable (e.g. sediment cover), the scores derived from the biomonitoring tools will diverge and provide valuable information on the likely cause of deviation from reference-condition community composition. In the case of LIFE and E-PSI, a similar approach to model development for LIFE could result in even greater decoupling of the two tools.

5.5.3 Sources of variation

Sources of variation in the relationship between E-PSI and fine sediment were discussed in Chapter 4 (Section 4.5.3), including error associated with the visual estimates of fine sediment and biological sampling, and the natural variation in impact-response relationships, which is likely to be influenced by the different climatic regions (Wasson et al. 2010), seasons (Lytle and Poff 2004) and biotic communities (Johnson et al. 2006, Holomuzki et al. 2010). In addition to these, at the agriculturally impacted sites, the invertebrate communities are also likely to be influenced by the presence of other pressures associated with agricultural practices (e.g. pesticides, organic pollution, nutrient enrichment, habitat modification), thus confounding the results (Folt et al. 1999, Matthaei et al. 2010).

5.5.3.1 Confounding pressures

As well as the potential for collinearity between sediment and variables associated with longitudinal gradients, streams in agricultural catchments are commonly subjected to potentially confounding pressures arising from pesticide and nutrient applications,

morphological changes, and modifications to riparian vegetation. Relatively few studies have considered the interactions and co-occurrence of in-stream pressures, which are likely to affect the performance of biomonitoring tools. A recent study of 9330 sites throughout 14 European countries, found 47% of rivers (90% of lowland rivers) throughout Europe to be subject to multiple pressures relating to hydrology, morphology, water quality and connectivity (Schinegger et al. 2012). This highlights the importance of a mechanistic linkage between indices and sediment conditions, given the potential for these multiple pressures to confound biomonitoring approaches. The extremely limited quantitative analyses of responses to multiple pressures in freshwater environments (Nöges et al. 2016), suggest that many pressures will interact synergistically or antagonistically, and their influences on the macroinvertebrate community are likely to vary alongside different environmental characteristics. For example, Folt et al. (1999) observed synergism and antagonism among anthropogenic pressures (e.g. sodium dodecyl sulfate), biotic interactions (e.g. food availability), and environmental characteristics (e.g. temperature). Townsend et al. (2008) and Wagenhoff et al. (2011) observed synergistic interactions between nutrients and fine sediment pressure in macroinvertebrate responses in agricultural catchments (32 study sites and nine experimental sites, and 43 study sites, respectively; New Zealand).

Of these multiple pressures, the influence of non-native invasive species on macroinvertebrate community composition and habitat may be an important consideration (Johnson et al. 2010, Mathers et al. 2016a). The family Astacidae, which in the UK includes one endangered native species (*Austropotamobius pallipes*; white clawed crayfish) and a number of invasive crayfish species, were recorded at some sites. Given the family-level resolution of the data, it was unclear whether these records were referring to native or invasive species. Furthermore, given the difficulties of detecting crayfish using single year samples, other sites may also have populations of native or non-native species. Of particular concern is the signal crayfish

(*Pacifastacus leniusculus*), which is widespread throughout the UK, and is thought to influence geomorphology and fine sediment dynamics in rivers and streams (Johnson et al. 2011, Rice et al. 2012, Harvey et al. 2014), and predate on a number of taxa that are included in the E-PSI index indicator weightings (e.g. taxa belonging to the orders of Diptera, Hirudinea, Gastropoda and Bivalvia) (Crawford et al. 2006, Mathers et al. 2016a).

5.6 Conclusion

The E-PSI index can be used alongside other indices to help identify the causes of ecological degradation in rivers and streams. Nevertheless, given the multitude of pressures both natural and anthropogenic that influence macroinvertebrate community compositions, it is likely that most pressure-specific indices will be limited in terms of their ability to make accurate predictions of the degree of impact across all river and stream ecosystems. Instead, perhaps a more achievable and realistic goal is to use a suite of different pressure-specific indices to identify potential areas of ecological degradation and the likely causes of any deviations, allowing for more targeted monitoring and investigation.

The common occurrence of diverse pressures in rivers and streams, places a great importance on a mechanistic linkage and understanding of biotic responses to the pressure targeted by a biomonitoring tool. By relying purely on statistical approaches for model development that can be influenced by the presence of confounding pressures, there is a risk drawing inaccurate conclusions from tool outputs. This chapter has demonstrated a robust approach to testing biomonitoring tools, and has evaluated the performance of the index over both minimally impacted and agriculturally impacted stream sites. The following chapter continues this testing and evaluation of the E-PSI index, by considering the influence of a widespread invasive species on the

outputs from biomonitoring tools. This type of rigorous testing is essential if these tools are to be used by regulatory agencies to aid in the monitoring and protection of freshwater ecosystems.

Chapter 6 The impacts of non-native signal crayfish (*Pacifastacus leniusculus*) on sediment-biomonitoring and fine sediment

Part of the content of this chapter is reproduced from the following:

Turley, M.D., G.S. Bilotta, A. Gasparrini, F. Sera, K.L. Mathers, I. Humphreyes, and J. England. 2017. The effects of non-native signal crayfish (*Pacifastacus leniusculus*) on fine sediment and sediment-biomonitoring. *Science of the Total Environment* 601-602:186-193.

Contributions: MT led the analysis and writing of this chapter (and publication), supported and supervised by GB (primary supervisor). AG, FS and KM provided training and advice on modeling techniques. KM also provided comments on crayfish ecology. JE and IH provided assistance obtaining data and advice on regulatory agency protocols.

6.1 Abstract

Non-native invasive species represent a significant threat to freshwater biodiversity. Signal crayfish (*Pacifastacus leniusculus*) are a North American crustacean species that has invaded freshwater ecosystems across Europe, and are widespread in England. Recent studies suggest that predation of macroinvertebrates by signal crayfish can lead to shifts in macroinvertebrate community composition, which in-turn could affect the performance of biomonitoring tools used to assess causes of ecological degradation in freshwater ecosystems. Given the reliance on biomonitoring tools by regulatory agencies globally, it is crucial that the potential influence of invasive species on tool outputs is better understood.

Crayfish are often considered to be biogeomorphic agents, and therefore the aim of this chapter was to investigate whether sediment-biomonitoring tool outputs changed following signal crayfish invasions, and whether these changes were reflecting real post-invasion changes in deposited fine sediment conditions, or changes to community compositions unrelated to fine sediment. The aim was addressed through a quasi-experimental study design involving interrupted time series (ITS) analysis of long-term routine environmental data, collected as part of national and international monitoring programmes. The ITS analysis of all sites displayed a small, but statistically significant increase between pre- and post-invasion index scores (4.1, $p < 0.001$, 95%CI: 2.1, 6.2) for the Proportion of Sediment-sensitive Invertebrates (PSI) index biomonitoring tool, but no statistically significant difference was observed for the Empirically-weighted PSI (0.4, $p = 0.742$, 95%CI: -2.1, 2.9) or fine sediment (-2.3, $p = 0.23$, 95%CI: -6.0, 1.4). Subgroup analyses of sites grouped by habitat heterogeneity and the percentage of coarse substrate, demonstrated changes in biomonitoring tool scores of similar magnitude to the overall analysis, ranging from four to 10 percentage points. Importantly, these subgroup analyses showed only relatively small changes to fine sediment, two of which were statistically significant (greatest effect estimate: -10.9, $p = 0.011$, 95%CI: -19.3, -2.6), but did not coincide with the expected responses from biomonitoring tools.

The results suggest that outputs from biomonitoring tools may be influenced by signal crayfish invasions, but that these impacts are likely to be context dependent, and perhaps not the result of the biogeomorphic activities of crayfish. Crucially, the highlighted changes to post-invasion biomonitoring scores were of low magnitude, and therefore unlikely to result in an incorrect diagnosis of sediment pressure, particularly as tools should be used alongside a suite of other pressure-specific indices when assessing for ecological degradation.

6.2 Introduction

Biological invasions of non-native species (herein invasive species) represent a significant threat to global biodiversity (Simberloff et al. 2013). With freshwater invasions expected to increase as a result of climate change and globalisation, invasive species have the potential to result in widespread ecological impacts; defined as measurable changes to the state of an ecosystem (Ricciardi et al. 2013, Kumschick et al. 2015). Invasive species can exert strong pressures on the resident native biota of invaded habitats, both directly, through predation, competition or displacement, and indirectly by disrupting trophic dynamics (Lodge et al. 2012, Early et al. 2016). Some invasive species have also been shown to alter the physical and chemical characteristics of the habitats that they invade (Johnson et al. 2011, Fei et al. 2014, Greenwood and Kuhn 2014).

In Europe, one widespread freshwater invasive species is the North American signal crayfish (*Pacifastacus leniusculus*). Their success is likely to be due partly to their opportunistic feeding, with an omnivorous and detritivorous diet; consuming algae, detritus, macrophytes, benthic macroinvertebrates, fish and other crayfish (Harvey et al. 2011). Crayfish have been described as displaying an ontogenetic shift with juveniles being predominantly carnivorous and adults becoming omnivorous, and therefore the population age structure may influence the effects on native communities, however studies of signal crayfish are conflicting (Guan and Wiles 1998, Bondar et al. 2005). Furthermore, population density and resource limitation can often influence the dietary sources of omnivores, although Bondar et al. (2005) found that density had little effect on the dietary composition of signal crayfish. Recent research has suggested that as a result of modifications to macroinvertebrate community composition (Mathers et al. 2016a), signal crayfish invasions can lead to changes to biomonitoring tool outputs (Mathers et al. 2016b). Given the reliance of regulatory agencies globally on biomonitoring tools to diagnose ecological degradation in freshwater ecosystems, it is

crucial that the potential for invasive species to influence tool outputs is better understood (MacNeil et al. 2013).

The Proportion of Sediment-sensitive Invertebrates (PSI) index has been shown to exhibit inflated scores following crayfish invasions (Mathers et al. 2016b). Higher PSI scores are normally indicative of lower fine sediment conditions, however Mathers et al. (2016b) suggested that the post-invasion inflation of PSI scores were likely the result of selective predation by crayfish. Other research has shown decreased abundance of Gastropoda, Bivalvia and Hirudinea (preferential prey of crayfish; Crawford et al. 2006, Dorn 2013), a shift in community composition towards more mobile taxa that are able to avoid predation (Mathers et al. 2016a), and reduced species diversity and densities, particularly when signal crayfish are present at high densities (Crawford et al. 2006). These taxa generally score highly in the PSI index, resulting in a higher overall PSI score being recorded.

However, crayfish are biogeomorphic agents, with the ability to rework substrate, increase suspended particulate matter and alter stream sediment dynamics, primarily due to their burrowing in river banks (increasing erosion and bank collapse), construction of pits and mounds, their large size, aggressive nature, and general movement and foraging on the river bed (Harvey et al. 2011, Johnson et al. 2011, Rice et al. 2012, Albertson and Daniels 2016, Faller et al. 2016). In their native range crayfish have been recorded at densities of 0.25 to 1.8 per m² (Reynolds et al. 2013). In comparison, in the UK, very high densities of signal crayfish (0.9 to 20 individuals per m²) and their burrows (10 – 20 burrows per m² in soft river banks) have been recorded (Harvey et al. 2011), highlighting the potential for crayfish to resuspend deposited material and introduce fine sediment through increased bank erosion and bank collapse. Therefore, whilst the effects on sediment-biomonitoring tool outputs may be the result of shifts in community composition from direct predation and/or the resulting changes to food web dynamics, they could also be partly the result of

alterations to fine sediment conditions (i.e. resuspension of deposited fine sediment) caused by the invasive species - a confounding factor that was not investigated by Mathers et al. (2016b).

The aim of this study was to utilise a quasi-experimental study design and interrupted time series (ITS) analysis to investigate whether inflation of sediment-biomonitoring tool (PSI and Empirically-weighted PSI; E-PSI) scores occurred following signal crayfish invasions, and whether this was associated with changes to deposited fine sediment over time, or shifts in macroinvertebrate community composition resulting from other effects of crayfish invasion (direct or indirect). Interrupted time series analysis is able to estimate the effects of an intervention (e.g. invasion), taking account of pre-intervention long-term and seasonal trends, and autocorrelation, which are common in ecological applications (Friberg et al. 2009). The application of such techniques in epidemiology, social sciences and clinical research is relatively common (Bernal et al. 2016, Gasparrini 2016), however its use within invasion ecology is rare (e.g. Brown et al. 2011), likely due to the challenges of obtaining long term data for pre- and post-invasion periods. Time series analysis (not ITS) has been used in a previous study of population dynamics of invasive and native crayfish in a single river basin (Almeida et al. 2014), but the authors did not investigate the effects on other taxa, biomonitoring tools or fine sediment, which are the focus of the present study.

Time since invasion is an important consideration when studying the impact of invasive species on the receiving ecosystem, as impacts may intensify over time, or may diminish, with native communities/ecosystems being able to recover (Strayer et al. 2006). Therefore, time series data are likely to provide important insights into the impacts of invasive species (Strayer et al. 2006, Kumschick et al. 2015), although the length of time required to observe acute and/or chronic impacts is unclear, but is likely to depend both on the attributes of the invader and the receiving ecosystem. Signal crayfish are highly mobile organisms with an ability to move both upstream and

downstream, with radio tagging studies showing variability among individuals, for example, Bubb et al. (2004) found tagged individuals to travel distances between <1 m and 790 m during tracking (in this case for a duration of 74 days) in upland streams. This motility contributes to the invasion success of signal crayfish, with the probability of successful establishment predicted to be > 50%, 4 years after first introduction (at sites with similar latitudes to the UK) (Sahlin et al. 2010).

A further aim of this study was to investigate the influence of stream characteristics; habitat heterogeneity and percentage of coarse substrate, on invader impacts. A stream with high habitat heterogeneity/complexity is likely to provide a greater variety of habitat for benthic macroinvertebrate refugia, than those with homogeneous habitat, potentially resulting in increased community stability and resilience to predation (Brown and Lawson 2010, Kovalenko et al. 2012). Substrate composition is a characteristic typically related to longitudinal gradients associated with channel gradient, stream power and flow (Church 2002), and is thought to be an important driver of macroinvertebrate community composition (Minshall 1984). Macroinvertebrate taxa have a variety of habitat preferences as a result of their biological traits (Extence et al. 2013), and as such, a stream with a high percentage of coarse substrate is likely to be inhabited by a different macroinvertebrate assemblage to one dominated by fine sediment. Signal crayfish invasions may impact these different assemblages to varying degrees, for example, due to the availability of preferential prey items. Furthermore, physical site characteristics may play an important role in determining the effects of crayfish invasions, with the lowland or mid-reaches of a river (often characterised by fine bed and bank material) being more sensitive to crayfish activity (Harvey et al. 2011).

This study was led by the following five hypotheses:

Hypothesis 1: Family-level PSI (PSI_{fam}) and E-PSI ($E-PSI_{fam09}$) index scores are inflated

after signal crayfish invasions.

Hypothesis 2: The percentage of fine sediment is lower at sites post-invasion compared with pre-invasion.

Hypothesis 3: The abundances of preferential crayfish prey taxa (e.g. Gastropoda and Hirudinea) are lower in the post-invasion periods.

Hypothesis 4: Changes to PSI_{fam} and $E-PSI_{fam69}$ index scores in post-invasion periods will be greatest at sites with low habitat heterogeneity.

Hypothesis 5: Changes to PSI_{fam} and $E-PSI_{fam69}$ index scores in post-invasion periods will be greatest at sites with low percentages of coarse substrate.

6.3 Methods

6.3.1 Site selection

The stream and river sites included in this chapter were selected from a database comprising all past macroinvertebrate samples collected by the Environment Agency of England. A systematic search of the entire database for "*Pacifastacus leniusculus*" returned all stream and river sites in England where this species was recorded between the year 1990 and 2014. The mostly family-level taxonomic data created uncertainty whether records of the family Astacidae were referring to the native white-clawed crayfish (*Austropotamobius pallipes*), signal crayfish, or other invasive crayfish species. Therefore, to avoid misidentifying the timing of the first record of signal crayfish, those sites with "Astacidae" recorded prior to the first record of "*Pacifastacus leniusculus*" were removed from the dataset. For each of the remaining sites, the midpoint between the first record of "*Pacifastacus leniusculus*" and the previous sample, was designated as the date of invasion; sites with fewer than four pre-invasion and four post-invasion samples were subsequently removed from the dataset. Finally,

for sites on the same watercourse, only the site with > 10 pre-invasion samples and the greatest number of post-invasion samples was retained, to ensure independence of sites. The 71 remaining sites (Figure 6.1) had an average (mean) of 22 pre-invasion samples, collected over an average period of 14 years, and 10 post-invasion samples, collected over an average period of 6.5 years.

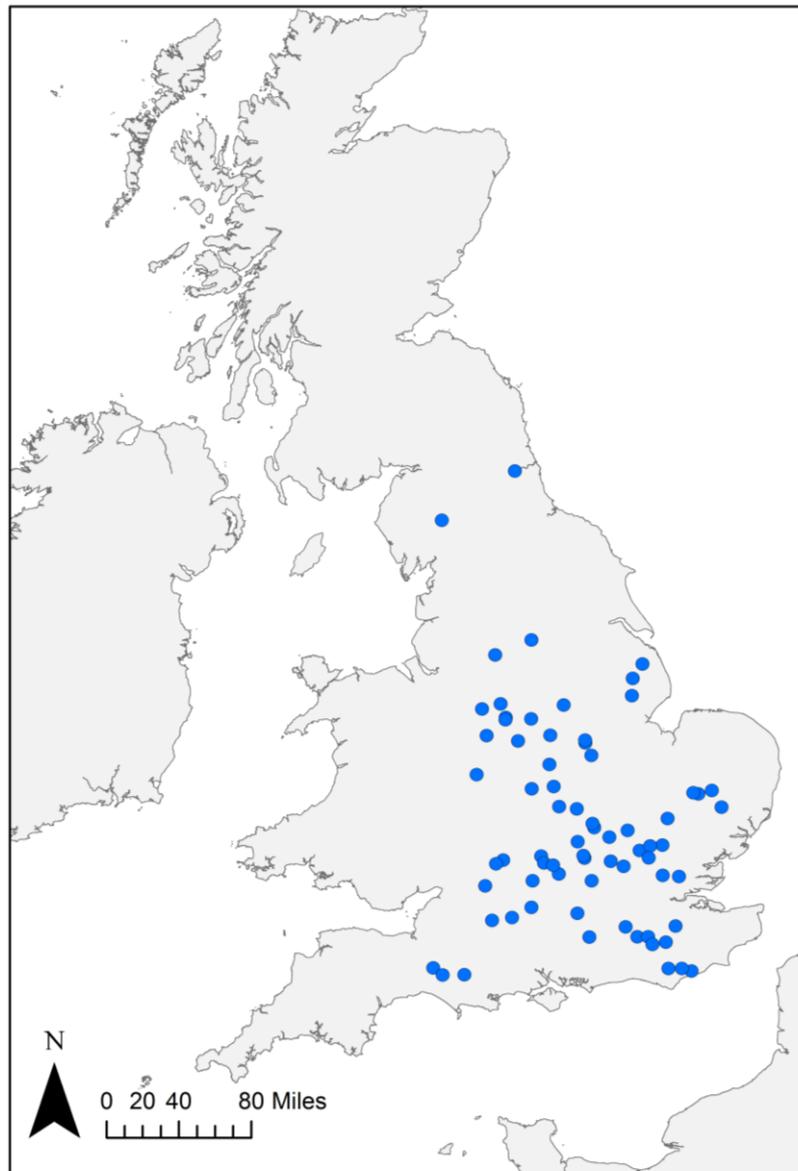


Figure 6.1. Locations of the selected river sites throughout England with records of signal crayfish (*Pacifastacus leniusculus*).

6.3.2 *Substrate composition data*

The substrate composition data consisted of visual estimates of the percentage of the substrate composed of bedrock, boulders (≥ 256 mm), cobbles (64 – 256 mm), pebbles/gravel (2 – 64 mm), sand (≥ 0.06 and < 2.00 mm), and silt and clay (< 0.06 mm), recorded at the time of each macroinvertebrate sample. Similarly to previous chapters, the size classes for sand, silt and clay were combined to form a substrate class referred to from this point forward as fine sediment. The visual estimate method used to collect these data is described in Chapter 3 (Section 3.3.1.2) of this thesis and in the Standardisation of River Classifications project protocol (EU-STAR 2004).

6.3.3 *Macroinvertebrate data and calculation of sediment biomonitoring indices*

Macroinvertebrate data were collected by the Environment Agency using the UK standard method; a three-minute kick-sample, followed by a one-minute manual search, as described in Chapter 3 (Section 3.3.1.1) and in the Standardisation of River Classifications Project (EU-STAR 2004). The family-level data were used to calculate PSI_{fam} and $E-PSI_{fam69}$ for each sample (see Supplementary Material D).

6.4 **Statistical analysis**

Interrupted time series analysis using segmented regression was employed to estimate the effects of crayfish invasions on biomonitoring tool outputs and fine sediment. A hierarchical modelling approach was applied to model differences in baseline levels and trends as random effects in *R* (version 3.3.2; R Core Team 2016). Linear mixed effect (*lme*) models (Pinheiro and Bates 2000) and linear quantile mixed models (*lqmm*) (Geraci 2014) were fitted to the time series data of $E-PSI_{fam69}$, PSI_{fam} , and fine sediment, from all 71 sites. Quantile regression can be particularly useful in ecological studies where unequal variance (heteroscedasticity) is common, which could result in

erroneous estimates of statistical significance (Cade and Noon 2003). Both mixed effect models included fixed (invasion progress, time, and seasonal variation) and random effects (time and site). Time was a linear variable used to model the average trend (fixed effects) and site-specific (random effects) deviations from this trend.

An *a priori* definition of the type of impact (e.g. step change, slope change, combination) was necessary to avoid the potential for statistical artefacts to occur when testing numerous models (Bernal et al. 2016). Invasion impacts typically increase rapidly in the early stages of establishment, leveling-off in the long term (Strayer et al. 2006, Ricciardi et al. 2013). Predictions of establishment time for signal crayfish suggest that ~50% of invaded sites (at similar latitudes) are successfully established within 4 years (Sahlin et al. 2010). Therefore, the post-invasion periods in this study were modelled as gradual step changes, and a four-year establishment period was assumed following invasions (Figure 6.2).

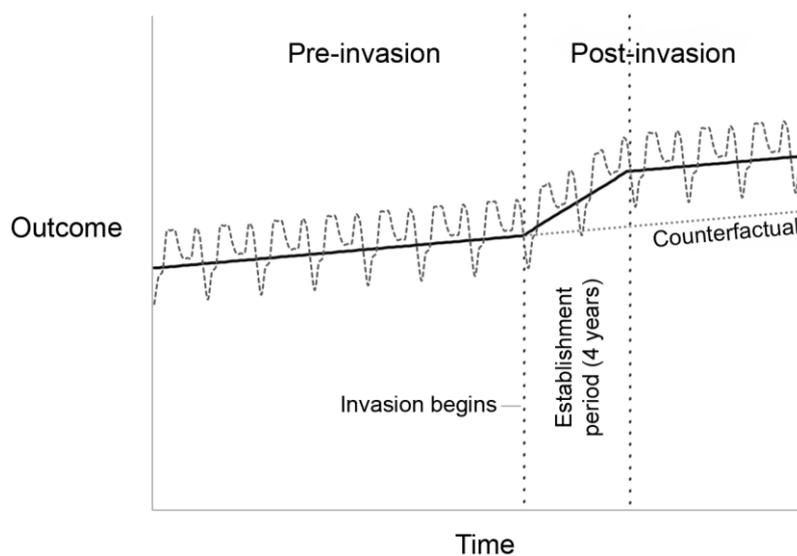


Figure 6.2. Conceptual diagram of the gradual step change modelling approach used in this study. Solid line represents the regression line (site-specific). Dashed line represents the long term and seasonal variation (based on data from all study sites). Dotted vertical lines mark the beginning and end of the crayfish establishment period.

Although the impacts of some invasive species can take decades to become apparent (Strayer et al. 2006), this ecologically relevant modelling approach could provide an insight into the relatively short-term potential impacts following crayfish invasions.

The seasonal variations of PSI_{fam} , $E-PSI_{fam69}$, and fine sediment, were modelled using harmonic functions of time (Hunsberger et al. 2002, Barone-Adesi et al. 2011). Invasion progress was coded between 0, prior to the invasion commencing (the midpoint between the first “invaded” sample and the previous sample), and 1, following the end of the 4-year “establishment period”, depending on the samples temporal position within the establishment period (e.g. a sample was coded as 0.5 if it occurred halfway through the establishment period).

Model assumptions were checked, and the residuals of the *lme* models showed some degree of heteroscedasticity. Despite this, they provide a useful indication of the magnitude of effects. The *lqmm* is less reliant on distributional assumptions, but in this study comes at the cost of precision, and therefore the *lqmm* results are only presented in Appendix D, to allow comparison of the effect estimates. After controlling for seasonality there was little evidence of autocorrelation of residuals.

The multiple associations tested were limited to a small number of comparisons and were based on specific *a priori* hypotheses. In these circumstances it has been suggested that adjustments for family-wise error rates (e.g. Bonferroni-Holm corrections) can be overly conservative (Moran 2003), and therefore in this chapter *p*-values were not adjusted.

6.4.1 Subgroup analyses

Subgroup analyses were conducted to investigate whether the effect of crayfish on biomonitoring tool scores and fine sediment conditions varied as a function of habitat

characteristics. The dataset of 71 sites was split into three roughly equal groups based on (i) substrate/habitat heterogeneity, and (ii) percentage of coarse substrate.

6.4.2 *Habitat heterogeneity*

The 71 sites were ranked and divided into three subgroups according to their median substrate Shannon diversity. This was calculated using the Shannon diversity of each samples' substrate composition in the pre-invasion period. The Shannon Diversity Index (H) has been previously used as a measure of habitat heterogeneity in ecological and geomorphological research (Yarnell et al. 2006), and is calculated using Equation 6.1. In the present study, Heterogeneity Group 1 – 3 represent low to high habitat heterogeneity with Shannon diversity values ranging from $H = 0.36$ to $H = 1.32$.

$$H = -\sum p_i \ln p_i$$

Equation 6.1. Formula to calculate the Shannon Diversity Index (H) for substrate composition, where p_i is the proportion of the streambed categorised as substrate size class i .

6.4.3 *Percentage of coarse substrate*

The 71 sites were ranked and divided into three subgroups based on the median of their pre-invasion estimates of coarse substrate (Substrate Group 1 – 3; low to high % coarse substrate), which ranged from 5% – 100% (boulders, cobbles, pebbles and gravel).

6.4.4 *Shifts in community composition*

Differences in the community composition between pre- and post-invasion periods

were examined in *PRIMER 7* software via non-metric multidimensional scaling (NMDS) centroid plots and Bray-Curtis similarity coefficients. Analysis of similarity (ANOSIM; 999 permutations) was conducted to examine if the differences in communities were statistically different (R values: <0.25, barely distinguishable; 0.25-0.75, separated but overlapping; >0.75, well separated; Clarke and Gorley 2001). To account for the variation in community composition over all 71 sites, ordination analyses were carried out on the subgroups. The similarity percentage function (SIMPER) was used to determine which taxa contributed most to the statistically significant differences between pre- and post-invasion community compositions. In order to use the available data, which was collected using a semi-quantitative technique, the raw abundance values were organised into ordinal classes (1 = ≤ 9, 2 = 10 – 32, 3 = 33 – 99, 4 = 100 – 332, 5 = 333 – 999, 6 = ≥1000).

6.5 Results

6.5.1 Sediment-biomonitoring and fine sediment

The results presented in Table 6.1 demonstrate that the Interrupted Time Series Analysis of all sites combined, showed a small, but statistically significant increase between pre- and post-invasion PSI_{fam} scores (4.1, $p < 0.001$, 95%CI: 2.1, 6.2), with no such evidence of differences for E- PSI_{fam69} (0.4, $p = 0.742$, 95%CI: -2.1, 2.9) or fine sediment (-2.3, $p = 0.227$, 95%CI -6.0, 1.4). Visualisations of the *lme* models are provided in Appendices E, F & G.

Table 6.1. Coefficients, p -values and confidence intervals for gradual step change linear mixed effect models of PSI_{fam} , $E-PSI_{fam69}$ and fine sediment, for all sites, and sites split into subgroups based on their (i) habitat heterogeneity and (ii) percentage of coarse substrate; (both groups 1-3: low-high).

		Coefficient	p -value	Confidence intervals
All sites $n = 71$	PSI_{fam}	4.130	< 0.001	2.090, 6.169
	$E-PSI_{fam69}$	0.423	0.742	-2.092, 2.938
	Sediment	-2.306	0.227	-6.046, 1.433
Heterogeneity Group 1 $n = 24$	PSI_{fam}	-0.445	0.816	-4.187, 3.297
	$E-PSI_{fam69}$	-5.323	0.027	-10.025, -0.620
	Sediment	-9.860	0.011	-17.480, -2.240
Heterogeneity Group 2 $n = 23$	PSI_{fam}	5.731	0.002	2.180, 9.282
	$E-PSI_{fam69}$	4.887	0.026	0.589, 9.185
	Sediment	-0.048	0.987	-5.916, 5.820
Heterogeneity Group 3 $n = 24$	PSI_{fam}	7.361	< 0.001	4.032, 10.690
	$E-PSI_{fam69}$	0.863	0.682	-3.275, 5.000
	Sediment	3.001	0.322	-2.944, 8.946
Coarse substrate Group 1 $n = 26$	PSI_{fam}	1.942	0.337	-2.029, 5.914
	$E-PSI_{fam69}$	0.095	0.971	-5.057, 5.246
	Sediment	-10.946	0.011	-19.333, -2.559
Coarse substrate Group 2 $n = 23$	PSI_{fam}	10.068	< 0.001	6.806, 13.330
	$E-PSI_{fam69}$	4.603	0.024	0.596, 8.610
	Sediment	1.129	0.716	-4.949, 7.207
Coarse substrate Group 3 $n = 22$	PSI_{fam}	-0.321	0.850	-3.659, 3.017
	$E-PSI_{fam69}$	-4.152	0.034	-7.989, -0.316
	Sediment	1.526	0.490	-2.812, 5.863

Bold text denotes outcome coefficient was statistically significant.

6.5.2 *Habitat heterogeneity*

Results from the analyses of sites grouped by their habitat heterogeneity highlight low magnitude changes to PSI_{fam} scores. Statistically significant increases were evident in post-invasion periods for sites with moderate (Heterogeneity Group 2; 5.7, $p = 0.002$, 95%CI: 2.2, 9.3) and high habitat heterogeneity (Heterogeneity Group 3; 7.4, $p < 0.001$, 95%CI: 4.0, 10.7).

E- PSI_{fam69} scores also displayed low magnitude changes in post-invasion periods. Changes in scores at sites with low and moderate habitat heterogeneity, were statistically significant (Heterogeneity Group 1: -5.3, $p = 0.027$, 95%CI: -10.0, -0.6; Heterogeneity Group 2: 4.9, $p = 0.026$, 95%CI: 0.6, 9.2).

A reduction in fine sediment was observed in post-invasion periods for sites in Heterogeneity Group 1 (-9.9, $p = 0.011$, 95%CI: -17.5, -2.2), but there was no evidence of changes to fine sediment in Heterogeneity Group 2 (0.0, $p = 0.987$, 95%CI: -5.9, 5.8) or Heterogeneity Group 3 (3.0, $p = 0.322$, 95%CI: -2.9, 8.9).

6.5.3 *Coarse substrate*

Analysis of the sites grouped by their percentage of coarse substrate demonstrated statistically significant increases in PSI_{fam} scores post-invasion compared with pre-invasion, at sites with intermediate percentages of coarse substrate (Substrate Group 2: 10.1, $p < 0.001$, 95%CI: 6.8, 13.3). Other subgroups of sites showed no evidence of changes to PSI_{fam} scores following crayfish invasions.

Low magnitude effect size estimates were shown for E- PSI_{fam69} scores, with statistically significant changes to post-invasion scores, in Substrate Group 2 (4.6, $p = 0.024$, 95%CI: 0.6, 8.6), and Substrate Group 3 (-4.2, $p = 0.034$, 95%CI: -8.0, -0.3).

The results from the analyses of fine sediment within the coarse substrate groups,

displayed a statistically significant decrease in fine sediment (-10.9 , $p = 0.011$, 95%CI: -19.3 , -2.6) for sites with a low percentage of coarse substrate (Substrate Group 1). Other subgroups demonstrated no evidence of changes to fine sediment following crayfish invasions.

6.5.4 *Shifts in community composition*

Centroid NMDS ordination plots of all sites indicated some dissimilarities in macroinvertebrate community composition (ANOSIM $p < 0.001$) associated with crayfish invasion, but with substantial overlapping (R value of 0.232). Subgroup analyses illustrated dissimilarities (with partial overlapping) between pre- and post-invasion communities, which coincided with those ITS subgroup analyses that were found to have statistically significant changes to their post-invasion PSI_{fam} or $E-PSI_{fam69}$ scores (Figure 6.3e and 6.3f).

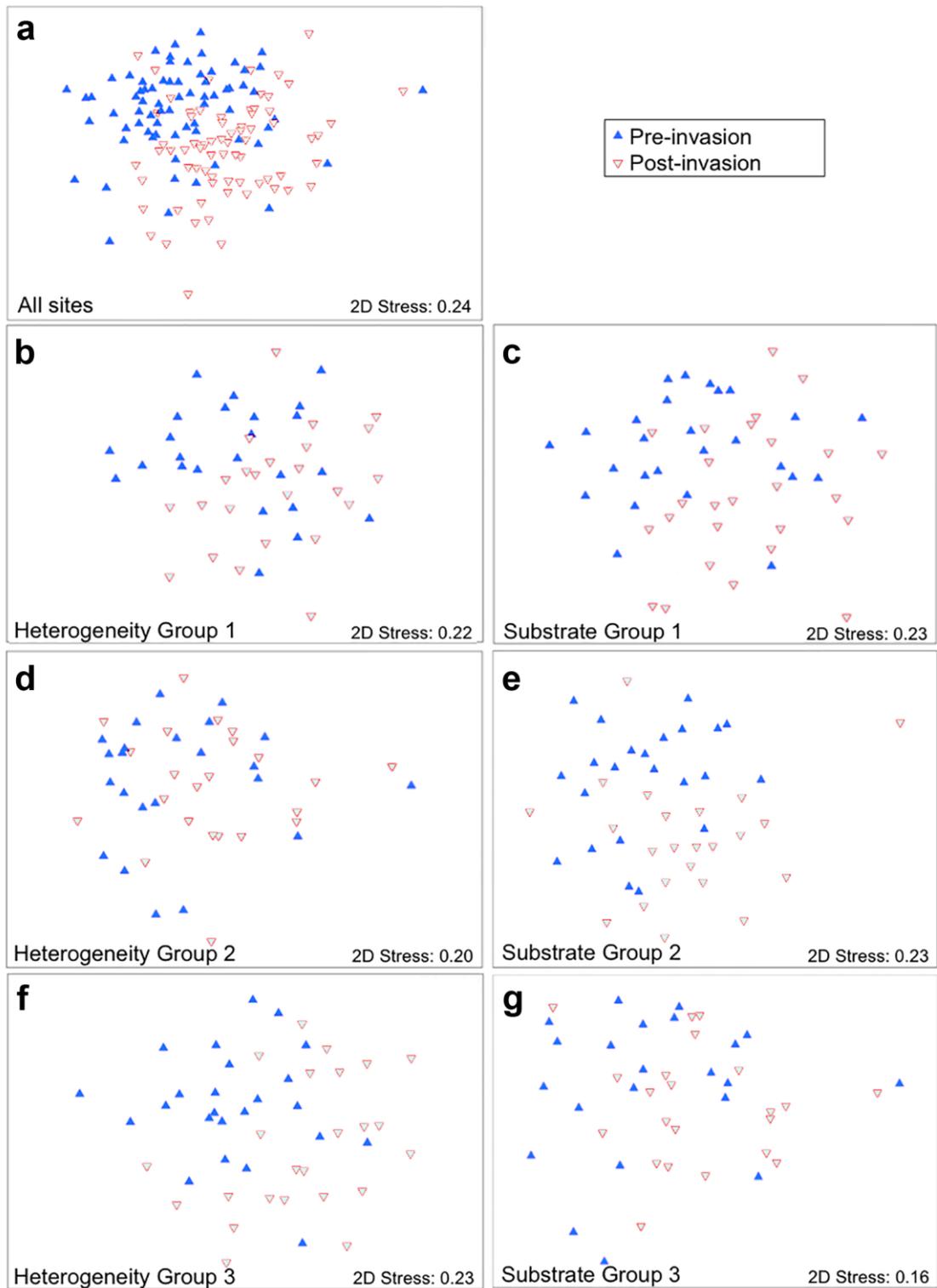


Figure 6.3a-g. Non-metric dimensional scaling centroid plots of benthic macroinvertebrate community composition for all sites, and sites split into subgroups based on their (i) habitat heterogeneity and (ii) percentage of coarse substrate (both groups: 1-3 represents low-high).

The degree of separation between pre- and post-invasion community compositions within subgroups indicated a statistically significant separation for Heterogeneity Group 3 (ANOSIM: $R = 0.333$, $p < 0.001$), and Substrate Group 2 (ANOSIM: $R = 0.329$, $p < 0.001$). Although the ITS analyses also highlighted differences in post-invasion index scores in Heterogeneity Group 1 and Heterogeneity Group 2, ANOSIM indicated that whilst there were statistically significant differences in community compositions in these subgroups, there was substantial overlapping ($R = 0.226$, $p < 0.001$ and $R = 0.152$, $p = 0.02$, respectively). A summary of all ANOSIM values is presented in Table 6.2.

Table 6.2. Analysis of Similarities (ANOSIM) of community composition pre-invasion versus post-invasion for all sites based on NMDS centroid plots using Bray-Curtis similarity coefficients (R), and sites split into subgroups based on their (i) habitat heterogeneity and (ii) percentage of coarse substrate; (both groups: 1-3: low-high).

	R	p
All sites	0.232	$p < 0.001$
Heterogeneity Group 1	0.226	$p < 0.001$
Heterogeneity Group 2	0.152	$p = 0.02$
Heterogeneity Group 3	0.333	$p < 0.001$
Substrate Group 1	0.199	$p < 0.001$
Substrate Group 2	0.329	$p < 0.001$
Substrate Group 3	0.165	$p = 0.02$

SIMPER identified that nine of the 10 taxa most responsible for causing the differences in the subgroups pre- and post-invasion community compositions, were identical, with consistent increases in abundance of Hydrobiidae, Gammaridae, Oligochaeta, Baetidae, Chironomidae, Simuliidae and decreases in Sphaeriidae, Asellidae and Hydropsychidae (Table 6.3).

Table 6.3. Results of SIMPER analyses of community composition pre-invasion versus post-invasion, based on NMDS centroid plots using Bray-Curtis similarity coefficients, for all sites, and sites split into subgroups based on (i) habitat heterogeneity and (ii) percentage of coarse substrate; (both groups 1-3: low-high). (+) and (-) indicate whether family abundance typically increased or decreased in post-invasion periods.

All sites	Heterogeneity Group 1	Heterogeneity Group 2	Heterogeneity Group 3	Substrate Group 1	Substrate Group 2	Substrate Group 3
Hydrobiidae (+)	Hydrobiidae (+)	Gammaridae (+)	Gammaridae (+)	Hydrobiidae (+)	Hydrobiidae (+)	Oligochaeta (+)
Gammaridae (+)	Gammaridae (+)	Hydrobiidae (+)	Hydrobiidae (+)	Gammaridae (+)	Gammaridae (+)	Gammaridae (+)
Oligochaeta (+)	Baetidae (+)	Oligochaeta (+)	Oligochaeta (+)	Oligochaeta (+)	Oligochaeta (+)	Baetidae (+)
Baetidae (+)	Oligochaeta (+)	Baetidae (+)	Elmidae (-)	Asellidae (-)	Chironomidae (+)	Hydrobiidae (-)
Chironomidae (+)	Chironomidae (+)	Chironomidae (+)	Baetidae (+)	Chironomidae (+)	Baetidae (+)	Simuliidae (+)
Sphaeriidae (-)	Simuliidae (+)	Asellidae (-)	Caenidae (-)	Sphaeriidae (-)	Caenidae (-)	Chironomidae (+)
Simuliidae (+)	Asellidae (-)	Sphaeriidae (-)	Chironomidae (+)	Caenidae (-)	Sphaeriidae (-)	Sphaeriidae (-)
Asellidae (-)	Sphaeriidae (-)	Simuliidae (+)	Sphaeriidae (-)	Baetidae (+)	Elmidae (-)	Hydropsychidae (-)
Elmidae (-)	Hydropsychidae (-)	Hydropsychidae (-)	Simuliidae (+)	Elmidae (+)	Simuliidae (+)	Elmidae (-)
Hydropsychidae (-)	Elmidae (+)	Elmidae (-)	Hydropsychidae (-)	Simuliidae (+)	Hydropsychidae (-)	Asellidae (-)

6.6 Discussion

6.6.1 *Fine sediment*

Despite crayfish being considered biogeomorphic agents, the results of this study provide limited evidence of changes to deposited fine sediment conditions following crayfish invasions. Nevertheless, in agreement with recent research focused on rusty crayfish (*Orconectes rusticus*), which observed reduced accumulation of fine sediment in invaded streams (Albertson and Daniels 2016); two of the subgroup analyses demonstrated statistically significant, low magnitude declines in fine sediment (approximately 10 percentage points). Declines in deposited fine sediment may be the result of crayfish activity (e.g. foraging, general movement) on the streambed, mobilising deposited fine sediment (Harvey et al. 2014, Albertson and Daniels 2016, Cooper et al. 2016, Rice et al. 2016). The lack of a consistent effect on fine sediment in the analysis of all sites, and across subgroup analyses, suggests that the influence of signal crayfish on fine sediment may be context dependent, perhaps confounded by site-specific characteristics, such as local bank biophysical properties (Faller et al. 2016), affecting fine sediment inputs associated with burrowing in river banks (Harvey et al. 2014). Other factors, such as site-specific changes to flow dynamics and catchment land use over time, may also be confounding the time series analysis of substrate composition (Allan 2004, Dewson et al. 2007). In order to understand these complex interactions between invaders and receiving ecosystems, beyond-“Before-After, Control-Impact (BACI) study designs may prove helpful, where suitable uninvaded control sites can be identified. Nevertheless, a more objective and standardised approach to quantifying fine sediment may be required to accurately reflect invader impacts (see Section 6.6.6). It may also be necessary to utilise more detailed data on multiple parameters, by conducting large scale targeted field studies, or utilising and combining opportunistic data analysis of multiple existing datasets.

6.6.2 *Biomonitoring tool outputs*

Results from this study suggest that signal crayfish invasions may influence the scores from sediment-biomonitoring tools. In agreement with previous work (Mathers et al. 2016b), the PSI_{fam} index was marginally inflated in post-invasion periods in the overall analysis, as well as in a number of subgroup analyses. The $E-PSI_{fam69}$ index is slightly less affected, showing no inflation in the overall analysis, and changes of lower magnitude (compared to PSI_{fam}) in the subgroup analyses. Importantly, the relatively low magnitude changes to both biomonitoring tool scores did not coincide with the expected alterations to fine sediment conditions. This suggests that changes to scores in post-invasion periods may not be the result of genuine geomorphic effects of crayfish. Instead, the changes to community composition (indicated by biomonitoring tool scores) may be the result of consumptive and/or non-consumptive effects of crayfish predation (Sih et al. 2010, Dorn 2013), and/or indirect effects, such as altering predator-prey dynamics of native fauna or modifying other aspects of the habitat (Byers et al. 2010). Nevertheless, the effect estimates for both indices were relatively small (maximum of 10.1 index points) and are unlikely to result in an incorrect diagnosis of sediment pressure (or lack of).

Similar to the fine sediment analyses, the lack of a consistent change to biomonitoring tool scores across all sites and subgroups, suggests that site-specific characteristics may influence the degree to which biomonitoring tools are affected by signal crayfish. This may be partly due to the longitudinal location of the site (i.e. upland to lowland), which is likely to influence the availability of food items, with lowland streams typically having a greater abundance of preferential prey (e.g. molluscs and leeches), compared to upland rivers (Vannote et al. 1980, Charvet et al. 2000), which may result in lowland rivers being more susceptible to biomonitoring tool score inflation. Similarly, Klose and Cooper (2012) also found crayfish impacts to be site-specific.

The disparity between post-invasion PSI_{fam} and $E-PSI_{fam69}$ scores may be the result of

the different methods of index development and calculation. The development of the E- PSI_{fam69} index also involved the removal of a number of “sensitive” families from its calculation (which are still included in PSI calculations), due to their indifference to reach scale estimates of fine sediment (Turley et al. 2016).

6.6.3 *Habitat heterogeneity*

The subgroup of sites with more homogeneous substrate was predicted to be the most probable to exhibit differences between pre- and post-invasion biomonitoring outputs as a result of crayfish predation. These sites are likely to afford the least resilience to crayfish predation, providing fewer refugia (Brown and Lawson 2010), and are likely inhabited by a community of fewer species (Tews et al. 2004). In partial agreement with this prediction, the subgroup had a small, but statistically significant decrease in post-invasion E- PSI_{fam69} scores, and analysis of community composition indicated dissimilarities between pre- and post-invasion periods. However, the effect estimate and confidence interval with a lower limit of almost zero, suggests that the magnitude of the effect on E- PSI_{fam69} is low.

The PSI_{fam} index exhibited inflated scores of low magnitude in the post-invasion period at sites with moderate and high habitat heterogeneity, but not at those with low heterogeneity. Heterogeneous substrate is often associated with zones of high velocity and well oxygenated water, areas that are typically inhabited by a high proportion of rheophilic and relatively fast-moving taxa (Dunbar et al. 2010a), many of which are rated as highly sensitive to fine sediment. The inflated post-invasion scores and observed shifts in community composition at these sites may be the result of the crayfish having difficulties capturing fast-moving taxa, and instead selectively preying on slower moving taxa (many of which are rated as tolerant of fine sediment) resulting in a higher PSI_{fam} score. A number of other studies have also suggested that more

mobile taxa dominate in areas where crayfish are abundant (Nyström et al. 1999, Usio and Townsend 2004, Grandjean et al. 2011).

6.6.4 *Coarse substrate*

Longitudinal gradients in rivers and streams, and the associated transition from coarse substrate to fine sediment are important influencing factors of macroinvertebrate community composition (Minshall 1984). As discussed in Section 6.6.2, the longitudinal position of the site is likely to influence the availability of preferred prey. Sites in this study with an intermediate percentage of coarse substrate appear to be the most affected by crayfish invasions in terms of their PSI_{fam} scores, $E-PSI_{fam69}$ scores, and community composition. This effect may be the result of similar processes to those hypothesised for the observed changes to PSI scores at sites with high habitat heterogeneity. The sites in this subgroup (Substrate Group 2) have relatively equal proportions of coarse substrate and fine sediment, and as a result, both sediment-sensitive and sediment-tolerant taxa are likely to be well represented in the macroinvertebrate community. Selective crayfish predation on slower moving, sediment-tolerant taxa would therefore result in inflated index scores.

6.6.5 *Community composition*

Invasive crayfish have been shown to alter native macroinvertebrate communities, reducing diversity and biomass, particularly of gastropods and bivalves (Klocker and Strayer 2004, Crawford et al. 2006, Dorn 2013). The consistent declines in Sphaeriidae (bivalve) abundance in post-invasion periods compared with pre-invasion periods in this study, agree with this previous research. The sedentary nature of this taxon is likely to result in a poor ability to evade predation, making them easy prey items. In contrast, a number of taxa (i.e. Hydrobiidae, Gammaridae, Oligochaeta, Baetidae,

Chironomidae, and Simuliidae) were consistently identified as having a greater abundance in post-invasion periods. These taxa are likely to have biological traits that allow them to persist in the presence of crayfish (e.g. high mobility, high fecundity, multivoltine), and/or have innate or inducible defence mechanisms. For example, *Gammarus pulex* (Gammaridae) have been shown to increase locomotion, vertical migration and drift in the presence of predators (Haddaway et al. 2014). *Potamopyrgus jenkinsi* (Hydrobiidae) have also been shown to increase vertical migration in the presence of foraging crayfish (Haddaway et al. 2014).

6.6.6 *Fine sediment quantification*

As discussed in previous chapters, deposited fine sediment is a challenging environmental characteristic to quantify. It is unclear which sediment quantification technique is the most biologically relevant (Sutherland et al. 2012), or at which spatial or temporal scale fine sediment should be quantified, to detect modifications arising from crayfish activity (Harvey et al. 2011). Fine sediment values used in this study were collected at the 'reach' scale, using the visual estimate technique, that is likely to have a degree of biological relevance as it relates to niche availability (Turley et al. 2017). The technique is intended as a rapid assessment approach, but has been criticised for its subjectivity and the associated operator error that can result in a low precision (Wang et al. 1996). In this study it was anticipated that the standardised training provided to the operators responsible for carrying out the visual estimate would have reduced the subjectivity and optimised the precision of the technique (Roper and Scarnecchia 1995, Poole et al. 1997).

6.6.7 Limitations

In addition to the challenges concerning the quantification of fine sediment conditions, there are other noteworthy limitations of this study. The modelling approach and structure may have resulted in an over- or under-estimation of differences between pre- and post-invasion periods. Nevertheless, it was necessary to define an *a priori* model, and the model utilised in this study was based on invasion ecology theory and available knowledge of signal crayfish invasion dynamics (Sahlin et al. 2010, Ricciardi et al. 2013). In addition, the objective approach to identifying the date of invasion may have resulted in an underestimation of the differences between pre- and post-invasion periods. Due to the challenges of detecting crayfish at low densities (Peay 2003), it is possible that the sites were invaded prior to the first detection, however, at low densities their impacts are likely to be less significant. Lastly, although the *lme* model residuals showed some signs of heteroscedasticity, which may have influenced estimates of statistical significance, the effect estimates are of greater interest, and were broadly similar to the *lqmm* results (which have less distributional assumptions) presented in Appendix D.

6.6.8 Reliability of biomonitoring in the presence of invasive species

With current water legislation placing a strong emphasis on the use of biomonitoring (Birk et al. 2012), and aquatic biological invasions expected to increase in the future (Early et al. 2016), an understanding of the influences of invasive species on native biodiversity and their effect on the performance of biomonitoring tools, is crucial. The context dependency shown in this study, highlights the need for investigation of the potential for site-specific effects caused by invasive species (Klose and Cooper 2012). Invader impacts are likely to be species-specific, impacting receiving communities and biomonitoring schemes to varying degrees. Knowledge of the invaders biological traits and ecological preferences (in their native range) may help focus research efforts, on

those species most likely to be impacting on biodiversity and biomonitoring (Pyšek et al. 2012). Additionally, investigation of the effects of other pressures on invader impacts and establishment rate/success (Didham et al. 2007, Diez et al. 2012) is important for determining the reliability of biomonitoring tools in invaded ecosystems.

In order for the impacts of invasions to be realised, data need to be available for both pre- and post-invasion periods at a suitable resolution to capture the natural community variation and sampling variation of the outcome variable of interest, and ideally for a length of time that exceeds the successful establishment of the invasive species. However, studies of this temporal scale are often considered prohibitively expensive. The use of regulatory agency data that spans wide geographic areas, and which is often collected over multiple years, represents a coarse, but comparatively rapid and low-cost approach that can help to inform the protection and management of freshwater ecosystems (Dafforn et al. 2016).

6.7 Conclusion

The results of this study highlight the potential context dependency and variability of invader impacts, with the effect of crayfish invasions on biomonitoring tool outputs, fine sediment, and community composition, appearing to vary between sites. It is recommended that pressure-specific biomonitoring approaches be utilised alongside the full range of biomonitoring tools available to the user, to assist with evaluating the most probable causes of ecological degradation in rivers and streams.

Further research is needed to disentangle the multitude of possible factors, such as the presence of multiple pressures (e.g. channel modification, water quality and climate change), and extreme events (e.g. droughts and floods), which may facilitate more severe impacts on biodiversity following invasions. Conversely, it is also important to identify the characteristics and mitigation measures that can increase ecosystem

resilience to invasions. Understanding the mechanisms by which invasion impacts are facilitated or mitigated is also crucial for the management and protection of aquatic ecosystems. Lastly, a more objective and standardised technique for quantifying fine sediment is likely to help improve our understanding of the geomorphic activities of crayfish on deposited fine sediments, as well as any potential influence these activities may have on sediment-biomonitoring. One such approach is developed and evaluated in the following chapter (Chapter 7) of this thesis.

Chapter 7 A novel technique for quantifying submerged deposited fine sediment in rivers and streams

Part of the content of this chapter is reproduced from the following:

Turley, M.D, G. Bilotta, G. Arbocute, R. Chadd, C. Extence, and R. Brazier. 2017. Quantifying Submerged Deposited Fine Sediments in Rivers and Streams Using Digital Image Analysis. River Research and Applications DOI: 10.1002/rra.3073.

Contributions: MT led the analysis and writing of this chapter (and publications), supported and supervised by GB (primary supervisor), CE & RB (external supervisors). GA was the independent operator for the Digital Image Analysis. RC helped with the identification of macroinvertebrates and gave advice on invertebrate ecology.

7.1 Abstract

The ability to quantify deposited fine sediment in rivers and streams is critical to understanding fine sediment dynamics and their ecological effects, which in turn, is required for the development of robust sediment-specific biomonitoring tools. One widely used method of quantifying fine sediment is the visual estimate technique, which relies on subjective estimates of particle size and percentage cover. A more objective quantification technique would be beneficial for regulatory agencies, and those involved in river restoration and the management of the anthropogenic activities responsible for modifying fine sediment. In this chapter, two novel alternative approaches are presented, based on non-automated Digital Image Analysis (DIA), designed to reduce the subjectivity of submerged, surficial fine sediment estimates and provide a non-destructive and verifiable record of the conditions at the time of sampling. The DIA methods were tested across ten systematically selected,

contrasting temperate stream and river typologies, over three seasons of monitoring. The resultant sediment metrics were strongly, positively correlated with visual estimates (both $r_s = 0.72$, $p < 0.01$), and moderately to strongly, negatively correlated with the Empirically-weighted Proportion of Sediment-sensitive Invertebrates (E-PSI_{mixed}) index ($r_s = -0.64$ and $r_s = -0.75$ $p < 0.01$); suggesting some degree of biological relevance. The spatial variation of fine sediment between transects is also investigated to provide an insight into the optimum number of transects over which to collect digital images. The chapter provides “proof of concept” for the DIA techniques, which have the potential to be valuable tools for application in numerous areas of river research and management, where a non-destructive, less subjective and verifiable method is desirable.

7.2 Introduction

As highlighted in Chapter 2, fine sediment (organic and inorganic particles; < 2mm) is an essential component of freshwater ecosystems (Owens et al. 2001). Nonetheless, anthropogenic activities can lead to modified levels of fine sediment delivery to surface waters, impacting the physical, chemical and biological characteristics of these ecosystems (Wood and Armitage 1997). The ability to quantify deposited fine sediment is critical for developing a sediment-specific biomonitoring tool. However, it is also necessary if we are to understand the impacts it can have, successfully manage the anthropogenic activities that are responsible for modifying it, and determine the effectiveness of mitigation and river restoration techniques. A number of methods for quantifying both surficial (Table 7.1) and subsurface fine sediment have been documented. Some of the most commonly used methods by both researchers and regulatory agencies are rapid assessments (Faustini and Kaufmann 2007), often comprising visual estimates of substrate composition (Sennatt et al. 2006, Descloux et al. 2010). These assessments involve operators estimating the surficial fine sediment

in terms of the percentage cover of different particle sizes across a site. Frequently, the particle size classifications follow the Wentworth system (Wentworth 1922): Boulders (≥ 256 mm), cobbles (64 – 256 mm), gravels/pebbles (2 – 64 mm), sand (< 2 mm, ≥ 0.06 mm), silt and clay (< 0.06 mm) (Bain and Stevenson 1999, Clapcott et al. 2011).

Table 7.1. Various approaches used globally to quantify submerged, surficial fine sediments in rivers and streams.

Approach	Description	Reference
Particle counts	Wolman pebble count. Particles are selected at random by sampling using toe-to-heel spacing and selecting the first particle touched by a finger at the toe of the operators boot. Transects between bankfull widths within habitats of interest (or a zig-zag pattern), are followed until 100 particles (or desired number) are selected.	(Wolman 1954, Bevinger and King 1995, Diplas and Lohani 1997)
	Wolman pebble count (modified). Grid-by-number methods using a measuring tape, wire mesh or frame with elastic bands to select particles.	(Kellerhals and Bray 1971, Bunte and Abt 2001)
	Wolman pebble count (modified). The toe-to-heel approach is used in conjunction with a clay disc and piston, which is pressed onto the streambed to sample fine sediment.	(Fripp and Diplas 1993)
In-stream visual estimate	A measuring tape is placed between the beginning and end of a transect. At 0.3 m increments, the dominant sediment class over the length is recorded.	(Platts et al. 1983)
	Using an underwater viewer, the percentage cover of fine sediment is estimated.	(Matthaei et al. 2006)
Bankside visual estimate	Substrate composition percentages (boulders, cobbles, gravels/pebbles, sand, silt and clay) are estimated following a visual inspection of the reach from the stream bank.	(EU-STAR 2004)

Approach	Description	Reference
	The percentage of fine sediment (< 2 mm) is estimated from the stream bank.	(Clapcott et al. 2011)
Photographic image analysis	Above water image capture. Areas of substrate are photographed using a photographic film camera, an underwater viewer, and a structure to isolate the area of streambed. Photographic transparencies were then projected at three times life size, onto a grid with 400 squares. The predominant particle size in every fourth square was then recorded.	(Gee 1979)
	Similar to the technique used by Gee (1979), with the addition of a digitizing program to obtain the particle size distribution.	(Ibbeken and Schleyer 1986)
	Areas of substrate are photographed from above the water using a photographic film camera, and underwater viewer. Photographic transparencies are digitized and analysed using Geographic Information Software.	(Whitman et al. 2003)
	Images are collected using a modified camera with underwater housing and a light ring. The resulting images are approx. 0.02 m across, and are analysed using an autocorrelation algorithm to determine grain sizes.	(Rubin et al. 2007)
Artificial mats	Artificial turf mats (0.15 m x 0.10 m) are fixed to the streambed and left for three weeks. Mats are carefully retrieved and placed in zip-lock bags, and returned to the laboratory where the sediment is washed out, sieved, and collected for drying and weighing.	(Von Bertrab et al. 2013)

Approach	Description	Reference
Adhesive plates	A plate is covered with a thin layer of adhesive material (e.g. clay) and is pressed onto the streambed. The sample is then wet-sieved to remove the clay.	(Fripp and Diplas 1993)
Re-suspension	A steel cylinder measuring 1 m is pushed 0.1 m into the streambed. The water within the cylinder is agitated, artificially suspending the surficial fine sediment, allowing for three 0.5 L samples to be collected. This process is carried out at three or more “representative” sites, and samples are used to determine the mean mass (g m^{-2}) of sediment released.	(Lambert and Walling 1988)
	Modified from Lambert and Walling (1988), using a steel cylinder measuring 0.75 m. Samples of 0.05 L are collected from two “depositional” and two “erosional” patches at each site.	(Duerdoth et al. 2015)
Embeddedness	Qualitative assessment using five categories relating to the percentage that large particles were covered by fine sediment.	(Platts et al. 1983)
	Qualitative assessment of embeddedness using three categories: 1 = lying loosely on top of the bed, 2 = partly covered by surrounding substratum, 3 = well buried in the surrounding substratum or firmly wedged in by surrounding stones.	(Matthaei et al. 1999)
	Assessment of embeddedness over 11 transects (55 particles) by estimating the percentage embeddedness of each particle.	(Peck et al. 2002)

Surficial percentage cover estimates often rely on the subjective ability of the operator to identify particle sizes and percentage cover of these particles (Faustini and Kaufmann 2007). Whilst not necessarily an indicator of subsurface fine sediment, percentage cover is likely to be important in terms of determining the biological implications of altered fine sediment dynamics, as it is concerned with the surface “drape”, which can lower the oxygen availability in the benthos, and reduce niche availability and the quantity of forage and refugial habitat (Sutherland et al. 2010).

Research has investigated the quality of these sediment quantification methods (Roper et al. 2002, Sennatt et al. 2006, Whitacre et al. 2007, Clapcott et al. 2011). In terms of precision (i.e. the degree of closeness between repeated measures), visual estimates by ten operators have been shown to vary by up to 40 percentage points for sites with the same Wolman pebble count (Clapcott et al. 2011); although this is likely to be an extreme example as operators received no prior training on the technique. Furthermore, the Wolman pebble count has been shown as biased against small particles (Marcus et al. 1995, Diplas and Lohani 1997). In terms of accuracy (i.e. closeness to the true value), the ‘true’ value for substrate composition in a stream is generally unknown (Sutherland et al. 2010), although some authors have used ‘measured’ methods in place of a ‘true’ value. However, it is important to remember that the ‘measured’ method is not necessarily closer to the ‘true’ value, and a difference between the estimated and ‘measured’ methods will always occur due to the different methodologies measuring slightly different aspects of the substrate and any fine sediment.

An alternative to the conventional method of visual estimates of substrate composition is the use of image-based techniques. Photography has been used to some extent by geomorphologists and sedimentologists for over 35 years (Adams 1979), with recent work utilising technological advances to determine grain size characteristics, using (i) automated grain size analysis of images and (ii) geostatistical techniques and empirical

calibration, mainly of exposed gravel bed rivers (Rice 1995, McEwan et al. 2000, Butler et al. 2001, Sime and Ferguson 2003, Carbonneau et al. 2004, Graham et al. 2005a, Graham et al. 2005b, Warrick et al. 2009). Both Rubin et al. (2007) and Buscombe et al. (2010) have applied their techniques underwater, the latter in controlled conditions. Whilst these techniques provide opportunities to process large numbers of images and obtain particle size distributions, the identification of fine sediments, particularly submerged, and/or cohesive sediments, is often limited due to technological limitations and image resolutions (Graham et al. 2005a, Bertoldi et al. 2012) as well as a requirement for well-defined grain boundaries (McEwan et al. 2000, Graham et al. 2005a).

In addition to the precision and accuracy of each technique, the biological relevance of the resulting sediment metric is often of great concern. In the EU, whilst sediment pressure is not directly legislated, as part of the EU Water Framework Directive member states are required to achieve “Good Ecological Status” in surface waters. Given the ecological impacts of fine sediment (reviewed in Bilotta and Brazier 2008), a method of quantifying fine sediment that provides a biologically relevant metric is therefore highly desirable.

The aim of this chapter is to provide “proof of concept” for two novel non-automated, Digital Image Analysis (DIA) methods for quantifying submerged, surficial deposited fine sediment, building on the work from previous authors, many of whom were attempting to characterize complete grain size distributions. Crucially the techniques have to be (i) applicable to fine sediments on submerged river/streambeds, (ii) more objective and verifiable than existing visual estimate methods, and (iii) simple to carry out requiring little specialist software or training. The results from these new procedures are compared with those based on visual estimates. The precision of each method is assessed using an independent operator, and the biological relevance is evaluated using the relationship between each sediment metric and the invertebrate

community composition, quantified using the sediment-specific biotic index that was developed in Chapter 4 and published in Turley et al. (2016). Between-transect and between-season variation is also assessed to provide an insight into fine sediment spatial variation, and the likely ability of the DIA sampling design to suitably characterize fine sediment conditions across the site.

7.3 Methods

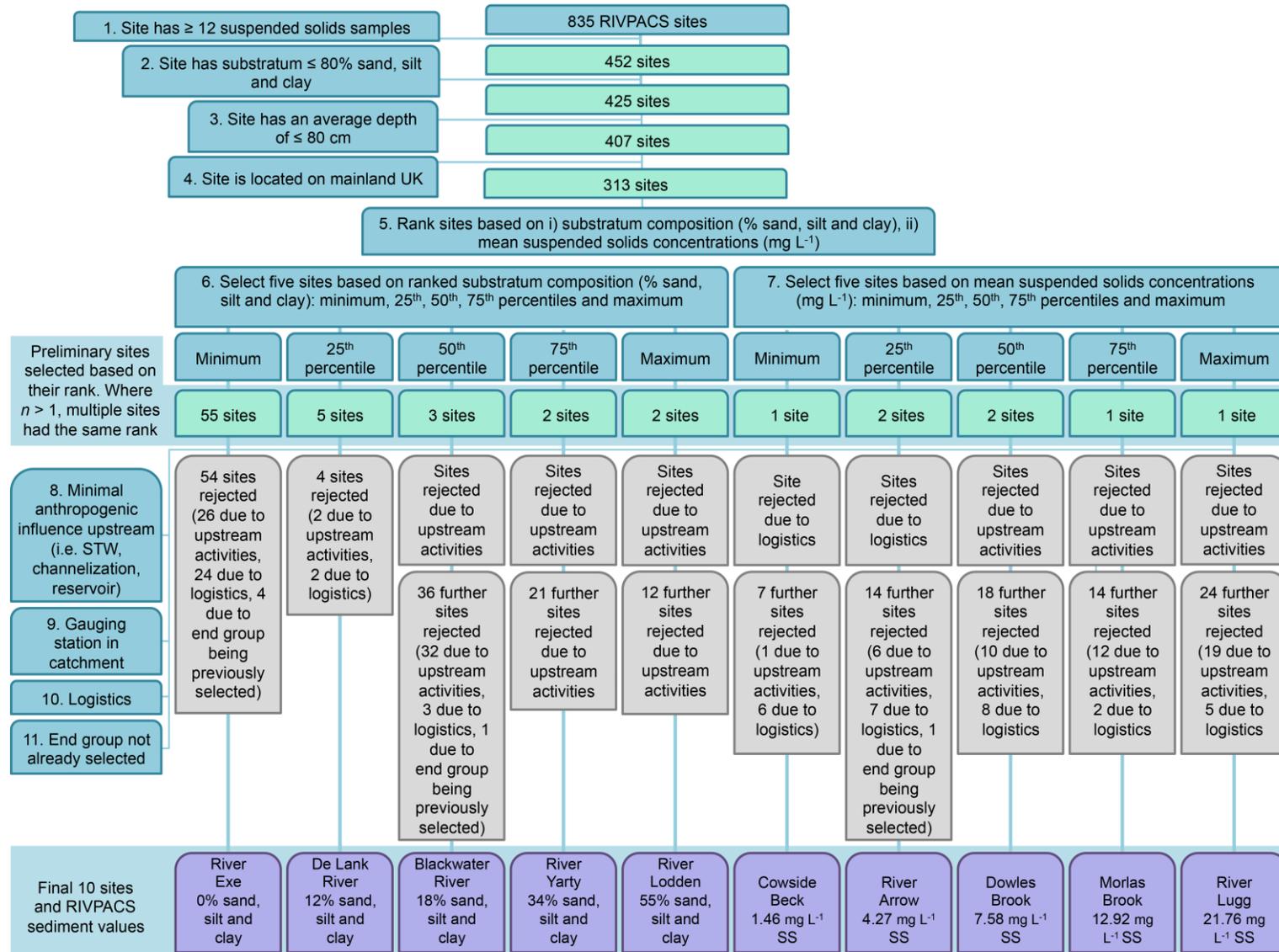
7.3.1 Site selection

Ten sites were selected from the RIVPACS database (described in Chapter 3 - Section 3.3.1 and Chapter 4 - Section 4.3.1) of 835 minimally impacted sites on rivers and streams located throughout the United Kingdom. The ten sites represented a range of different river types, with contrasting environmental characteristics; depths between 0.05 m and 0.80 m and importantly, a range of fine sediment cover (0% - 55%, based on visual estimates).

The site selection process involved both stratified and systematic stages to ensure sites with a range of different deposited fine sediment conditions and suspended solids concentrations, were selected with minimal bias (Figure 7.1). Briefly, this involved removing those sites with average depths > 0.80 m (wadeable limit), > 80% fine sediment (due to the difficulties of carrying out the standardised invertebrate sampling in such conditions), and annual averages of suspended solids based on fewer than 12 measurements. The remaining sites were ranked in terms of (i) the percentage of the substrate comprised fine sediment (based on previous visual estimates), and (ii) their mean annual suspended solids concentrations. Those sites that represented the minimum, 25th, 50th, 75th and 100th percentile values were primarily selected to progress to the next stage in the selection process (i.e. five sites for deposited fine sediment, five sites for suspended solids). Next, beginning with the sites that were selected for having

the highest sediment values, satellite imagery was used to determine whether the sites met a number of criteria. These criteria were applied to the preliminary sites to minimise selection bias, and to ensure that sites were relatively unimpacted by point source organic pollution (e.g. no sewage treatment works, significant urban/rural dwellings upstream), physical modifications (e.g. no obvious channelization) or arable agriculture (and the associated fertilisers and pesticides), to reduce the number of pressures with the potential to influence the macroinvertebrate community. Sites that failed to meet the criteria were rejected, and the next ranked site was put through the same criteria, until ten sites were found that met all criteria.

Figure 7.1. Schematic diagram of the stratified and systematic site selection process, highlighting the criteria applied and the ten selected sites.



The characteristics of these systematically selected sites are shown in Table 7.2, and their locations are illustrated in Figure 7.2. In this study, each site consisted of a short river reach approximately 10 metres in length, over which the visual estimates, digital images, and macroinvertebrates were collected.

Table 7.2. Characteristics of the ten stream sites selected for their deposited fine sediment conditions (white cells), and suspended solids concentrations (grey cells) based on data from the RIVPACS IV database.

Environmental variables	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9	Site 10
Altitude ^a (m)	364	205	75	25	66	220	325	70	50	97
Slope ^a (m m ⁻¹)	0.01	0.008	0.008	0.004	0.003	0.02	0.02	0.007	0.03	0.003
Average width ^b (m)	1.9	5.8	3.9	8.0	11.8	7.5	1.1	7.0	2.7	15
Average depth ^b (m)	0.08	0.30	0.14	0.25	0.65	0.28	0.09	0.08	0.15	0.47
Alkalinity ^c	24.4	6.8	61.8	111.7	247.0	103.3	66.9	32	154.1	174.3
Fine sediment ^b (%)	0	12	18	34	55	0	9	4	7	16
Average suspended solids concentration (mg L ⁻¹) ^c	5.1	2.4	14.1	17.2	5.2	1.5	4.3	7.6	12.9	64.4

Environmental variables derived from: ^a Map data, ^b mean of three seasonal measurements, and ^c averages of 12 monthly measurements.

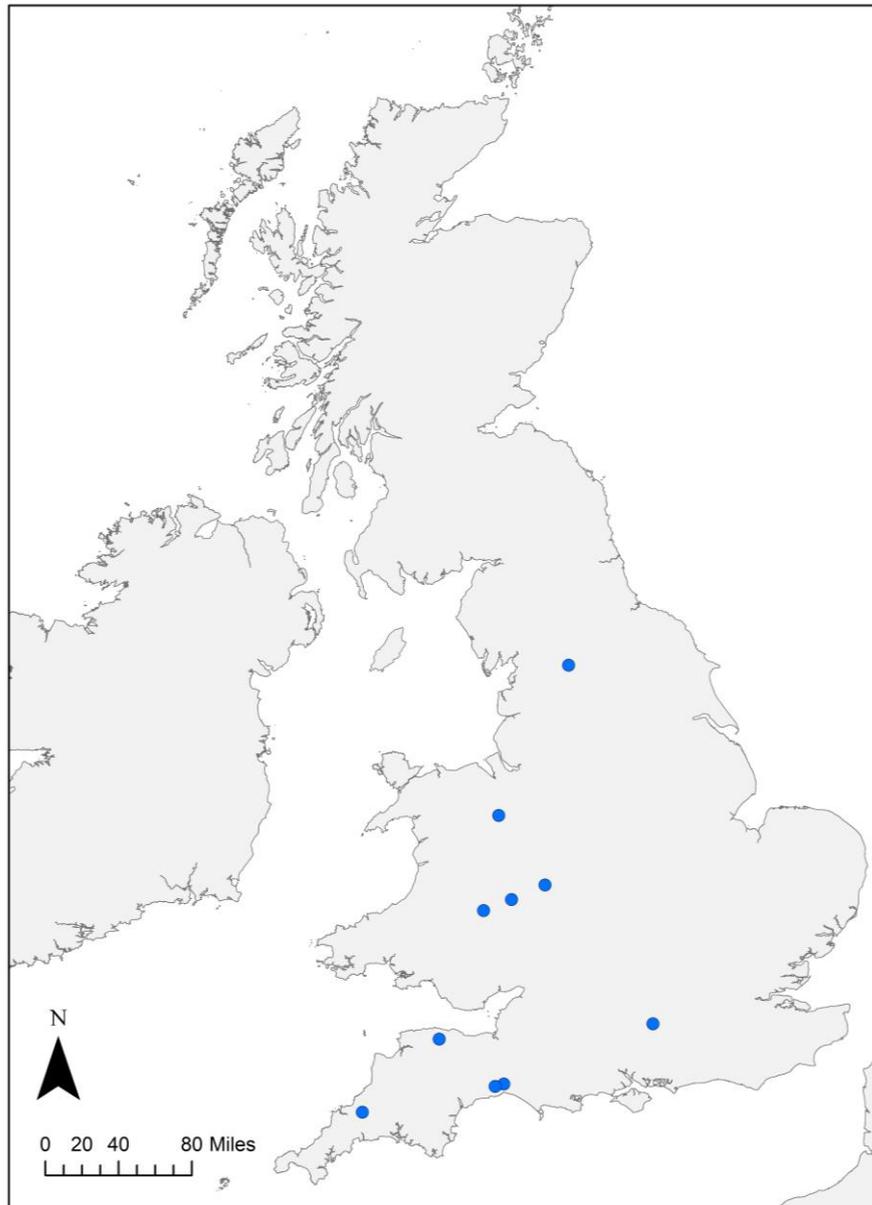


Figure 7.2. Locations of the ten sampling sites throughout the UK, which were selected using a stratified and systematic process.

7.3.2 *Visual estimates*

The percentage of fine sediment cover was estimated over the site, using the visual estimate technique, following the UK standard protocol (EU-STAR 2004) described in Chapter 3 (Section 3.3.1.2). Briefly, this involved carrying out observations from the stream bank, where necessary probing areas of substrate, and estimating the

percentages of substrate composition. The overall percentage of fine sediment (< 2 mm) was recorded to the nearest 5%.

7.3.3 Digital image collection

In order to reduce the potential for sampling bias (i.e. selecting areas of high or low levels of fine sediment), each site was photographed systematically using equidistant transects. Five transects were setup at each site, beginning at the downstream end of the site and positioned every two metres (Figure 7.3), incorporating riffles, runs, and glides where present. Three sampling locations were positioned on each transect at $\frac{1}{4}$, $\frac{1}{2}$, and $\frac{3}{4}$ of the wetted channel width. Each site was sampled over three seasons (spring, summer and autumn) in the same year (2014), with the exception of Site 8 during summer and autumn visits when the water was extremely turbid and image collection was not possible.

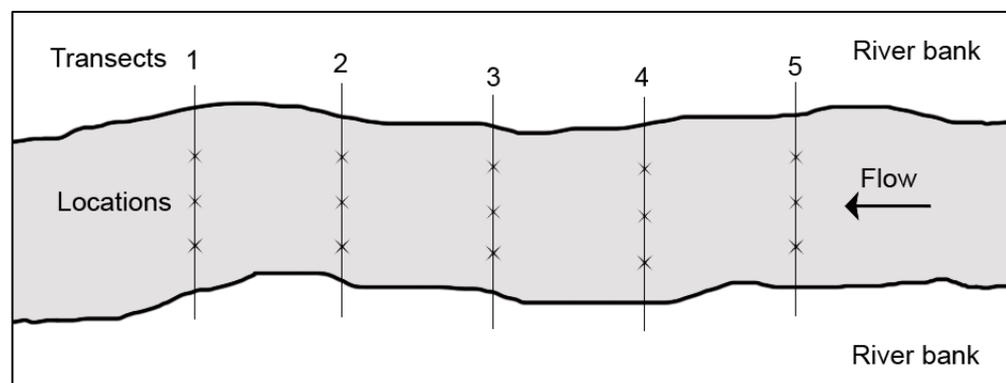


Figure 7.3. Schematic diagram of the sample design (five equidistant transects perpendicular to the flow direction, each with three equidistant survey points) used for collecting digital images of deposited fine sediment on streambeds.

A relatively low cost (< 500 GBP) 16.1 megapixel waterproof digital camera (*Panasonic DMC-FT5*) was used to eliminate the need for a structure to protect the camera from water. In addition to being waterproof, another important feature was the camera's reported performance under low light conditions, and its minimum focal distance. Both of these were important considerations as photographs were to be taken within the water column, which would result in reduced natural light and a limited distance between the camera and the streambed.

At each site and on each visit the camera was submerged and test photographs were taken and reviewed in-situ to determine the approximate, optimum distance between the camera and the streambed. The camera was held from a vertical position to capture images of the substrate in plan view. The optimum distance was deemed as that which resulted in an image, which captured the maximum area of the streambed (limited by the water depth) whilst providing a suitably clear image for later analysis. In streams that are relatively turbid this can result in a small area (< 0.01 m²) of streambed being captured in each image. Beginning at the downstream end of the site, so as not to disturb the substrate prior to image capture, an image was taken at each sampling location. Image quality was briefly reviewed before moving on to the next sampling location. The distance between the streambed and the camera lens was measured using a graduated measuring pole with 0.01 m increments, and was recorded for each image. The minimum and maximum distances between the camera lens and the streambed in this study were 0.02 m and 0.76 m, respectively. Once the images had been collected, they were returned to the laboratory and uploaded for processing.

7.3.4 Image processing

The image processing was kept to a minimum in comparison with the automated processes described by previous authors, and was carried out using Adobe Photoshop Elements 13, but can also be conducted using image editing freeware (e.g. Pixlr 3.0). The relationship between camera height, and the area (m²) of streambed captured in the image was determined using a tripod setup in the laboratory. Images of a one-metre rule were captured with the camera positioned at 0.05 m height increments between 0.02 m and 0.80 m, and the length of the rule captured in each image, at each height was recorded. Using these data, a formula was derived using linear regression to calculate the length of streambed captured at each camera height increment between 0.02 m and 0.80 m using the Panasonic DMC-FT5, assuming a planar bed (Equation 7.1). The length of each image collected in the field was then resized so that they represented the actual captured area of the streambed, and their resolution was set to 180 pixels per inch (ppi).

$$L = 1.223h + 0.018$$

Equation 7.1. Formula used to calculate the length of streambed (L ; metres) captured at a known camera height (h ; metres).

7.3.5 Estimate-based Digital Image Analysis (eDIA)

Images collected for Digital Image Analysis were used by the operator to estimate the percentage of fine sediment. This involved a grid layout (10 x 10) being placed over the image in Photoshop (each square of the grid representing 1% of the area in the image) to aid a systematic summation. In order to assist in the identification of fine sediment, the *Brush Tool* was set to 14 pixels as this size brush represents approximately 2 mm at the resolution of the saved images, allowing a comparison between the cursor and particles. Where necessary, the contrast and brightness of the image was manipulated

to improve the clarity of dark areas of substrate (e.g. interstices). The percentage of fine sediment was then estimated to the nearest 1% and recorded. This process required less than five minutes per image (i.e. < 75 minutes per site).

7.3.6 Software-based Digital Image Analysis (sDIA)

Using the images collected in the field, areas of fine sediment were highlighted and quantified. This involved firstly opening the resized images in Photoshop and selecting the foreground colour of 'ff0000' along with the Brush Tool set at 14 pixels. Moving the Brush Tool over the image, any particles that were less than the size of the cursor were then highlighted with the foreground colour (Figure 7.4) using either the *Brush Tool*, *Polygonal Lasso*, or in some cases the *Magic Wand Tool*. The *Magic Wand Tool* could only be used at a low tolerance (< 25 %) when the areas of fine sediment were suitably contrasted to the other particles surrounding it.

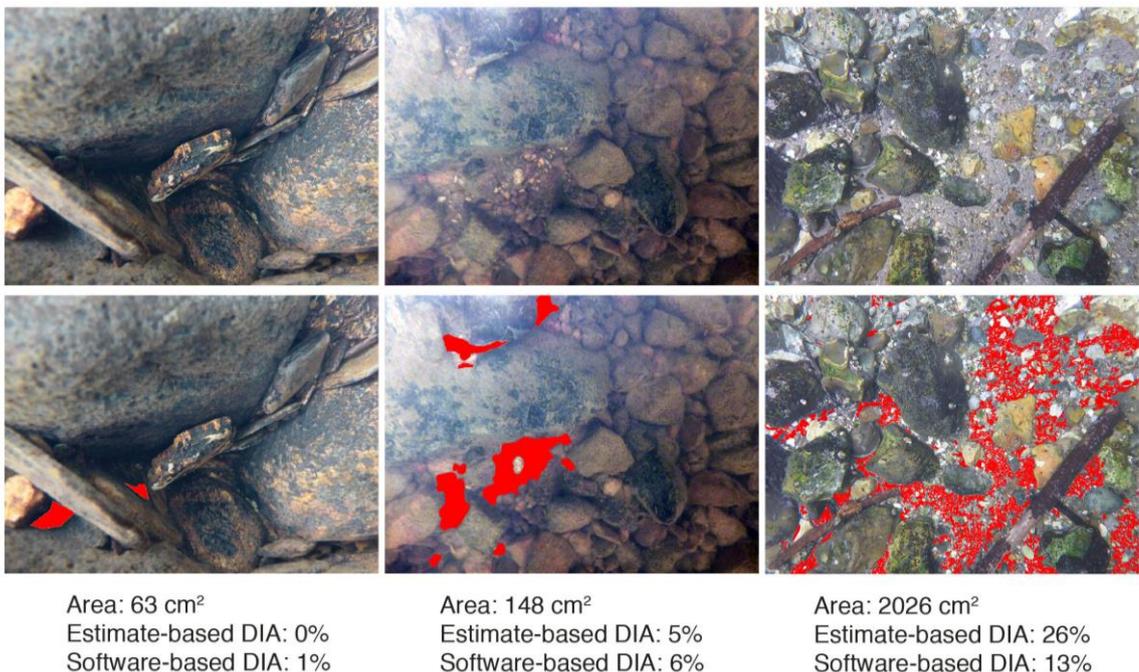


Figure 7.4. Examples of DIA images (top row) and these images with fine sediment highlighted (bottom row). Area of streambed, and fine sediment estimates using estimate-based DIA and software-based DIA are detailed.

Once the fine sediment had been identified and highlighted (requiring up to 30 minutes per image), the images were “saved for web”, as Portable Network Graphics (PNGs). The images were then uploaded to *PixelCount*, a freeware Google Chrome application that was developed to count the number of pixels of a specific colour (ff0000 - which is set prior to uploading the images). The application outputs the proportion of each image that matches the chosen colour. By converting this proportion to an area (m²) using the known area of the streambed in each image, the total area (m²) of fine sediment in all 15 images was divided by the total overall area in the images, and multiplied by 100 to obtain the overall percentage cover of fine sediment (Equation 7.2).

$$\% \text{ fine sediment} = \frac{\sum(a * p)}{\sum a} * 100$$

Equation 7.2. Formula to calculate the overall percentage cover of fine sediment in digital images, where *a* is the total area (m²) of streambed in the image, and *p* is the proportion of the image that is highlighted. In the numerator of the equation, the area in each image is multiplied by the proportion of highlighted fine sediment in the image, and the products are then summed. In the denominator, the areas of each image are summed. The equation takes into consideration that each image can capture a different size area of streambed, and as such, the same proportion of fine sediment in any two images may not represent the same size area.

7.3.7 Precision of Digital Image Analysis techniques

In order to evaluate the precision of the DIA techniques, an independent operator carried out duplicate analyses for a single season for the five sites that were selected based on their recorded substrate composition in the RIVPACS database. The exact seasonal sample that was analysed was randomly selected, and inter-operator

comparisons were made between the same sites and seasons. Whilst the first operator had knowledge of the sites (having visited the location and conducted visual estimates), the independent operator had no prior knowledge of the site characteristics. Brief training (1 hour) was provided using 10 trial images with a range of sediment covers, to guide the independent operator through the process of identifying, estimating and highlighting fine sediment in the images.

7.3.8 Fine sediment spatial variation

To evaluate the effectiveness of the image collection sampling design, as well as the spatial variation in fine sediment at the study sites, the between-transect variation in fine sediment values were assessed in terms of the 95% confidence intervals for the mean percentage of fine sediment based on five transects. This required the percentage of fine sediment cover in each one of the sites five transects to be calculated individually (using the three images in each transect) and averaged over the five transects.

7.3.9 Biological relevance

The biological relevance of the methods are assessed by comparing their Spearman's rank correlation coefficients with a sediment-specific biotic index; the mixed taxonomic level, Empirically-weighted Proportion of Sediment-sensitive Invertebrates (E-PSI_{mixed}) index (Turley et al. 2016). The E-PSI index is designed to identify fine sediment conditions in streams, using the benthic invertebrate community composition. Invertebrates were initially rated by their sensitivity to fine sediment, based on expert knowledge and an assessment of biological and ecological traits that result in a sensitivity or tolerance of fine sediment (Extence et al. 2013, Turley et al. 2014). The E-PSI index maintains this biological basis, and assigns more detailed indicator weights

based on empirical data of invertebrate community compositions and visual estimates of fine sediment (Turley et al. 2016). For this reason, the index may be more strongly correlated with visual estimates than with the other methods.

The macroinvertebrate data used to calculate this index was collected using the UK standard method described in Chapter 3 (Section 3.3.1.1); a standardised three-minute active kick-sample, followed by a one-minute manual search (EU-STAR 2004). Sampling was carried out at each of the ten sites, over three seasons (with the exception of Site 7, which was sampled for macroinvertebrates only in spring due to the stream drying up and being too shallow to sample in the summer and autumn). Macroinvertebrates were recorded to the lowest practicable taxonomic level, mostly to species or genus, with the exception of Oligochaeta (class) and Diptera (family).

7.3.10 Statistical analysis

The data were compiled in Microsoft Excel (see Supplementary Material E) and analysed using SPSS statistical software (IBM SPSS Statistics 24). Fine sediment data were aligned with E-PSI_{mixed} scores that were calculated using mixed-level taxonomic data collected contemporaneously. The fine sediment data did not satisfy the assumption of bivariate normality for Pearson's correlation coefficient and could not be successfully transformed. As such, the results from the DIA methods were compared to the visual estimates using Spearman's rank correlation coefficients, to identify any relationships between sDIA, eDIA and visual estimates, as well as their relationships to the sediment-specific biotic index. All correlations were interpreted using the Dancey and Reidy (2007) classifications of correlations; 0.1 – 0.39 = weak, 0.4 – 0.69 = moderate, 0.7 – 0.99 = strong.

7.4 Results

7.4.1 Comparison of methods

Software-based DIA and eDIA were strongly and positively correlated with visual estimates (both $r_s = 0.72$, $p < 0.01$) (Figure 7.5). Software-based DIA was also strongly correlated to eDIA ($r_s = 0.96$, $p < 0.01$). The mean difference between an individual DIA value and the corresponding (same site and season) visual estimate was 12.4 percentage points (for both sDIA and eDIA), whilst the largest difference was 33 percentage points (37% fine sediment compared to 70% fine sediment).

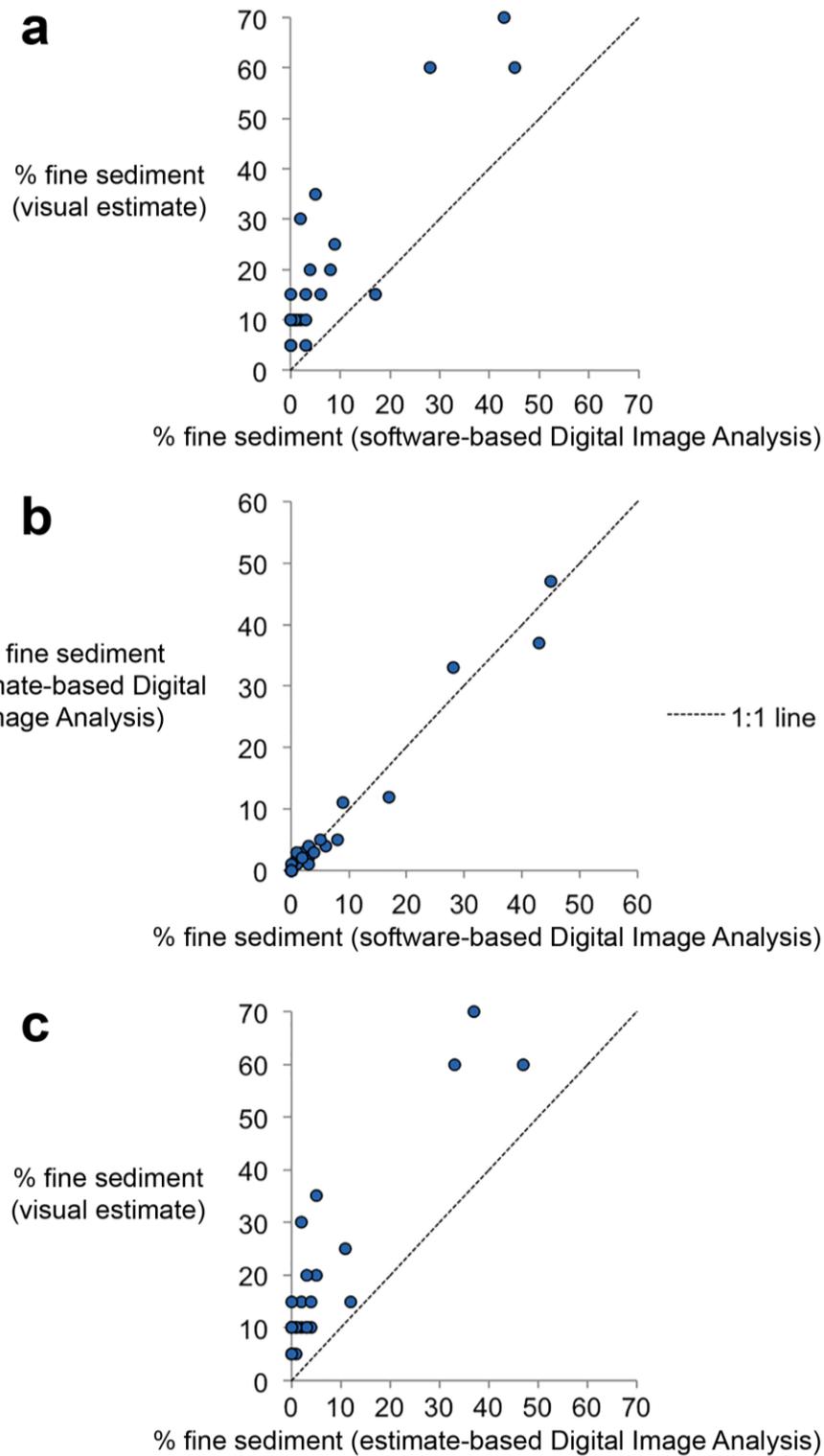


Figure 7.5. Comparison between a) visual estimates and software-based DIA, b) software-based DIA and estimate-based DIA, and c) visual estimates and estimate-based DIA, at ten sites sampled over three seasons (with the exception of one site sampled in spring only; i.e. $n = 28$).

7.4.2 Precision of software-based and estimate-based Digital Image Analysis

The mean difference between the first operator's and the independent operator's sDIA results over the five sites was 1.8 percentage points, with the maximum difference being three percentage points. Similarly, the mean difference between the eDIA from each operator was 1.8 percentage points, with a maximum difference of five percentage points (Figure 7.6).

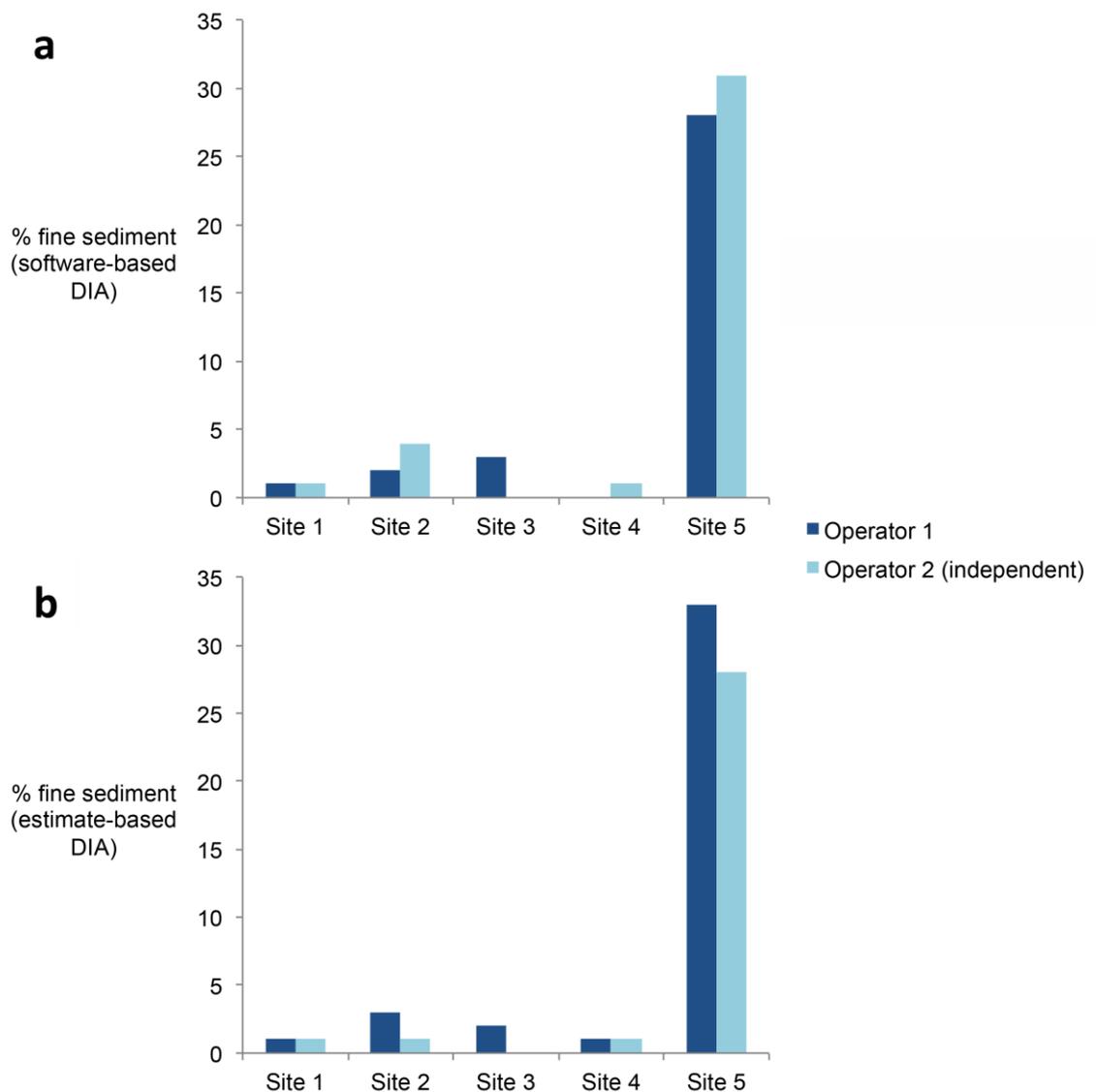


Figure 7.6. Differences between fine sediment values from two operators using a) software-based DIA, and b) estimate-based DIA techniques.

7.4.3 Fine sediment spatial variation

The variation between transects is illustrated by Figure 7.7 using data from the sDIA. Many of the sites had narrow confidence intervals (< 5 percentage points). Some sites had relatively large confidence intervals, mainly those with mean fine sediment cover of > 10%. The largest confidence interval was 35 percentage points (Site 9 – summer).

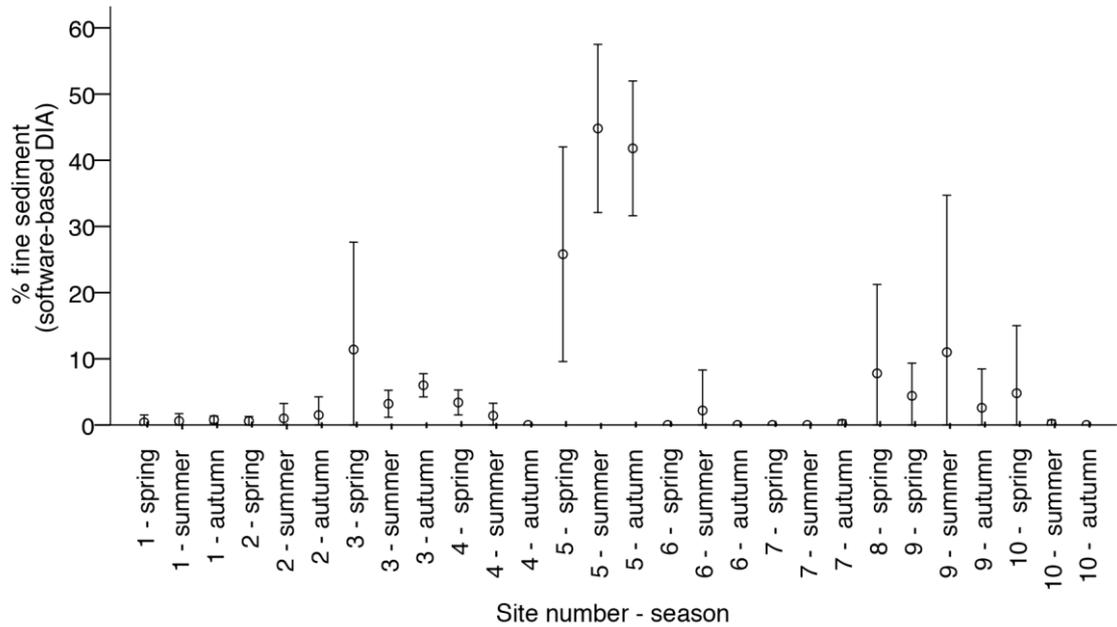


Figure 7.7. Mean percentage fine sediment values from software-based Digital Image Analysis of five transects (each comprising three images), from ten sites over three seasons (spring, summer, autumn - only spring data for Site 8 due to high turbidity). Error bars show 95% confidence intervals.

7.4.4 Biological relevance

All three methods of quantifying fine sediment were strongly negatively correlated to the E-PSI_{mixed} index. The visual estimates and sDIA were similarly strongly correlated to the E-PSI_{mixed} index ($r_s = - 0.72$ and $r_s = - 0.64$, $p < 0.01$, respectively) with the most strongly correlated method being the eDIA ($r_s = - 0.75$, $p < 0.01$) (Figure 7.8).

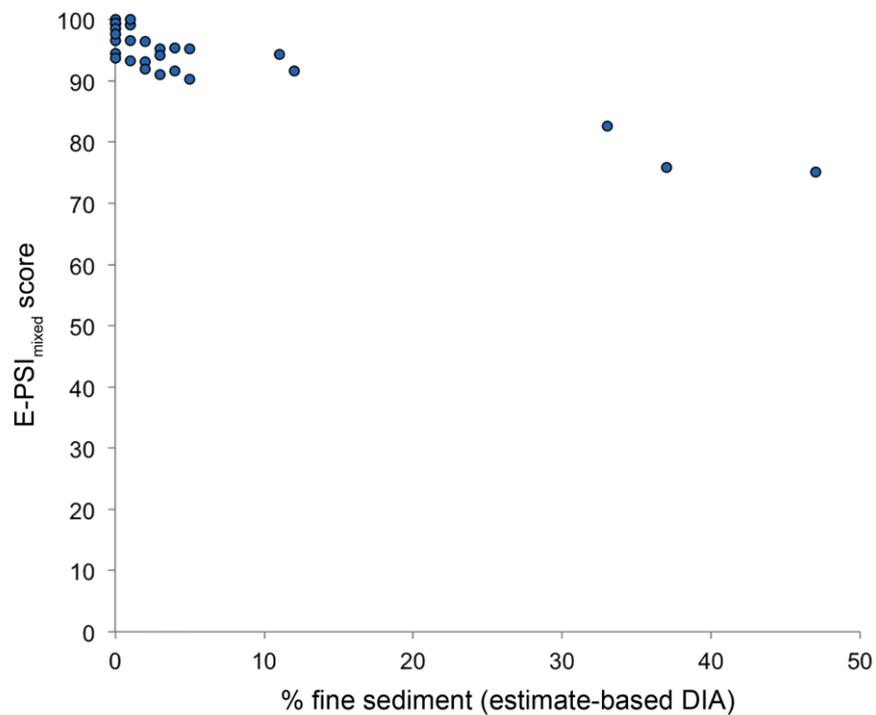


Figure 7.8. Relationship between % fine sediment (estimate-based Digital Image Analysis) and the mixed-level Empirically-weighted PSI (E-PSI_{mixed}) index.

7.5 Discussion

7.5.1 Comparison of methods

The results of this study show that both the software-based DIA and estimate-based DIA methods were strongly correlated with the visual estimates. Similar to (McHugh and Budy 2005), the visual estimates conducted for this study, resulted in higher values for percentage fine sediment, an overestimation of the 'measured' method (in their case a hoop-based embeddedness technique). Whilst the differences between DIA and visual estimates may be the result of one method being more accurate than the other, this cannot be tested, as the 'true' value for fine sediment is unquantifiable. The differences between the data from the two methods could also be due to different methodologies, with DIA attempting to represent the percentage of fine sediment

across the site using sample patches, and visual estimates summarising the entire study site. Some of the differences are also likely to be due to the subjectivity of the visual estimate method (Roper et al. 2002, Sennatt et al. 2006, Descloux et al. 2010), as well as the sampling design used in this study for image collection. However, using systematically selected transects and fixed image locations, reduces the subjectivity of the technique in comparison to other commonly used methods of quantifying fine sediment. For example, the practicalities of deploying numerous pieces of equipment and/or removing and processing large amounts of sediment, mean that many methods result in a limited number of locations being sampled. These locations are often selected based on operator judgements, which may introduce operator error or bias. Techniques that utilise bucket traps, artificial mats, adhesive plates or re-suspension techniques often require subjective decisions to be made regarding selection of sampling locations. For example, application of the resuspension technique can require the identification of “erosional” and “depositional” sampling locations (Duerdoth et al. 2015) necessitating a subjective evaluation of stream characteristics, which may vary with flow conditions. Furthermore, it is likely that methods that require such sampling location decisions are influenced by the nature of the substrate or channel itself, with substrates containing large boulders, and/or deeper waters (deeper than sampling equipment), and/or narrow braided channels (narrower than sampling equipment) being omitted from sampling due to operational constraints. These common limitations impact the ability of methods to represent spatial variation in substrate composition accurately. In contrast, a systematic approach, such as that used in this study, may more accurately represent spatial variation, although the number of transects/images required to do this is likely to be site-specific. Increasing the number of transects/images requires a minimal investment of time, particularly if opting for the estimate-based approach, which entails less than five minutes processing per image (compared to up to 30 minutes for sDIA).

Digital Image Analysis is more objective than visual estimates as it allows for particles to be measured and percentages to be computed. The operational limitations associated with DIA are similar to those that are experienced for visual estimates. In conditions where water clarity is poor, the method can prove problematic, however, moving the camera closer to the streambed can in many cases overcome this issue. Similarly, low water clarity can also prevent visual estimates. One of the benefits of using digital photography is that photographs can be reviewed instantaneously, something which is not possible for film photography. Other conditions (e.g. low light, rapid flow) leading to poorly lit/focused images, require some level of subjectivity in order to identify areas of fine sediment, though digital image contrast can be altered as part of the post-processing to mitigate these problems. The method is restricted to streams that are wadeable unless a boat mounted system could be used. Nevertheless, one of the significant strengths of the approach is the ability for images to be archived for verification, therefore providing a means of quality assurance. It is also a non-destructive technique, a characteristic that is particularly desirable when being carried out alongside biological sampling and/or in conservation areas (Naden et al. 2003, Woolsey et al. 2007).

Perhaps unsurprisingly, the sDIA was more strongly correlated to eDIA than to the visual estimates. This is likely to be due to the two methods quantifying fine sediment over the same areas of streambed (captured in the images), whereas the visual estimates consider the site as a whole. This strong correlation ($r_s = 0.96$, $p < 0.01$) suggests that the comparatively rapid approach (up to five minutes per image) of estimating the amount of fine sediment in the images using eDIA could negate the requirement of manually highlighting fine sediment for sDIA, which is a time-intensive procedure (up to 30 minutes per image). All three methods are likely to be subject to 'fabric errors', which are the result of misclassifications of particle size due to the orientation of particles in relation to the plane of the image, as well as the potential for

particles to be partially hidden (Graham et al. 2005b). However, as the focus of the analyses was fine sediment, this error is likely to be minimal.

7.5.2 Precision of software-based and estimate-based Digital Image Analysis

The inter-operator comparison for both methods of DIA yielded relatively small differences between fine sediment values (maximum five percentage points) across the five samples. This is despite the site/season being randomly selected for verification, and the independent operator having no prior knowledge or expectations of the site. Although the precision of visual estimates carried out in this study is not considered, previously published work on this topic has shown the method to have a lower level of precision. For example, a study using ten different operators, showed visual estimates to vary by up to 40 percentage points for sites with the same Wolman counts (Clapcott et al. 2011). However, the operators did not receive any specific training prior to the observations, and so this example is likely to be an extreme case. Wang et al. (1996) found the precision of visual estimates between six operators to be “moderate”, with confidence intervals of between 5 and 15 percentage points. It is thought that recent standardised training of operators is likely to improve the precision of visual estimates of stream habitat (Roper and Scarnecchia 1995, Poole et al. 1997). A high level of inter-operator precision was shown for the DIA methods across sites with a range of substrate compositions, however this analysis involved fewer operators. The sDIA method has a potential advantage over the eDIA method, which introduces some subjectivity to DIA, requiring operators to estimate the percentage of fine sediment in the images (using a 10 x 10 grid), which is likely to lower its accuracy and precision. Nonetheless, eDIA offers substantial time and cost-savings in comparison to sDIA, requiring approximately one-sixth of the time commitment.

7.5.3 *Fine sediment spatial variation*

In application the sampling design used to collect digital images for DIA should be designed to provide suitable representation of fine sediment spatial variation. The use of five transects here, is merely an example of how the method could be applied. The relatively narrow confidence intervals for Site 1, 2, 4 and 7, suggest that the sites exhibited little variation in terms of fine sediment coverage, and that five transects or less, may provide sufficient representation of spatial variation in some situations. The larger confidence intervals for the remaining sites (not all seasons) suggest that a greater number of transects/larger areas of streambed may need to be sampled to suitably represent fine sediment conditions over certain sites. Environmental factors are likely to influence the number of images required in order to provide an accurate representation of spatial variation. These include the water depth, water clarity and light availability, which limit the area of the streambed captured in each image, potentially necessitating the collection of a greater number of images. Furthermore, sites with a heterogeneous and poorly sorted substrate are likely to require a greater number of images in comparison with more homogeneous, well-sorted streambeds, in order to capture images that accurately represent the fine sediment conditions throughout the site.

The positioning of image locations for Digital Image Analysis is not influenced by the presence of boulders, cobbles or bedrock, and so may be able to suitably capture the 'true' characteristics of stream and river substrate. In contrast, techniques such as adhesive plates, artificial mats or re-suspension sampling devices are restricted by the diameter of the device, and the ability to attach or insert the device into the substrate, although they do benefit from enabling analysis of geochemical properties of the sediment, as part of the post-processing.

7.5.4 *Biological relevance*

As the 'true' value for the percentage of fine sediment in a stream is unknown, the accuracy of the methods cannot be determined. However, a potentially more meaningful measure (depending on the methods intended application) is its biological relevance. The E-PSI_{mixed} index was moderately to strongly correlated to all three methods of quantifying deposited fine sediment. These results suggest that all three methods have some degree of biological relevance, particularly given the biological basis of the index, which provides a mechanistic linkage for the observed correlations. These correlations were observed at systematically selected sites that greatly varied in their environmental characteristics, suggesting that the techniques are able to provide metrics indicative of fine sediment conditions across a range of different temperate river and stream types. Although the DIA approach does not consider the quality of fine sediment (geochemical and particle size distribution), the percentage cover of fine sediment is likely to be biologically relevant as it relates to niche theory and habitat suitability throughout the reach (Hirzel and Le Lay 2008).

7.6 Conclusion

Although visual estimates of fine sediment have been found to be some of the most correlated metrics to both land use and macroinvertebrate biotic indices, the subjectivity of these methods have the potential to result in incorrect conclusions as to the sediment conditions at a site. Digital Image Analysis presents an opportunity to reduce the subjectivity involved in characterising streambed fine sediment conditions. Such an approach is highly desirable for monitoring and research applications, as well as for river restoration and management projects, which require non-destructive, reliable and ecologically meaningful habitat indicators. Given the budget constraints often placed on these types of application, the eDIA approach may provide a more suitable means of characterising fine sediment conditions. Further work should be

conducted to determine the optimum number of transects or area of streambed that is necessary to provide a good representation of fine sediment conditions across sites with differing environmental characteristics.

Chapter 8 Synthesis and Conclusions

8.1 The rise of biomonitoring for environmental assessment

Biomonitoring has been practiced to some extent for ca. 100 years (e.g. Kolkwitz and Marsson 1909), but only recently has water legislation placed a strong emphasis on its use for determining the ecological condition of freshwater environments. The benefits of biomonitoring tools, as discussed in Chapter 2, include the potential for them to indicate antecedent pressures, their relatively low cost and greater degree of biological relevance in comparison to conventional physicochemical monitoring. If regulatory agencies are to rely more upon biomonitoring tools to assign ecological status and diagnose the causes of impairment, the tools need to be grounded in ecological science and be thoroughly tested across the range of environmental characteristics to which they will be applied, as well as along the full gradient of the pressure of concern (Friberg et al. 2011). The development of biomonitoring tools is therefore an important area of research that is crucial for the protection of freshwater ecosystems.

8.2 Developing biomonitoring tools: Expert opinion versus statistical approaches

The development of biomonitoring tools in the literature has largely relied on empirical data and statistical techniques to assign sensitivity weights (Zweig and Rabeni 2001, Davy-Bowker et al. 2003, Relyea et al. 2012, Murphy et al. 2015, Hubler et al. 2016), with some notable exceptions that use expert knowledge and judgement to assign sensitivity weights (Hawkes 1998, Extence et al. 1999, Liess and Ohe 2005, Extence et al. 2013). The benefits and drawbacks of each approach are discussed in Chapter 4 and Chapter 5. Briefly, expert knowledge and judgement allows for the current state of knowledge to be utilised, by drawing together existing research of varying temporal and

spatial scales, and can also assist modelling when there is a paucity of data, or when dealing with complex environmental systems and processes (Kuhnert et al. 2010, Krueger et al. 2012). The main drawback of using expert judgement relates to the subjectivity of the process, and the potential for bias to be introduced, and it is therefore recommended that best practice be followed throughout the process (Krueger et al. 2012, Drescher et al. 2013). Purely statistical approaches to biomonitoring tool development benefit from the use of empirical data for modelling the relationship of concern, but risk being influenced by statistical artifacts and/or confounding factors (Altman and Krzywinski 2015).

Although our knowledge of the complexity of freshwater ecosystems is far from complete, in order for biomonitoring tools to be based on sound ecological science, techniques using available knowledge to develop tools, require investigation. The research in this thesis represents an important contribution to the literature, demonstrating a novel intermediate approach that utilises both expert knowledge and expert judgement to broadly classify taxa as “sensitive” and “insensitive”; and empirical data to assign more detailed indicator weights within these categories, to each taxon. By classifying macroinvertebrates in this way, the newly developed E-PSI index has a biological basis, thus providing a mechanistic linkage between the index and the pressure of concern (Turley et al. 2016). The main statistical technique used to assign the detailed indicator weights (non-linear optimisation) is just one example of the kind of method that can allow the use of prior knowledge alongside empirical data. An alternative technique that may also be useful in some situations is the use of Bayesian networks (Uusitalo 2007, Kuhnert et al. 2010).

8.3 Best-practice evaluation of biomonitoring tools

Gathering sufficient high quality data for the sole purpose of developing or testing biomonitoring tools is often prohibitively expensive. Perhaps for this reason, a review of lotic biomonitoring tools in use throughout Europe, found that 31% of tools had no documented testing of the pressure-response relationship (Birk et al. 2012). Nevertheless, biomonitoring tools should be tested across the range of environmental conditions to which they are to be applied, and along the full gradient of the pressure of concern. Chapter 4 discusses the numerous reasons why such testing is necessary, given the likelihood that pressure-response relationships will vary in different rivers and streams as a result of differing environmental characteristics, as well as due to biotic interactions. Furthermore, the presence of confounding factors (Chapter 5 & 6), relevant to the specific pressure, should be investigated to ensure that the tool is indicating the pressure of concern. As approximately half of European rivers are exposed to multiple pressures (biological, chemical and physical) (Schinegger et al. 2012), biomonitoring tools also need to be tested alongside these additional pressures to ensure that output from such tools is reliable in these complex situations.

The testing of the E-PSI index in Chapters 4, 5 and 6 makes use of existing data collected by the UK regulatory agencies as part of their routine monitoring. This opportunistic data analysis allowed ecologically relevant and hypothesis driven testing of the index (i) over minimally impacted sites, (ii) alongside physical and chemical pressures typical of agriculturally impacted catchments, (iii) in the presence of an invasive species, and (iv) with consideration of potentially confounding factors relating to longitudinal gradients. Such testing is essential if biomonitoring tools are to be used by regulatory agencies to help diagnose the causes of ecological degradation.

8.4 Uncertainties in the development and testing of biomonitoring tools

Uncertainties are introduced at every stage of biomonitoring tool development and testing, and are likely to be partly responsible for the variation that remains in the relationship between E-PSI and fine sediment. The uncertainties have been discussed throughout this thesis, but include those related to the accuracy and precision of the sediment quantification technique and the sampling technique for macroinvertebrates.

Adopting opportunistic data analysis restricts the parameters, and dictates the sampling methods that are available to the user, but can provide access to data with a wide spatial and/or long term temporal distribution. In this thesis the available fine sediment data were derived from visual estimates of the percentage of substrate composition. This commonly used method of rapid habitat assessment (Sennatt et al. 2006, Descloux et al. 2010) provides a reach scale estimate of fine sediment that aligns well with the macroinvertebrate sampling. Although the technique is often considered subjective, the UK regulatory agencies provide staff with specific training on the method, which is likely to reduce the errors associated with operator estimates. Nevertheless, a more objective, verifiable and standardised technique (such as the DIA method developed in Chapter 7; Turley et al. 2017), if available at similar spatial and temporal resolution, would be highly desirable. In contrast to many techniques for quantifying fine sediment, DIA benefits from being non-destructive, a feature that is likely to be particularly important in sensitive areas of conservation.

Similarly, although a quantitative technique (e.g. Surber sampler) for sampling macroinvertebrates would allow the use of higher resolution abundance data (Everall et al. 2017), which may prove advantageous when investigating pressure-response relationships, it was essential that the E-PSI index was compatible with the UK regulatory agencies' standard techniques, and the requirements of RICT.

8.5 Biomonitoring tools are only as good as their reference-condition models

At present the UK regulatory agencies rely on the River Invertebrate Classification Tool (RICT), to predict the “expected” macroinvertebrate community composition, and the subsequent “expected” biomonitoring tool scores. In application, the E-PSI index should be applied in this observed/expected framework, to determine whether the observed E-PSI scores reflect an impacted or unimpacted condition. However, as discussed in Chapter 2, at present, the current version of RICT uses substrate composition as a predictor variable, and therefore there is in-built circularity in the resulting predictions of expected community composition. Whilst it is possible to bypass the entry of this predictor variable, substratum is a strong predictor of macroinvertebrate community composition in the model (Davy-Bowker et al. 2006, Clarke et al. 2011), and RICT has not been tested without it. A new version of RICT with predictor variables independent of hydromorphological impacts (i.e. excluding substratum, width and depth) is currently under development (at the time of writing this thesis).

As temperatures rise as a result of climate change, the RICT model will likely require further updating to reflect modern day temperatures, as predictions currently rely on mean annual air temperature between 1961 and 1990. Updates and continual testing of RICT and other predictive models throughout Europe, is critical if they are to continue to form the basis of bioassessment approaches, and inform conservation and management decisions.

8.6 The future of biomonitoring

With a growing global population placing ever-greater demand on freshwater environments, increasing globalisation facilitating the introduction of invasive species and disease, alongside the effects of climate change, the interpretation of

biomonitoring tool outputs, and therefore the protection of freshwater biodiversity will become even more challenging.

A lack of understanding and the complex nature of freshwater ecosystems can constrain the development of biomonitoring tools with strong diagnostic capabilities. Although further research is needed, it is perhaps unrealistic to expect to understand these ecosystems fully, and diagnose the causes of their degradation accurately, particularly using a single metric, or groups of metrics, and considering the associated uncertainties and presence of multiple pressures. However, when indices have been developed and tested using best practice, they can be used alongside a host of other tools to provide an insight into the ecological condition and the most likely causes of any degradation.

Although family-level tools are considered inferior by some, particularly for identifying subtle environmental change, the family-level E-PSI ($E\text{-PSI}_{\text{fam69}}$) index performed similarly to the mixed-level version, in terms of its correlation with deposited fine sediment. Furthermore, family-level tools are typically required when analysing historic datasets (often only recorded at family-level), and in regions where resources and/or taxonomic expertise are limited. Coarse taxonomic resolution tools may also become increasingly important if the resources available to regulatory agencies are further reduced, or if taxonomic expertise continue to decline (Hopkins and Freckleton 2002) resulting in mixed-level identification becoming infeasible. Furthermore, the growing prevalence of citizen science groups (Silvertown 2009, Kobori et al. 2016) represents a potentially important end-user of family-level biomonitoring tools. If these groups can coordinate and standardise their methods, the resulting data may be an important source of information in the future, contributing to regional and even global river health assessments (Jackson et al. 2016).

As discussed in Chapter 2, biomonitoring tools that take a purely trait-based approach (ignoring taxonomic composition) have been proposed as an alternative means of biomonitoring (Charvet et al. 1998, Menezes et al. 2010). These approaches appear promising as traits are more stable over large geographic areas (less variation in trait composition) (Charvet et al. 2000) and they can provide a mechanistic linkage between index scores and the pressure of concern (Menezes et al. 2010). However, trait-based approaches are currently hindered by a lack of understanding of trait-environment-stressor interactions, and uncertainties regarding the influence of co-occurring traits (Poff et al. 2006, Culp et al. 2011).

Technological advances such as next-generation sequencing for environmental DNA (eDNA) may increase the feasibility of detecting a range of indicator species using eDNA (Shokralla et al. 2012), and if costs are reduced (Thomsen and Willerslev 2015), the 'burden' of traditional taxonomic identification may be reduced. The eDNA approach benefits from being non-destructive, non-invasive, and could soon provide a more rapid assessment of biodiversity (Thomsen and Willerslev 2015). However, significant challenges remain if eDNA is to assist with biomonitoring (Goldberg et al. 2015), including the need for more research linking eDNA to quantitative estimates of species abundance, and the difficulties of determining the location of a species (Thomsen and Willerslev 2015). If eDNA becomes a practicable approach for regulatory agencies, it may allow a greater number of sites to be monitored each year and at a higher frequency. Rather than replacing the role of taxonomists, it is likely that they will be required for verification of samples and to identify specimens in more targeted sampling activities, perhaps allowing time to increase the taxonomic resolution of previously coarse identifications.

8.7 Further research

To ensure that freshwater scientists are able to meet the demands for biomonitoring tools in water legislation, there are numerous opportunities and requirements for further research. In Europe, interpretation of the majority of biomonitoring tools, and therefore the assessment of ecological status, relies on predictions of reference condition or minimally impacted community composition. It is therefore essential that researchers review and periodically enhance the predictive models to ensure that predictions accurately reflect reference conditions. Similarly, existing biomonitoring tools should be evaluated and where possible enhanced, to meet the requirements of water legislation, and to ensure their ability to diagnose the causes of ecological degradation is maximised.

In order to support the development of macroinvertebrate-based biomonitoring tools with mechanistic linkages to biotic responses, further investigation and documentation of the biological traits and ecological preferences of taxa is required. This would facilitate research into how these characteristics may be used in biomonitoring to provide a mechanistic basis for existing and emerging pressures. Additional research of trait independencies and trait interactions will also be an important area of work, allowing consideration of the implications that these may have on taxonomic (with biological basis) and trait-based biomonitoring tools. Future biomonitoring tools may require novel, advanced statistical techniques that allow the inclusion of knowledge of biological traits and ecological preferences in their development stages. In many instances, this will require interdisciplinary research, and provide opportunities for collaboration.

Finally, and more broadly, management and protection of freshwater ecosystems will likely necessitate better understanding of the effects of multiple pressures on biotic communities. Therefore, continued research into the prevalence of multiple pressures will be required, and investigation of the potential for synergistic or antagonistic effects

will be crucial for assigning ecological status, diagnosing the causes of any degradation, and enabling the implementation of truly effective management.

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Appendices

Appendix A. Indicator weights (W) for the family-level, Empirically-weighted Proportion of Sediment-sensitive Invertebrates (E-PSI) index.

Sensitive taxa	W
Aphelocheiridae	0.99
Baetidae	0.75
Brachycentridae	0.75
Capniidae	0.75
Chloroperlidae	0.99
Elmidae	0.5
Ephemerellidae	0.5
Glossosomatidae	0.51
Heptageniidae	1
Hydraenidae	0.75
Hydropsychidae	0.75
Lepidostomatidae	0.99
Leptophlebiidae	0.51
Leuctridae	0.99
Niphargidae	0.5
Odontoceridae	0.5
Perlidae	1
Perlodidae	0.99
Philopotamidae	0.5
Planorbidae (genus <i>Ancylus</i> only)	0.51
Potamanthidae	0.5
Rhyacophilidae	0.51

Sensitive taxa (continued)	<i>W</i>
Sericostomatidae	0.5
Simuliidae	0.5
Taeniopterygidae	0.75
Tipulidae, Limoniidae, Cylindrotomidae and Pediciidae	0.5
Insensitive taxa	<i>W</i>
Aeshnidae	0.49
Asellidae	0.24
Bithyniidae	0.49
Caenidae	0
Calopterygidae	0.49
Cordulegastridae	0.49
Corixidae	0.49
Corophiidae	0
Crangonyctidae	0.25
Dendrocoelidae	0.01
Dryopidae	0.01
Dugesiidae	0
Dytiscidae	0.25
Ephemeraidae	0.49
Erpobdellidae	0.01
Glossiphoniidae	0.24
Haliplidae	0.48
Hirudinidae	0.01
Hydrobiidae	0.48

In insensitive taxa (continued)	<i>W</i>
Hydrophilidae, Helophoridae, Georissidae and Hydrochidae	0.49
Libellulidae	0.49
Lymnaeidae	0.01
Molannidae	0.01
Nemouridae	0
Nepidae	0.48
Neritidae	0.48
Noteridae	0.49
Phryganeidae	0.48
Physidae	0.25
Planariidae	0.25
Planorbidae (excluding genus <i>Ancylus</i>)	0
Platycnemididae	0.48
Psychodidae	0
Ptychopteridae	0.48
Sialidae	0.48
Siphonuridae and Ameletidae	0.25
Sphaeriidae	0.48
Stratiomyidae	0.49
Syrphidae	0.48
Tabanidae	0.48
Unionidae	0.48
Valvatidae	0.25
Viviparidae	0.24

Appendix B. Indicator weights (*W*) for the mixed-level, Empirically-weighted Proportion of Sediment-sensitive Invertebrates (E-PSI) index.

Sensitive taxa		<i>W</i>
<i>Agabus guttatus</i>	(Paykull, 1798)	0.5
<i>Agapetus</i> sp.		0.5
<i>Alainites muticus</i>	(Linnaeus, 1758)	0.88
<i>Allotrichia pallicornis</i>	(Eaton, 1873)	0.5
<i>Ameletus inopinatus</i>	(Eaton, 1887)	0.5
<i>Amphinemura sulcicollis</i>	(Stephens, 1836)	1
<i>Ancylus fluviatilis</i>	(O.F. Müller, 1774)	0.5
<i>Apatania muliebris</i>	(McLachlan, 1866)	1
<i>Aphelocheirus aestivalis</i>	(Fabricius, 1794)	0.5
<i>Athripsodes albifrons</i>	(Linnaeus, 1758)	0.5
<i>Athripsodes bilineatus</i>	(Linnaeus, 1758)	0.5
<i>Athripsodes cinereus</i>	(Curtis, 1834)	0.5
<i>Athripsodes commutatus</i>	(Rostock, 1874)	0.5
<i>Baetis buceratus</i>	(Eaton, 1870)	0.5
<i>Baetis rhodani</i>	(Pictet, 1843-1845)	1
<i>Baetis scambus</i> group		1
<i>Baetis vernus</i>	(Curtis, 1834)	0.5
<i>Beraea maurus</i>	(Curtis, 1834)	0.87
<i>Beraea pullata</i>	(Curtis, 1834)	0.5
<i>Beraeodes minutus</i>	(Linnaeus, 1761)	0.87
<i>Brachycentrus subnubilus</i>	(Curtis, 1834)	1
<i>Brachyptera putata</i>	(Newman, 1838)	1
<i>Brachyptera risi</i>	(Morton, 1896)	0.5

Sensitive taxa (continued)		W
<i>Calopteryx virgo</i>	(Linnaeus, 1758)	0.5
<i>Capnia atra</i>	(Morton, 1896)	0.5
<i>Capnia bifrons</i>	(Newman, 1839)	0.5
<i>Ceraclea albimacula</i>	(Rambur, 1842)	1
<i>Ceraclea annulicornis</i>	(Stephens, 1836)	1
<i>Ceraclea dissimilis</i>	(Stephens, 1836)	0.5
<i>Ceraclea fulva</i>	(Rambur, 1842)	0.5
<i>Ceraclea nigronervosa</i>	(Retzius, 1783)	0.5
<i>Ceraclea senilis</i>	(Burmeister, 1839)	0.75
<i>Cheumatopsyche lepida</i>	(Pictet, 1834)	1
<i>Chimarra marginata</i>	(Linnaeus, 1761)	1
<i>Chloroperla tripunctata</i>	(Scopoli, 1763)	1
<i>Crenobia alpina</i>	(Dana, 1766)	1
<i>Crunoecia irrorata</i>	(Curtis, 1834)	0.5
<i>Cyrnus trimaculatus</i>	(Curtis, 1834)	0.5
<i>Deronectes latus</i>	(Stephens, 1829)	0.5
<i>Dinocras cephalotes</i>	(Curtis, 1827)	1
<i>Diplectronea felix</i>	(McLachlan, 1878)	1
<i>Diura bicaudata</i>	(Linnaeus, 1758)	0.5
Dixidae		0.5
<i>Drusus annulatus</i>	(Stephens, 1837)	0.5
<i>Ecclisopteryx guttulata</i>	(Pictet, 1834)	1
<i>Ecdyonurus</i> sp.		1
<i>Electrogena lateralis</i>	(Curtis, 1834)	1
<i>Elmis aenea</i>	(Müller, 1806)	0.5

Sensitive taxa (continued)		W
<i>Elodes</i> sp.		0.5
<i>Ephemerella notata</i>	(Eaton, 1887)	0.87
<i>Gammarus duebeni</i>	(Liljeborg, 1852)	0.5
<i>Gammarus pulex</i>	(Linnaeus, 1758)	0.5
<i>Glossosoma</i> sp.		1
<i>Goera pilosa</i>	(Fabricius, 1775)	0.5
<i>Heptagenia sulphurea</i>	(Müller, 1776)	0.88
<i>Hydraena gracilis</i>	(Germar, 1824)	1
<i>Hydraena nigrita</i>	(Germar, 1824)	1
<i>Hydraena riparia</i>	(Kugelann, 1794)	0.5
<i>Hydraena rufipes</i>	(Curtis, 1830)	0.5
<i>Hydroporus ferrugineus</i>	(Stephens, 1829)	1
<i>Hydropsyche angustipennis</i>	(Curtis, 1834)	0.5
<i>Hydropsyche contubernalis</i>	(McLachlan, 1865)	1
<i>Hydropsyche fulvipes</i>	(Curtis, 1834)	0.5
<i>Hydropsyche instabilis</i>	(Curtis, 1834)	0.5
<i>Hydropsyche pellucidula</i>	(Curtis, 1834)	1
<i>Hydropsyche saxonica</i>	(McLachlan, 1884)	0.5
<i>Hydropsyche siltalai</i>	(Döhler, 1963)	0.88
<i>Isoperla grammatica</i>	(Poda, 1761)	1
<i>Labiobaetis atrebatinus</i>	(Eaton, 1870)	0.5
<i>Lepidostoma basale</i>	(Kolenati, 1848)	0.5
<i>Lepidostoma hirtum</i>	(Fabricius, 1775)	1
<i>Leuctra fusca</i>	(Linnaeus, 1758)	1
<i>Leuctra geniculata</i>	(Stephens, 1836)	0.63

Sensitive taxa (continued)		W
<i>Leuctra hippopus</i>	(Kempny, 1899)	1
<i>Leuctra inermis</i>	(Kempny, 1899)	1
<i>Leuctra moselyi</i>	(Morton, 1929)	1
<i>Leuctra nigra</i>	(Olivier, 1811)	0.5
<i>Limnius volckmari</i>	(Panzer, 1793)	0.5
Limoniidae		0.5
<i>Macronychus quadrituberculatus</i>	(Müller, 1806)	1
<i>Margaritifera margaritifera</i>	(Linnaeus, 1758)	0.5
<i>Melampophylax mucoreus</i>	(Hagen, 1861)	0.62
<i>Metalype fragilis</i>	(Pictet, 1834)	0.5
<i>Micronecta</i> sp.		0.5
<i>Micropterna</i> group		0.5
<i>Nemoura cambrica</i> group		0.5
<i>Neureclipsis bimaculata</i>	(Linnaeus, 1758)	1
<i>Nigrobaetis digitatus</i>	(Bengtsson, 1912)	1
<i>Nigrobaetis niger</i>	(Linnaeus, 1761)	0.5
<i>Niphargus aquilex</i>	(Schiodte, 1855)	0.5
<i>Normandia nitens</i>	(Müller, 1817)	1
<i>Odontocerum albicorne</i>	(Scopoli, 1763)	0.5
<i>Oecetis notata</i>	(Rambur, 1842)	1
<i>Oecetis testacea</i>	(Curtis, 1834)	0.63
<i>Orectochilus villosus</i>	(O.F. Müller, 1776)	0.87
<i>Oreodytes davisii</i>	(Curtis, 1831)	1
<i>Oreodytes sanmarkii</i>	(C.R. Sahlberg, 1826)	0.5
<i>Oreodytes septentrionalis</i>	(Gyllenhal, 1826)	1

Sensitive taxa (continued)		W
<i>Paraleptophlebia cincta</i>	(Retzius, 1835)	0.5
<i>Paraleptophlebia submarginata</i>	(Stephens, 1835)	0.63
Pediciidae		0.5
<i>Perla bipunctata</i>	(Pictet, 1833)	1
<i>Perlodes microcephalus</i>	(Pictet, 1833)	1
<i>Phagocata vitta</i>	(Duges, 1830)	0.5
<i>Philopotamus montanus</i>	(Donovan, 1813)	0.5
<i>Piscicola geometra</i>	(Linnaeus, 1761)	0.5
<i>Platambus maculatus</i>	(Linnaeus, 1758)	0.5
<i>Plectrocnemia conspersa</i>	(Curtis, 1834)	0.5
<i>Plectrocnemia geniculata</i>	(McLachlan, 1871)	1
<i>Polycentropus flavomaculatus</i>	(Pictet, 1834)	1
<i>Polycentropus irroratus</i>	(Curtis, 1835)	0.5
<i>Polycentropus kingi</i>	(McLachlan, 1881)	1
<i>Potamanthus luteus</i>	(Linnaeus, 1767)	0.5
<i>Potamophylax</i> group		0.5
<i>Procloeon pennulatum</i>	(Eaton, 1870)	0.87
<i>Protonemura meyeri</i>	(Pictet, 1841)	0.88
<i>Protonemura montana</i>	(Kimmins, 1941)	0.5
<i>Protonemura praecox</i>	(Morton, 1894)	1
<i>Psychomyia pusilla</i>	(Fabricius, 1781)	1
<i>Rhithrogena</i> sp.		1
<i>Rhyacophila dorsalis</i>	(Curtis, 1834)	0.88
<i>Rhyacophila fasciata</i>	(Hagen, 1859)	0.5
<i>Rhyacophila munda</i>	(McLachlan, 1862)	0.5

Sensitive taxa (continued)		W
<i>Rhyacophila obliterata</i>	(McLachlan, 1863)	0.5
<i>Riolus cupreus</i>	(Müller, 1806)	0.5
<i>Riolus subviolaceus</i>	(Müller, 1817)	0.5
<i>Sericostoma personatum</i>	(Kirby & Spence, 1826)	0.5
<i>Serratella ignita</i>	(Poda, 1761)	0.5
<i>Sialis fuliginosa</i>	(Pictet, 1836)	0.5
<i>Silo nigricornis</i>	(Pictet, 1834)	0.5
<i>Silo pallipes</i>	(Fabricius, 1781)	0.5
Simuliidae		0.5
<i>Siphonoperla torrentium</i>	(Pictet, 1841)	1
<i>Stictonectes lepidus</i>	(Olivier, 1795)	0.5
<i>Tinodes dives</i>	(Pictet, 1834)	0.5
<i>Tinodes unicolor</i>	(Pictet, 1834)	0.5
<i>Tinodes waeneri</i>	(Linnaeus, 1758)	0.5
Tipulidae		0.63
<i>Wormaldia</i> sp.		1

Insensitive taxa		W
<i>Aeshna</i> sp.		0.49
<i>Agabus bipustulatus</i>	(Linnaeus, 1767)	0
<i>Agabus didymus</i>	(Olivier, 1795)	0.49
<i>Agabus paludosus</i>	(Fabricius, 1801)	0.49

Insensitive taxa (continued)		W
<i>Agabus sturmii</i>	(Gyllenhal, 1808)	0
<i>Agrypnia obsoleta</i> group		0.25
<i>Alboglossiphonia heteroclita</i>	(Linnaeus, 1761)	0.49
<i>Amphinemura standfussi</i>	(Ris, 1902)	0.37
<i>Anabolia nervosa</i>	(Curtis, 1834)	0.49
<i>Anacaena bipustulata</i>	(Marsham, 1802)	0.25
<i>Anacaena globulus</i>	(Paykull, 1829)	0.49
<i>Anacaena limbata</i>	(Fabricius, 1792)	0
<i>Anacaena lutescens</i>	(Stephens, 1829)	0.12
<i>Anisus (Anisus) leucostoma</i>	(Millet, 1813)	0.49
<i>Anisus (Disculifer) vortex</i>	(Linnaeus, 1758)	0.49
<i>Anodonta</i> group		0.12
<i>Aplexa hypnorum</i>	(Linnaeus, 1758)	0.25
<i>Asellus aquaticus</i>	(Linnaeus, 1758)	0.37
<i>Athripsodes aterrimus</i>	(Stephens, 1836)	0.37
<i>Bathyomphalus contortus</i>	(Linnaeus, 1758)	0
<i>Bdellocephala punctata</i>	(Pallas, 1774)	0.49

Insensitive taxa (continued)		W
<i>Bithynia (Bithynia) tentaculata</i>	(Linnaeus, 1758)	0.49
<i>Bithynia (Codiella) leachii</i>	(Sheppard, 1823)	0.49
<i>Brachycercus harrisellus</i>	(Curtis, 1834)	0.12
<i>Brachytron pratense</i>	(Müller, 1764)	0.25
<i>Brychius elevatus</i>	(Panzer, 1793)	0.49
<i>Caenis horaria</i>	(Linnaeus, 1758)	0.49
<i>Caenis luctuosa</i> group		0.12
<i>Caenis pseudorivulorum</i> group		0
<i>Caenis pusilla</i>	(Navás, 1913)	0
<i>Caenis rivulorum</i>	(Eaton, 1884)	0
<i>Caenis robusta</i>	(Eaton, 1884)	0.49
<i>Callicorixa praeusta</i>	(Fieber, 1848)	0.49
<i>Callicorixa wollastoni</i>	(Douglas & Scott, 1865)	0.37
<i>Calopteryx splendens</i>	(Harris, 1782)	0.49
<i>Centroptilum luteolum</i>	(Müller, 1776)	0.49
<i>Cloeon dipterum</i>	(Linnaeus, 1761)	0.49
<i>Cloeon simile</i>	(Eaton, 1870)	0.49

Insensitive taxa (continued)		W
<i>Cordulegaster boltonii</i>	(Donovan, 1807)	0.49
<i>Corixa affinis</i>	(Leach, 1817)	0
<i>Corixa dentipes</i>	(Thomson, 1869)	0.49
<i>Corixa panzeri</i>	(Fieber, 1848)	0.25
<i>Corixa punctata</i>	(Illiger, 1807)	0.49
<i>Corophium</i> sp.		0.49
<i>Crangonyx pseudogracilis</i>	(Bousfield, 1958)	0
<i>Cyphon</i> sp.		0
<i>Dendrocoelum lacteum</i>	(O.F.Müller, 1774)	0
<i>Dina lineata</i>	(O.F.Müller, 1774)	0
<i>Dryops</i> sp.		0
<i>Dugesia polychroa</i> group		0
<i>Dugesia tigrina</i>	(Girard, 1850)	0
<i>Dytiscus marginalis</i>	(Linnaeus, 1758)	0.25
<i>Dytiscus semisulcatus</i>	(O.F. Müller, 1776)	0.49
<i>Ecnomus tenellus</i>	(Rambur, 1842)	0.25
<i>Enochrus testaceus</i>	(Fabricius, 1801)	0.24

Insensitive taxa (continued)		W
<i>Ephemera danica</i>	(Müller, 1764)	0.49
<i>Ephemera lineata</i>	(Eaton, 1870)	0.25
<i>Ephemera vulgata</i>	(Linnaeus, 1758)	0.47
<i>Erpobdella octoculata</i>	(Linnaeus, 1758)	0.12
<i>Erpobdella testacea</i>	(Savigny, 1812)	0.49
<i>Esolus parallelepipedus</i>	(Müller, 1806)	0
<i>Galba truncatula</i>	(O.F. Müller, 1774)	0.49
<i>Gammarus lacustris</i>	(Sars, 1863)	0
<i>Gammarus tigrinus</i>	(Sexton, 1939)	0
<i>Gammarus zaddachi</i>	(Sexton, 1912)	0
<i>Glossiphonia complanata</i>	(Linnaeus, 1758)	0.48
<i>Glossiphonia paludosa</i>	(Carena, 1824)	0
<i>Glossiphonia verrucata</i>	(Fr. Müller, 1844)	0
<i>Glyphotaelius pellucidus</i>	(Retzius, 1783)	0.49
<i>Gomphus vulgatissimus</i>	(Linnaeus, 1758)	0.49
<i>Graptodytes pictus</i>	(Fabricius, 1787)	0.49
<i>Gyraulus (Armiger) crista</i>	(Linnaeus, 1758)	0.12

Insensitive taxa (continued)		W
<i>Gyraulus (Gyraulus) albus</i>	(O.F. Müller, 1774)	0.12
<i>Gyraulus (Torquis) laevis</i>	(Alder, 1838)	0
<i>Habrophlebia fusca</i>	(Curtis, 1834)	0.49
<i>Haemopsis sanguisuga</i>	(Linnaeus, 1758)	0.49
<i>Halesus</i> sp.		0.49
<i>Haliplus confinis</i>	(Stephens, 1828)	0
<i>Haliplus flavicollis</i>	(Sturm, 1834)	0.49
<i>Haliplus fluviatilis</i>	(Aubé, 1836)	0.49
<i>Haliplus heydeni</i>	(Wehncke, 1875)	0.25
<i>Haliplus immaculatus</i>	(Gerhardt, 1877)	0
<i>Haliplus laminatus</i>	(Schaller, 1783)	0.12
<i>Haliplus lineatocollis</i>	(Marsham, 1802)	0.12
<i>Haliplus lineolatus</i>	(Mannerheim, 1844)	0.25
<i>Haliplus ruficollis</i>	(DeGeer, 1774)	0.49
<i>Haliplus sibericus</i>	(Motschulsky, 1860)	0.49
<i>Helobdella stagnalis</i>	(Linnaeus, 1758)	0.12
<i>Helophorus (Helophorus) flavipes</i>	(Fabricius, 1792)	0

Insensitive taxa (continued)		W
<i>Helophorus (Helophorus) minutus</i>	(Fabricius, 1775)	0.49
<i>Helophorus (Helophorus) obscurus</i>	(Mulsant, 1884)	0
<i>Helophorus (Helophorus) strigifrons</i>	(Thomson, 1868)	0.25
<i>Helophorus (Meghelophorus) aequalis</i>	(Thomson, 1868)	0.49
<i>Helophorus (Meghelophorus) grandis</i>	(Illiger, 1798)	0.49
<i>Helophorus (Rhopalohelophorus) arvernicus</i>	(Mulsant, 1846)	0
<i>Helophorus (Rhopalohelophorus) brevipalpis</i>	(Bedel, 1881)	0.49
<i>Hemiclepsis marginata</i>	(O.F.Müller, 1774)	0.12
<i>Hesperocorixa linnaei</i>	(Fieber, 1848)	0.49
<i>Hesperocorixa sahlbergi</i>	(Fieber, 1848)	0.49
<i>Hippeutis complanatus</i>	(Linnaeus, 1758)	0.12
<i>Hydatophylax infumatus</i>	(McLachlan, 1865)	0.49
<i>Hydraena pulchella</i>	(Germar, 1824)	0.49
<i>Hydraena testacea</i>	(Curtis, 1831)	0.49

Insensitive taxa (continued)		W
<i>Hydrobius fuscipes</i>	(Linnaeus, 1758)	0.49
<i>Hydrochus angustatus</i>	(Germar, 1824)	0
<i>Hydroporus discretus</i>	(Fairmaire & Brisout, 1859)	0
<i>Hydroporus memnonius</i>	(Nicolai, 1822)	0.49
<i>Hydroporus nigrita</i>	(Fabricius, 1792)	0
<i>Hydroporus obscurus</i>	(Sturm, 1835)	0.37
<i>Hydroporus palustris</i>	(Linnaeus, 1761)	0.12
<i>Hydroporus planus</i>	(Fabricius, 1782)	0
<i>Hydroporus pubescens</i>	(Gyllenhal, 1808)	0.49
<i>Hydroporus tessellatus</i>	(Drapiez, 1819)	0.49
<i>Hygrotus (Hygrotus) inaequalis</i>	(Fabricius, 1777)	0.49
<i>Hygrotus (Hygrotus) versicolor</i>	(Schaller, 1783)	0.49
<i>Hyphydrus ovatus</i>	(Linnaeus, 1761)	0.49
<i>Ilybius chalconatus</i>	(Panzer, 1796)	0.12
<i>Ilybius</i> sp.		0.49
<i>Kageronia fuscogrisea</i>	(Retzius, 1783)	0.49
<i>Laccobius (Laccobius) colon</i>	(Stephens, 1829)	0.25

Insensitive taxa (continued)		W
<i>Laccobius (Laccobius) minutus</i>	(Linnaeus, 1758)	0.49
<i>Laccobius (Macrolaccobius) atratus</i>	(Rottenburg, 1874)	0
<i>Laccobius (Macrolaccobius) sinuatus</i>	(Motschulsky, 1849)	0.25
<i>Laccobius (Macrolaccobius) striatulus</i>	(Fabricius, 1801)	0.25
<i>Laccobius (Macrolaccobius) ytenensis</i>	(Sharp, 1910)	0
<i>Laccophilus hyalinus</i>	(DeGeer, 1774)	0
<i>Laccophilus minutus</i>	(Linnaeus, 1758)	0.01
<i>Leptophlebia marginata</i>	(Linnaeus, 1767)	0.12
<i>Leptophlebia vespertina</i>	(Linnaeus, 1758)	0.12
<i>Limnebius nitidus</i>	(Marsham, 1802)	0.49
<i>Limnebius truncatellus</i>	(Thunberg, 1794)	0
<i>Limnephilus binotatus</i>	(Curtis, 1834)	0.25
<i>Limnephilus bipunctatus</i>	(Curtis, 1834)	0
<i>Limnephilus decipiens</i>	(Kolenati, 1848)	0.49
<i>Limnephilus extricatus</i>	(McLachlan, 1865)	0.49

Insensitive taxa (continued)		W
<i>Limnephilus flavicornis</i>	(Fabricius, 1787)	0.49
<i>Limnephilus fuscicornis</i>	(Rambur, 1842)	0
<i>Limnephilus lunatus</i>	(Curtis, 1834)	0.49
<i>Limnephilus marmoratus</i>	(Curtis, 1834)	0
<i>Limnephilus politus</i>	(McLachlan, 1865)	0.25
<i>Limnephilus rhombicus</i>	(Linnaeus, 1758)	0.49
<i>Limnephilus vittatus</i>	(Fabricius, 1798)	0.25
<i>Lymnaea stagnalis</i>	(Linnaeus, 1758)	0.49
<i>Molanna angustata</i>	(Curtis, 1834)	0.49
<i>Mystacides azurea</i>	(Linnaeus, 1761)	0.37
<i>Mystacides longicornis</i>	(Linnaeus, 1758)	0.49
<i>Mystacides nigra</i>	(Linnaeus, 1758)	0.37
<i>Nebrioporus assimilis</i>	(Paykull, 1798)	0.24
<i>Nebrioporus depressus</i>	(Fabricius, 1775)	0.49
<i>Nemoura avicularis</i>	(Morton, 1894)	0.12
<i>Nemoura cinerea</i>	(Retzius, 1783)	0.49
<i>Nemurella pictetii</i>	(Klapálek, 1900)	0.49

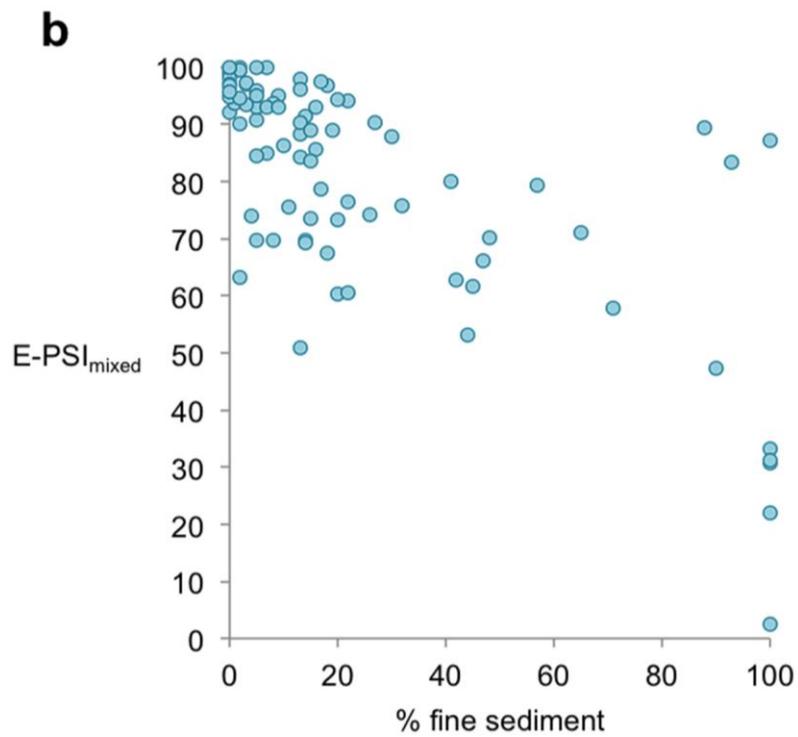
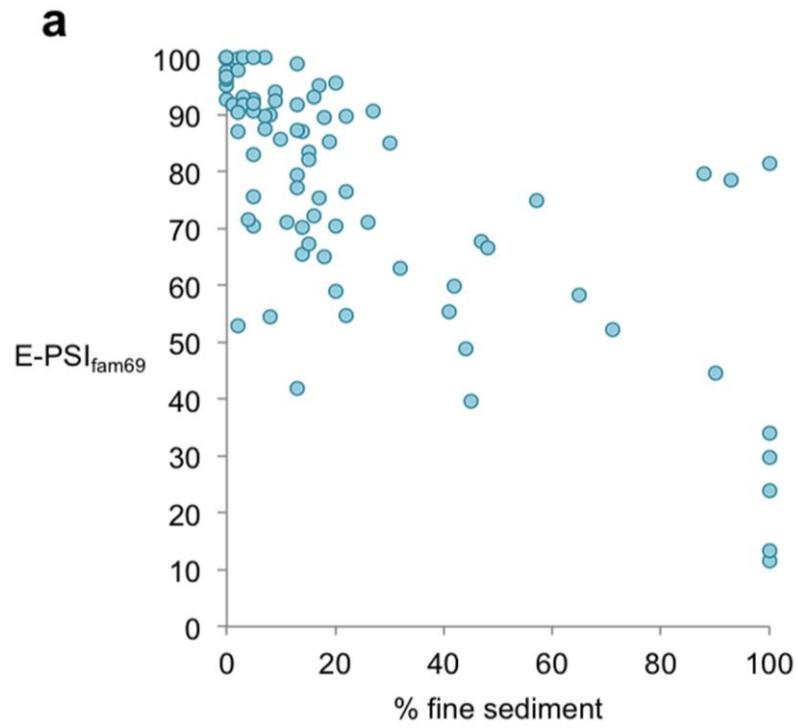
Insensitive taxa (continued)		W
<i>Nepa cinerea</i>	(Linnaeus, 1758)	0
<i>Noterus clavicornis</i>	(DeGeer, 1774)	0.49
<i>Notidobia ciliaris</i>	(Linnaeus, 1761)	0.49
<i>Ochthebius bicolon</i>	(Germar, 1824)	0
<i>Ochthebius dilatatus</i>	(Stephens, 1829)	0.49
<i>Ochthebius exsculptus</i>	(Germar, 1824)	0
<i>Ochthebius minimus</i>	(Fabricius, 1792)	0.49
<i>Oecetis lacustris</i>	(Pictet, 1834)	0.49
<i>Oecetis ochracea</i>	(Curtis, 1825)	0.49
<i>Orthetrum</i> sp.		0
<i>Oulimnius major</i>	(Rey, 1889)	0.12
<i>Oulimnius rivularis</i>	(Rosenhauer, 1856)	0.49
<i>Oulimnius troglodytes</i>	(Gyllenhal, 1827)	0
<i>Oulimnius tuberculatus</i>	(Müller, 1806)	0.12
<i>Paracymus scutellaris</i>	(Rosenhauer, 1856)	0.25
<i>Paraleptophlebia weneri</i>	(Ulmer, 1919)	0.49
<i>Phryganea</i> sp.		0.49

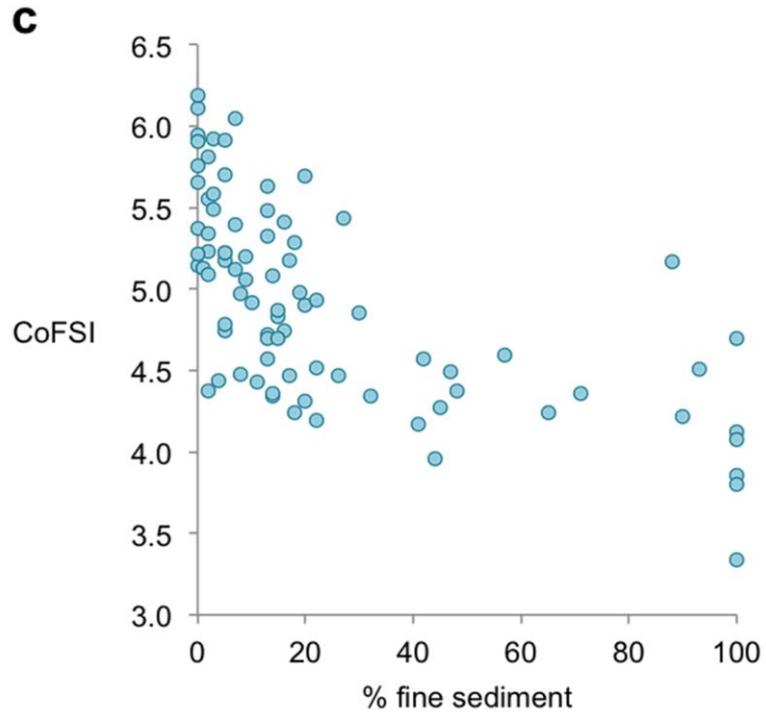
Insensitive taxa (continued)		W
<i>Physa fontinalis</i>	(Linnaeus, 1758)	0.49
<i>Physella (Costatella) acuta</i>	(Draparnaud, 1805)	0
<i>Pisidium</i> sp.		0.49
<i>Planaria torva</i>	(Müller, 1774)	0
<i>Planorbarius corneus</i>	(Linnaeus, 1758)	0.49
<i>Planorbis (Planorbis) carinatus</i>	(O.F. Müller, 1774)	0.49
<i>Planorbis (Planorbis) planorbis</i>	(Linnaeus, 1758)	0.49
<i>Platycnemis pennipes</i>	(Pallas, 1771)	0.49
<i>Polycelis felina</i>	(Dalyell, 1814)	0.37
<i>Polycelis nigra</i> group		0
<i>Porhydrus lineatus</i>	(Fabricius, 1775)	0.25
<i>Potamopyrgus antipodarum</i>	(J.E.Gray, 1843)	0.37
<i>Proasellus meridianus</i>	(Racovitza, 1919)	0.49
<i>Procloeon bifidum</i>	(Bengtsson, 1912)	0.49
Psychodidae		0.37
Ptychopteridae		0.49
<i>Radix auricularia</i>	(Linnaeus, 1758)	0

Insensitive taxa (continued)		W
<i>Radix balthica</i>	(Linnaeus, 1758)	0
<i>Scarodytes halensis</i>	(Fabricius, 1787)	0.49
<i>Segmentina nitida</i>	(O.F. Müller, 1774)	0.49
<i>Sialis lutaria</i>	(Linnaeus, 1758)	0.49
<i>Sialis nigripes</i>	(Pictet, 1865)	0.12
<i>Sigara (Pseudovermicorixa) nigrolineata</i>	(Fieber, 1848)	0.49
<i>Sigara (Retrocorixa) semistriata</i>	(Fieber, 1848)	0
<i>Sigara (Retrocorixa) venusta</i>	(Douglas & Scott, 1869)	0
<i>Sigara (Sigara) sp.</i>		0.49
<i>Sigara (Subsigara) distincta</i>	(Fieber, 1848)	0.12
<i>Sigara (Subsigara) falleni</i>	(Fieber, 1848)	0.49
<i>Sigara (Subsigara) fossarum</i>	(Leach, 1817)	0
<i>Sigara (Vermicorixa) lateralis</i>	(Leach, 1817)	0
<i>Siphonurus lacustris</i>	(Eaton, 1870)	0
<i>Sphaerium sp.</i>		0.49
<i>Stagnicola palustris</i>	(O.F. Müller, 1774)	0.49

Insensitive taxa (continued)		W
<i>Stictotarsus duodecimpustulatus</i>	(Fabricius, 1792)	0.12
Stratiomyidae		0.49
Syrphidae		0.49
Tabanidae		0.37
<i>Taeniopteryx nebulosa</i>	(Linnaeus, 1758)	0
<i>Theodoxus fluviatilis</i>	(Linnaeus, 1758)	0.49
<i>Theromyzon tessulatum</i>	(O.F.Müller, 1774)	0
<i>Trocheta bykowskii</i>	(Gedroyc, 1913)	0
<i>Trocheta subviridis</i>	(Dutrochet, 1817)	0
<i>Unio</i> sp.		0.49
<i>Valvata (Cincinna) piscinalis</i>	(O.F. Müller, 1774)	0.37
<i>Valvata (Tropidina) macrostoma</i>	(Morch, 1864)	0
<i>Valvata (Valvata) cristata</i>	(O.F. Müller, 1774)	0.49
<i>Viviparus viviparus</i>	(Linnaeus, 1758)	0.49

Appendix C. Relationship between % fine sediment and a) $E\text{-PSI}_{\text{fam69}}$ b) $E\text{-PSI}_{\text{mixed}}$ and c) CoFSI, across 84 minimally impacted river and stream sites.





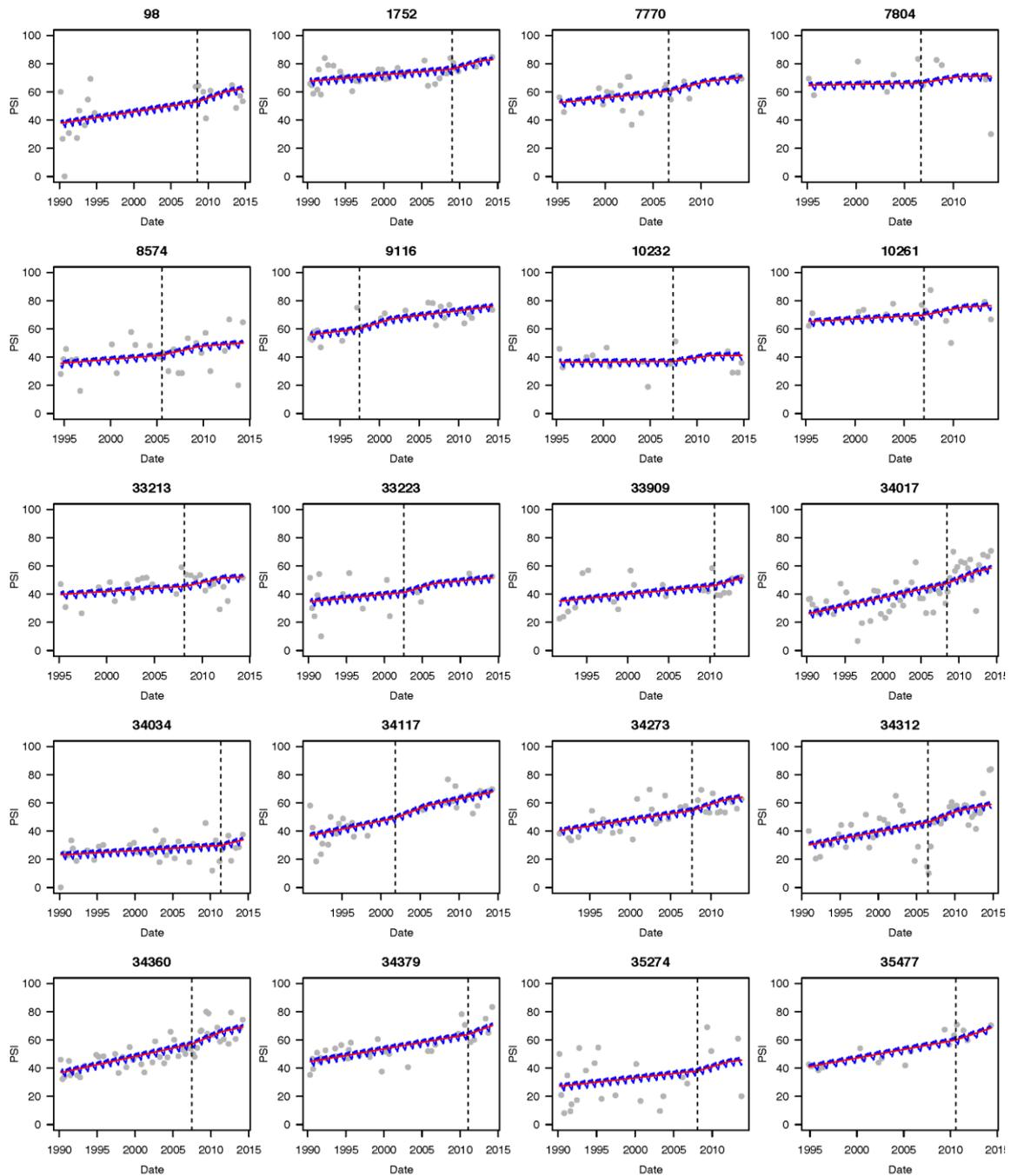
Appendix D. Coefficients, p -values and confidence intervals for gradual step change linear mixed effect models and linear quantile mixed models of PSI_{fam} , $E-PSI_{fam69}$ and fine sediment, for all sites, and sites split into subgroups based on their (i) habitat heterogeneity and (ii) percentage of coarse substrate; (Both groups 1-3: low-high).

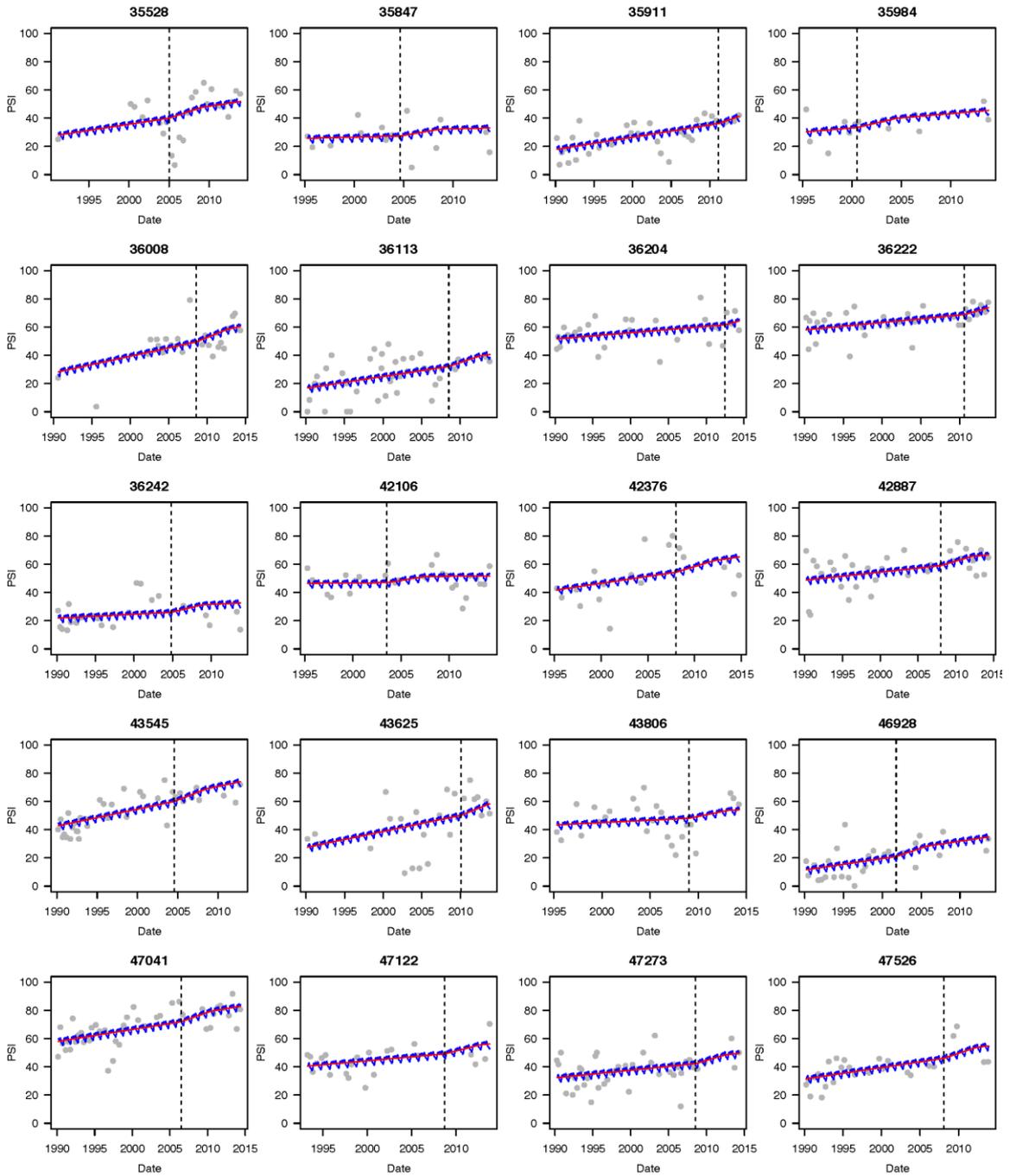
		Linear mixed effects model			Linear quantile mixed model		
		Coefficient	p -value	Confidence intervals	Coefficient	p -value	Confidence intervals
All sites $n = 71$	PSI_{fam}	4.130	< 0.001	2.090, 6.169	4.409	0.079	-0.521, 9.340
	$E-PSI_{fam69}$	0.423	0.742	-2.092, 2.938	0.454	0.819	-3.503, 4.411
	Sediment	-2.306	0.227	-6.046, 1.433	-5.601	0.134	-12.997, 1.795
Heterogeneity Group 1 $n = 24$	PSI_{fam}	-0.445	0.816	-4.187, 3.297	-2.552	0.377	-8.305, 3.201
	$E-PSI_{fam69}$	-5.323	0.027	-10.025, -0.620	-10.360	0.003	-17.066, -3.654
	Sediment	-9.860	0.011	-17.480, -2.240	-14.903	0.122	-33.909, 4.104
Heterogeneity Group 2 $n = 23$	PSI_{fam}	5.731	0.002	2.180, 9.282	3.549	0.349	-3.990, 11.089
	$E-PSI_{fam69}$	4.887	0.026	0.589, 9.185	-1.043	0.755	-7.728, 5.642
	Sediment	-0.048	0.987	-5.916, 5.820	-0.130	0.980	-10.427, 10.167
Heterogeneity Group 3	PSI_{fam}	7.361	< 0.001	4.032, 10.690	10.460	0.012	2.357, 18.564

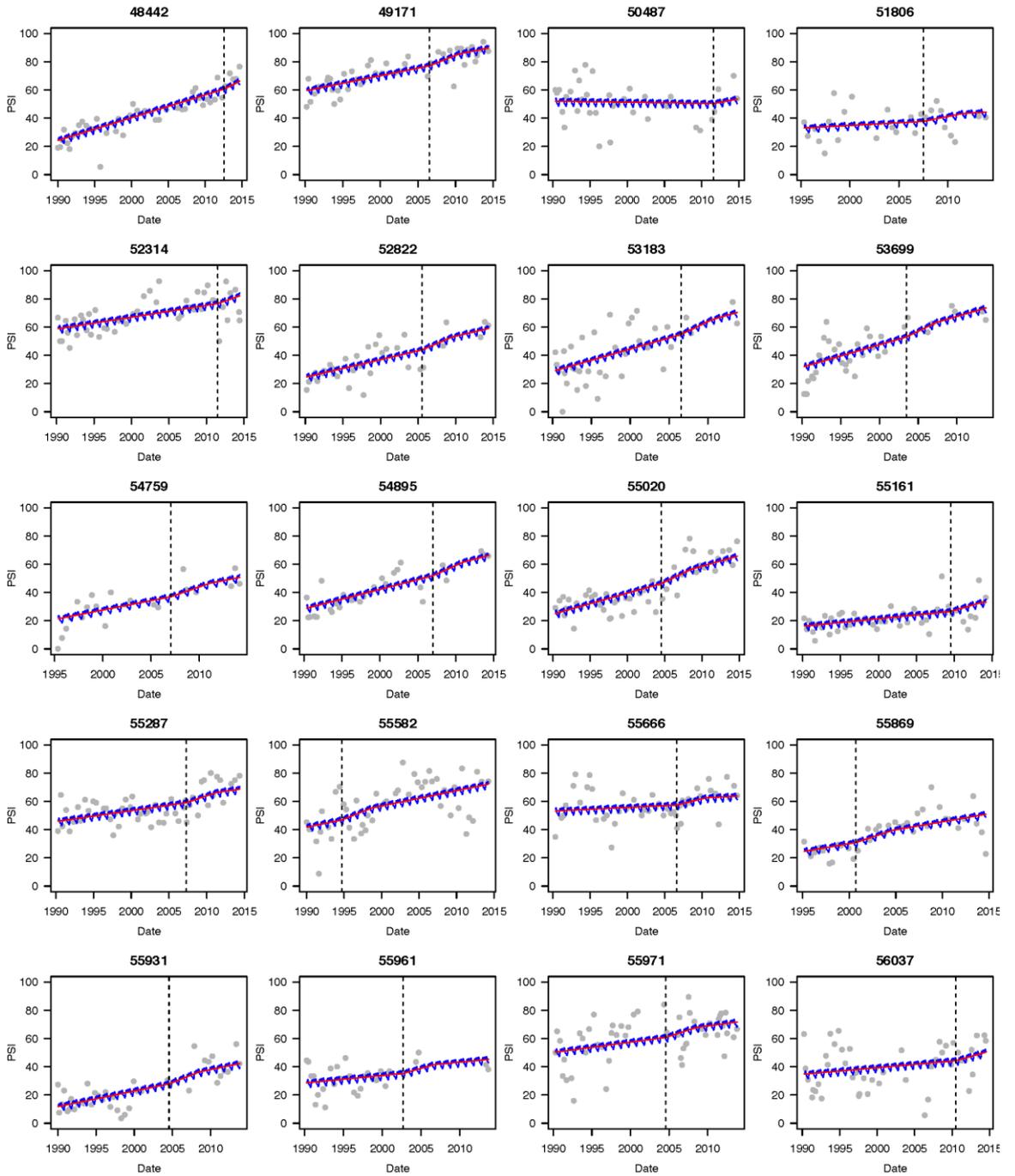
		Linear mixed effects model			Linear quantile mixed model		
		Coefficient	p-value	Confidence intervals	Coefficient	p-value	Confidence intervals
<i>n</i> = 24	E-PSI _{fam69}	0.863	0.682	-3.275, 5.000	2.004	0.658	-7.042, 11.050
	Sediment	3.001	0.322	-2.944, 8.946	-0.926	0.849	-10.634, 8.782
Coarse substrate Group 1 <i>n</i> = 26	PSI _{fam}	1.942	0.337	-2.029, 5.914	0.736	0.834	-6.264, 7.735
	E-PSI _{fam69}	0.095	0.971	-5.057, 5.246	-3.226	0.468	-12.088, 5.636
	Sediment	-10.946	0.011	-19.333, -2.559	-12.864	0.143	-30.221, 4.492
Coarse substrate Group 2 <i>n</i> = 23	PSI _{fam}	10.068	< 0.001	6.806, 13.330	11.487	< 0.001	5.397, 17.577
	E-PSI _{fam69}	4.603	0.024	0.596, 8.610	4.226	0.123	-1.181, 9.634
	Sediment	1.129	0.716	-4.949, 7.207	0.467	0.930	-10.227, 11.161
Coarse substrate Group 3 <i>n</i> = 22	PSI _{fam}	-0.321	0.850	-3.659, 3.017	0.416	0.889	-5.550, 6.382
	E-PSI _{fam69}	-4.152	0.034	-7.989, -0.316	-6.347	0.107	-14.112, 1.419
	Sediment	1.526	0.490	-2.812, 5.863	-2.768	0.366	-8.868, 3.333

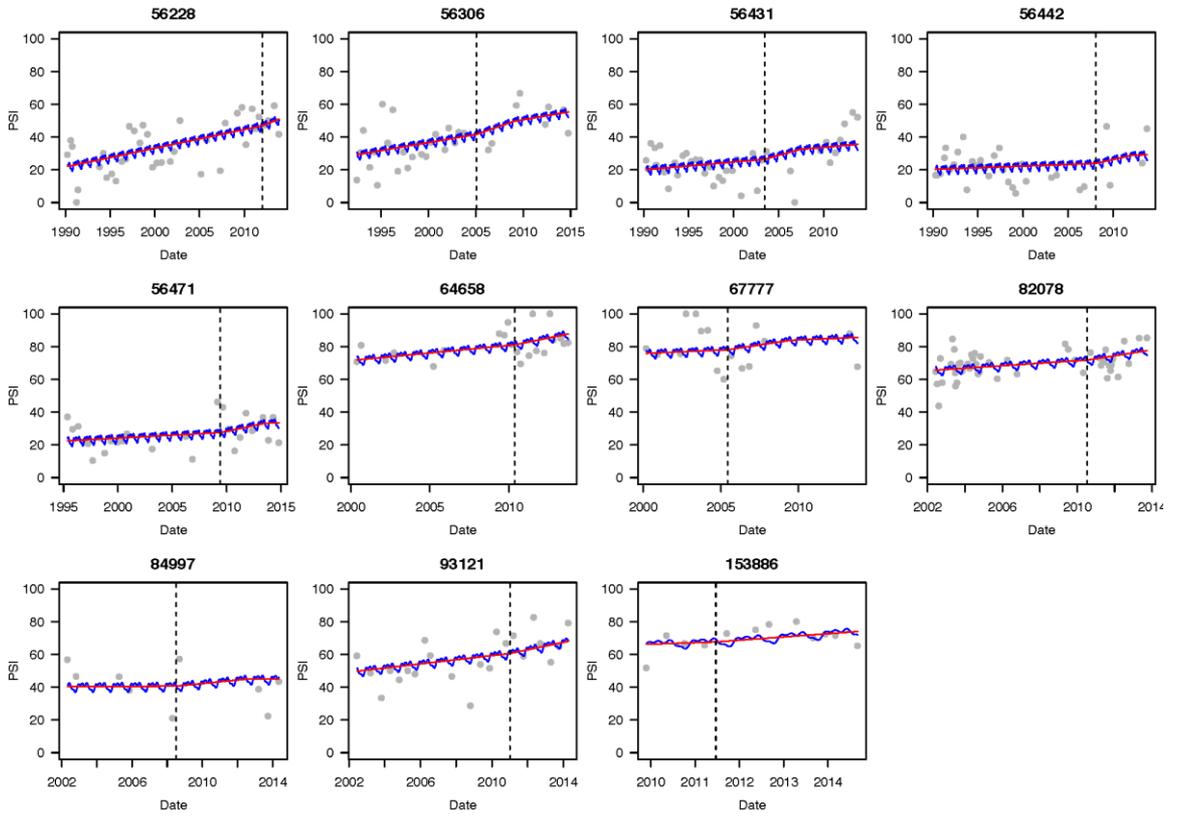
Bold text denotes outcome coefficient was statistically significant.

Appendix E. Plots of linear mixed effects models for time series data of the Proportion of Sediment-sensitive Invertebrates (PSI) index scores, across 71 sites (site numbers above plots). Dotted vertical line indicates invasion date.

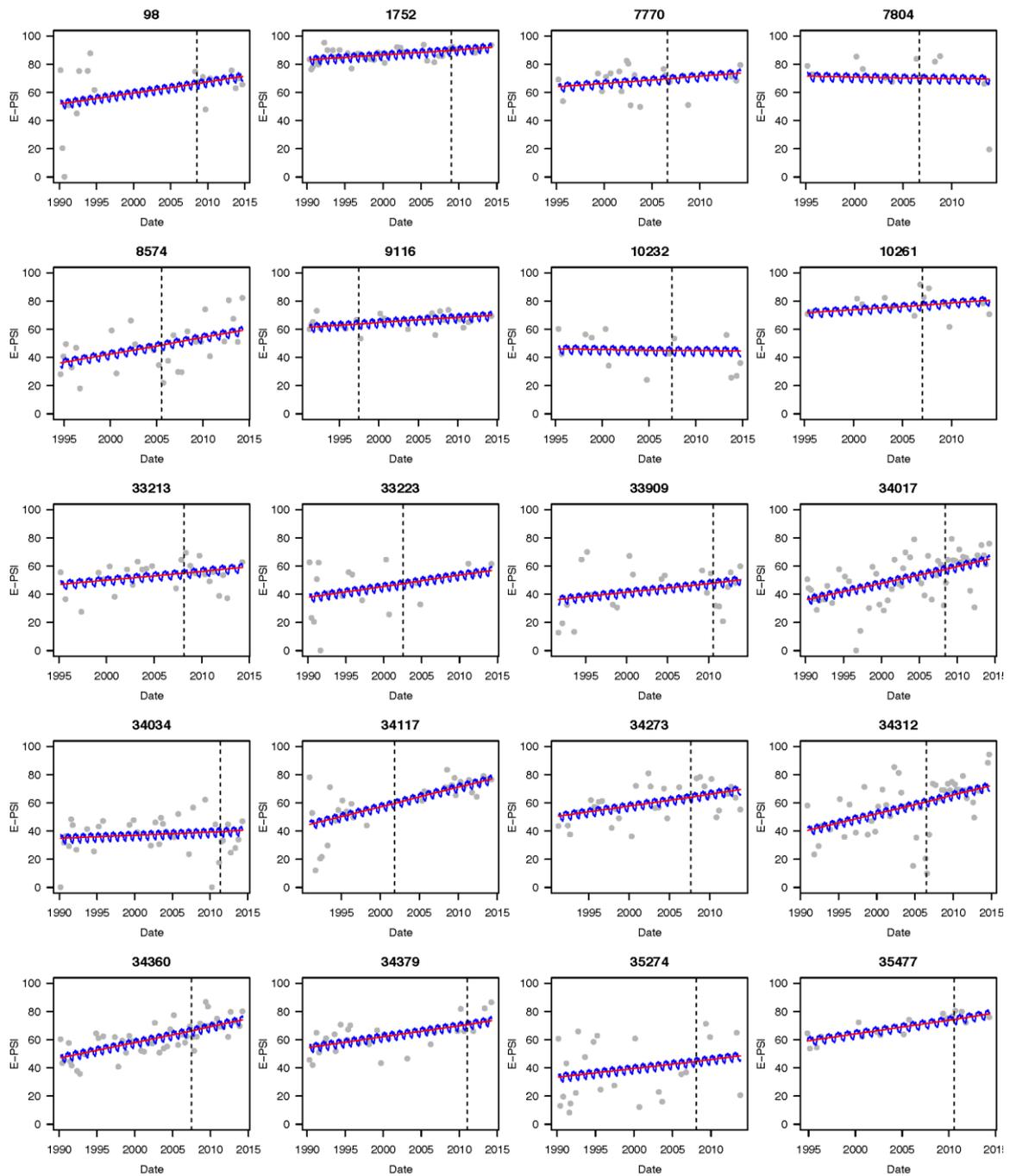


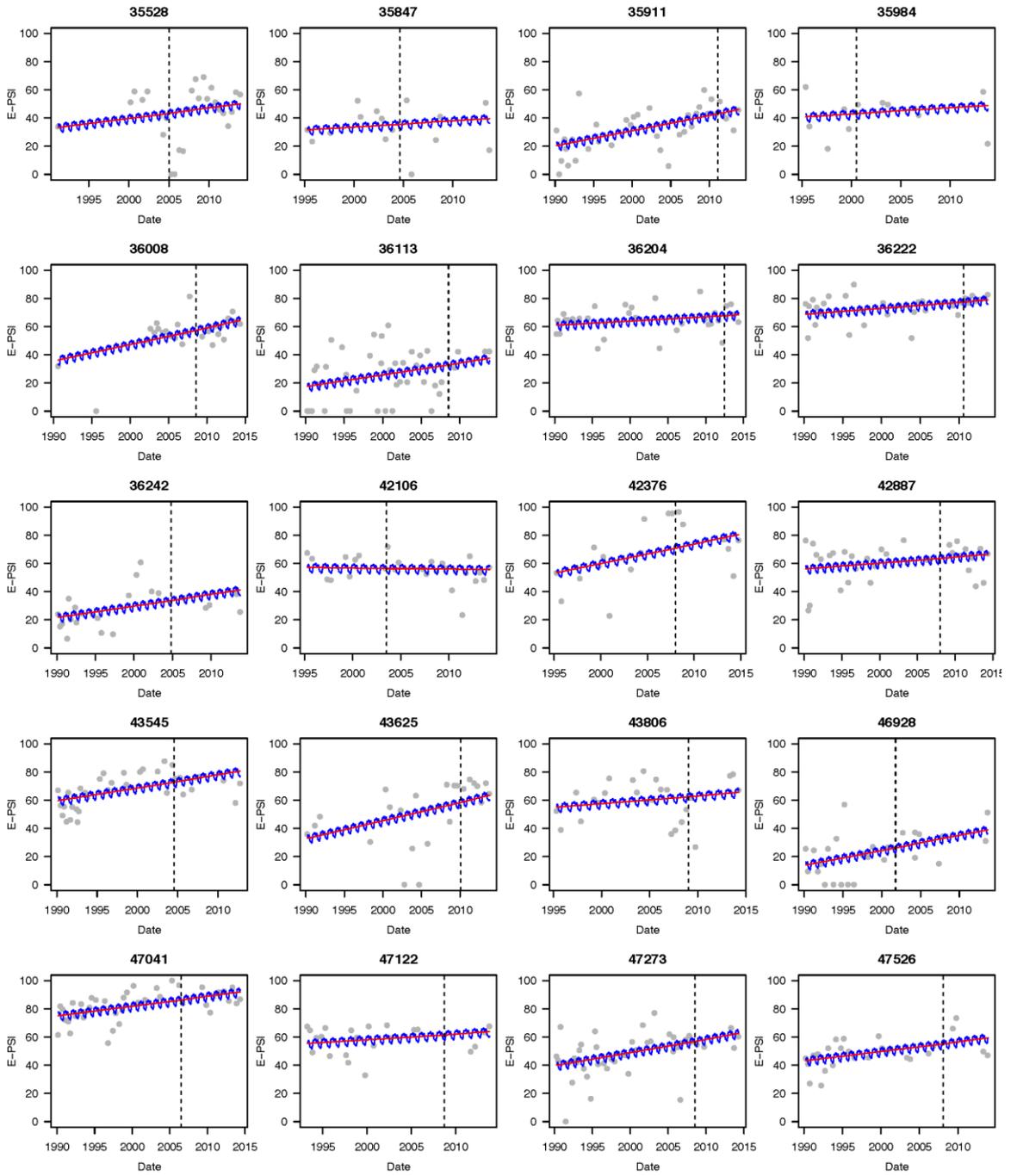


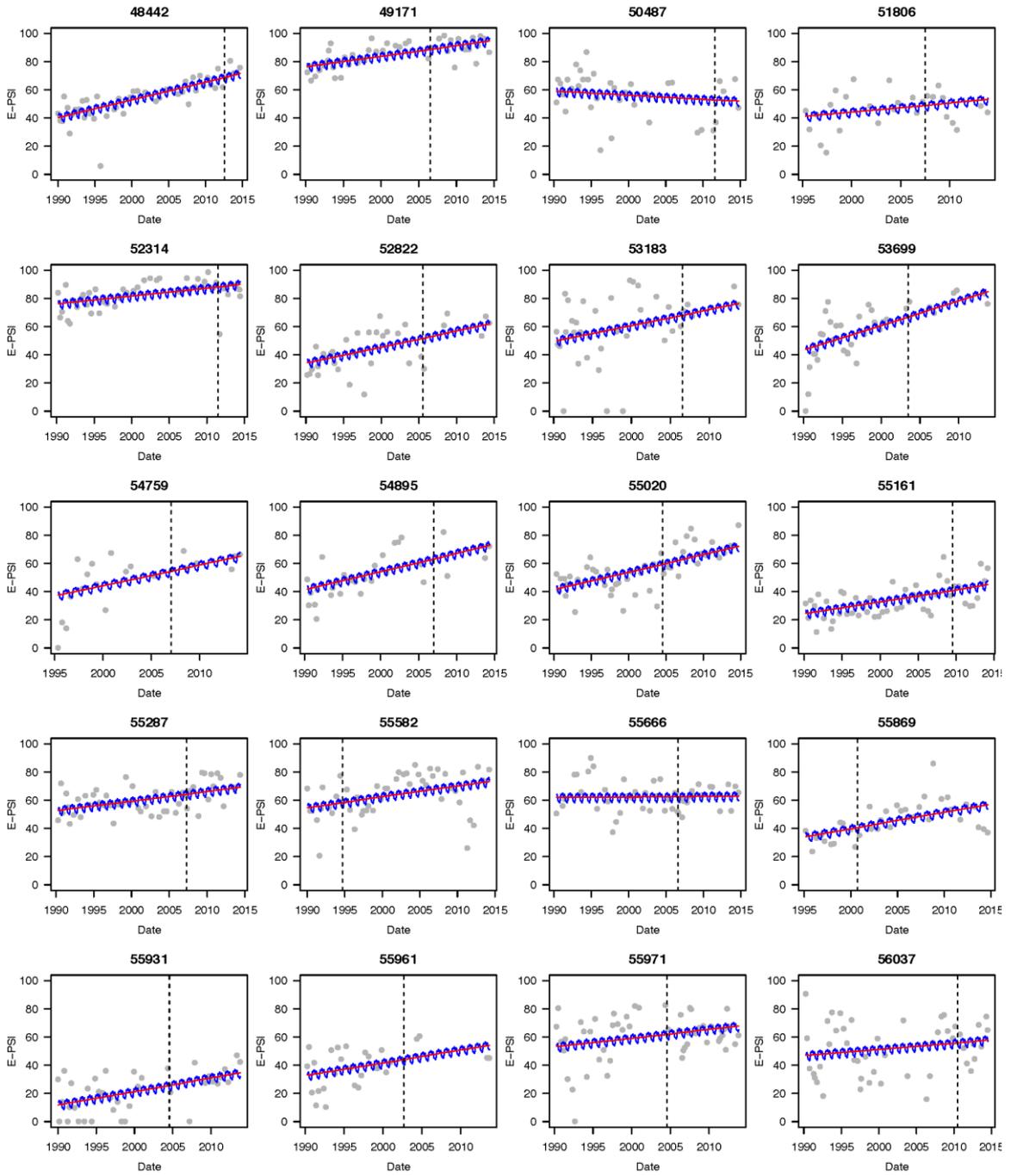


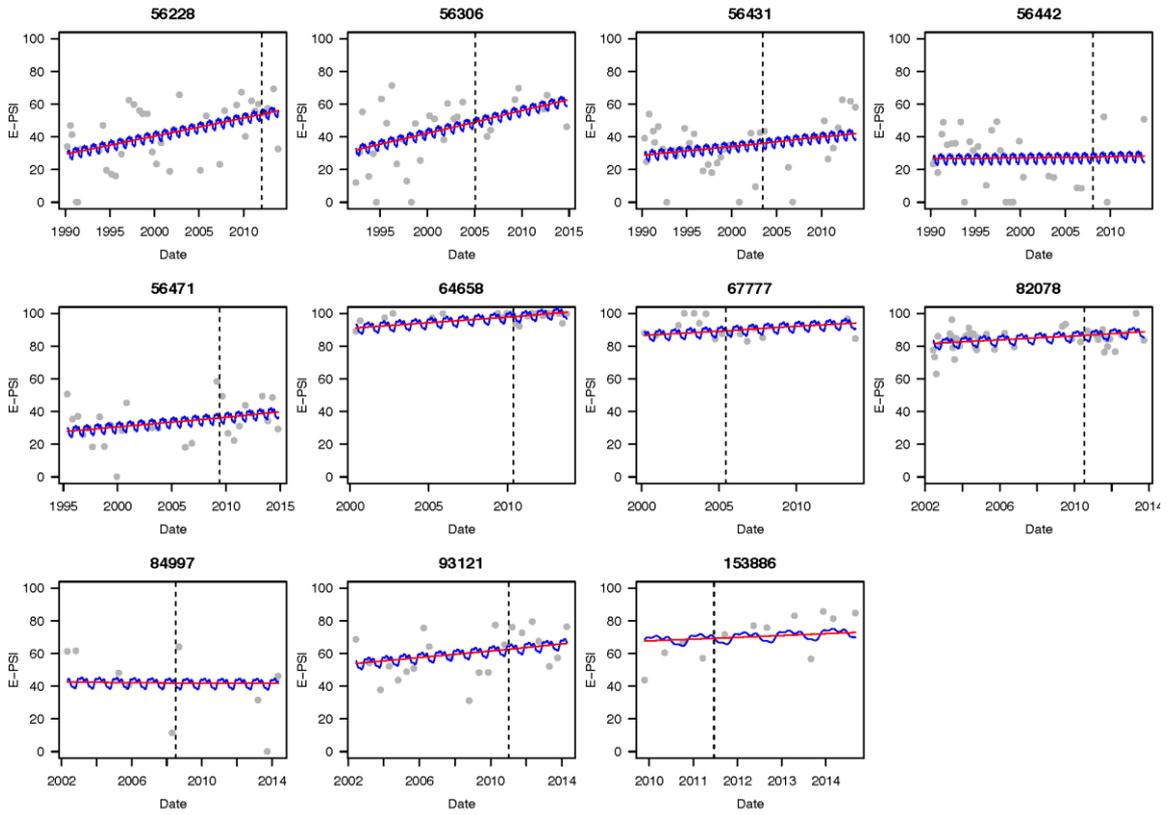


Appendix F. Plots of linear mixed effects models for time series data of Empirically-weighted Proportion of Sediment-sensitive Invertebrates (E-PSI) index scores, across 71 sites (site numbers above plots). Dotted vertical line indicates invasion date.









Appendix G. Plots of linear mixed effects models for time series data of percentage

fine sediment, across 71 sites (site numbers above plots). Dotted vertical line

indicates invasion date.

