Macroinvertebrate community structure as an indicator of phosphorus enrichment in rivers

Nicholas C. Everall[1*], Matthew F. Johnson[2], Paul Wood[3], Martin F. Paisley[4] and David J. Trigg[5] and Andrew Farmer[1]

[1*] Aquascience Consultancy Limited, 18 Hawthorne Way, Ashgate, Chesterfield, Derbyshire S42 7JS, UK.

[2] School of Geography, University of Nottingham, NG7 2RD, UK

[3] Centre for Hydrology and Ecosystem Science, Geography and Environment, Loughborough University, Loughborough, Leicestershire, LE11 3TU, UK.

[4] School of Creative Arts and Engineering, Staffordshire University, Stoke on Trent, ST4 2DE, UK

[5] School of Computing and Digital Technologies, Staffordshire University, Stoke on Trent, ST4 2DE, UK

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Abstract (223 words):

Nutrient enrichment represents one of the most important causes of detriment to river ecosystem health globally. Monitoring nutrient inputs can be particularly challenging given the spatial and temporal heterogeneity of nitrogen and phosphorus concentrations and the indirect and often lagged effects on instream faunal communities. In this paper we utilise existing and new datasets to explore the association between family level macroinvertebrate community data and Total Reactive Phosphorus (TRP). To achieve this, a biological index for phosphorus sensitivity (Total Reactive Phosphorus Index -TRPI) was developed and tested utilising data from 88 sites across England. There was a significant association between TRPI and TRP concentrations that was stronger than other macroinvertebrate indices currently available in the UK to characterise nutrient enrichment. Additional testing and validation are presented via local case studies, where results indicate that macroinvertebrate family sensitivity is dependent upon a range of abiotic factors including season (time of year), benthic substrate composition, altitude, and water alkalinity. At a national and local scale, TRPI offers additional information not currently available using other biological metrics.

1. Introduction

Nutrient enrichment represents one of the most pervasive and detrimental threats to water quality globally (Bennett et al. 2001; Withers et al. 2014). Agricultural intensification and application of fertilizers, including manure, onto arable and pastoral land, potentially increases nutrient loads delivered to rivers, as can wastewater treatment and urban runoff. Elevated phosphorus (P) is considered the leading cause of failure to meet EU Water Framework target status in England (Environment Agency 2012) and one of the main pressures on waterbodies globally (Evans-White et al. 2013; Javie et al. 2013; Mekonnen & Hoekstra, 2018). Widespread recognition of the historic detrimental impacts of elevated P has resulted in targeted management of its application across Europe and the USA over the last 20 years (Bouraoui and Grizzetti, 2011; Schoumans et al. 2015), but levels still regularly exceed those known to negatively affect the wider environment (Worrell et al. 2016; Everall *et al*. 2018). Monitoring P is logistically challenging given the temporal variability in concentrations known to occur (Bieroza & Heathwaite 2015; Bowes et al. 2015; Dupas et al. 2015). In addition, the identification of ecological effects of P are sometimes difficult to detect because of interactions among all trophic levels, lagged ecological responses and inherent differences associated with river type (e.g. altitude, geology, soil type) and other 'stressors' (Javie et al. 2013; Emelko et al. 2016). As a result, there is currently no standard methodology available to characterise or identify P pressures on instream communities that can be used to inform freshwater management or to determine if reductions in P lead to the expected / anticipated ecological recovery.

Freshwater algae and macrophytes require several macronutrients for growth, particularly nitrogen and P (Conley *et al.*, 2009). As P is usually limited in riverine systems, excessive nutrient loading can lead to excessive development of plant life (Evans-White et al. 2013; Azevedo et al. 2015; Javie et al. 2015), with interactive effects on the availability of faunal trophic resources, habitat availability and wider implications for ecosystem functioning and faunal community structure (Tessier et al. 2008; Binzer et al. 2015). Therefore, the mechanisms by which nutrient enrichment and particularly P affect instream communities can be complex.

It is widely acknowledged that nutrient enrichment reduces instream faunal biodiversity (Smith, 2003; Hilton *et al.*, 2006; Bini et al. 2014) and, in particular, decreases richness of macroinvertebrates through a reduction in the diversity of aquatic insect orders such as Ephemeroptera, Plecoptera and Trichoptera (Ortiz & Puig, 2007; Friberg *et al.*, 2010; Yuan, 2010). Specific responses to nutrient enrichment have been examined and community responses found to be complex (e.g. Piggot et al. 2012). There is evidence that invertebrate communities respond to strong nutrient gradients (Smith et al. 2007; Yuan, 2010; Heiskary & Bouchard Jr, 2015), potentially enabling biomonitoring techniques to be used to assess and quantify P pressures. The classic approach used for over 40-years is the Saprobic Index, widely used in continental Europe to assess nutrient stress on macroinvertebrates associated with reduced dissolved oxygen and increasing ammonia concentrations, which are often associated with eutrophication (Pantle & Buck, 1955; Zelinka & Marvan 1961).

The use of freshwater macroinvertebrates as biological indicators is well established, and a range of indices have been developed based on macroinvertebrate community responses to a range of environmental pressures and gradients (see Friberg *et al*. 2010). Macroinvertebrate biomonitoring across Europe is routinely used as one of the key indicators for compliance with national and international standards, such as ‘Good Ecological Status’ under the European Union Water Framework Directive (WFD) (WFD, 2000).

In the UK, the impact of Total Reactive Phosphorus (TRP – the biologically available P contribution) is currently assessed using the response and community change of diatoms (Trophic Diatom Index - TDI) (Kelly and Whitton, 1995; Kelly, 1998) or macrophytes (Mean Trophic Rank – MTR) (Holmes et al. 1999), in conjunction with monthly water chemistry measurements. There have been relatively few attempts internationally to use macroinvertebrates within indices of nutrient pressure, probably because the effects are largely considered indirect when compared to those experienced by macrophytes and algae (Maidstone and Parr, 2002). One exception is the research of Smith et al. (2007) who successfully developed a biomonitoring index for Total P and Total Nitrate using macroinvertebrates in New York State, USA.

Therefore, the development of a biomonitoring tool for quantifying the degree to which riverine TRP concentrations impact upon the macroinvertebrate community in the UK would be beneficial. Such a metric would complement existing eutrophication indicators for WFD classification (e.g. TDI, MTR) and align with other macroinvertebrate community base indices developed for other stressors (e.g. Proportion of Sediment-sensitive Invertebrates [PSI]; Extence et al. 2013). Ideally, such a tool could be applied to routinely collected macroinvertebrate data and retrospectively applied to historic data sets. In this paper, we detail the development and testing of a new family-level macroinvertebrate index, the Total Reactive Phosphorus Index (TRPI), and assess its ability to characterise the effects of TRP on riverine ecosystems. Specifically, we:

1. Explore whether there is a statistical relationship between family-level macroinvertebrate community data and TRP nationally;
2. To compare the strength of macroinvertebrate relationships with TRP to traditional biological measures of eutrophication, including diatom and macrophyte community composition;
3. Using case studies and national data, to assess whether a TRP macroinvertebrate biomonitoring index provides additional information unavailable using existing metrics;
4. To assess the ability of macroinvertebrate biomonitoring to identify changing TRP pressures using specific case studies;

2. Methodology

2.1. Background work on invertebrate family sensitivity to TRP

TRPI was developed utilising historic datasets which identified macroinvertebrate taxa that had strong statistical associations with TRP (Paisley *et al*., 2003; Everall, 2010; Paisley *et. al.*, 2011). Paisley et al. (2003) used chemical, environmental and biological data collected by the Environment Agency (EA) in 1995 covering a range of nutrient concentrations across England, Wales and Northern Ireland, to determine which invertebrate families were potential indicators of P status. Chemical data comprising monthly spot-measures of the concentration of 34 chemical variables, including TRP, were averaged over the three-month period prior to the collection of biological samples. Biological data comprised the abundance of spring and autumn macroinvertebrate samples based on the 76 BMWP scoring families. Paisley et al. (2003; 2011) used Mutual Information theory (MI) and impact analysis to quantify the association between macroinvertebrate families and 34 chemical measurements and 11 environmental measurements. This was corroborated by neural network analysis which demonstrated good statistical agreement with MI analysis (discussed further in Paisley *et al*. 2003).

Paisley *et. al.* (2011) attempted to minimise the effect of other environmental factors on invertebrate community composition by differentiating indicators of TRP for both spring and autumn and for different river habitat/morphology types. Specifically, they categorised each site into one of five river types using neural network analysis, which identified altitude, alkalinity and substrate composition as key controls on macroinvertebrate community response to TRP (Paisley *et. al.,* 2011). The five site typology represents a progression from fast-flowing upland streams to slow-flowing lowland streams, with generally increasingly alkalinity and fining of substrate particle size (Table 1).

Table 1: *Characteristics of the 5 site types that differentiate TRP indicator invertebrates after Paisley et al. (2011). Descriptions and TRP levels are only included as indications. To determine site type, focus should be given first to the composition of the substrate, then the alkalinity and finally to the altitude.*

Site type	Description	Composition of substrate (% by area)				Alkalinity (mg L^{-1})	Altitude (m)	TRP level
		Boulders	Pebbles	Sand	Silt			
1	Upland, fast-flow	50	40	5	5	30	> 100	Low
2		40	50	5	5	90	30 – 100	
3	↓	30	50	10	10	180	30 – 100	↓
4		10	50	20	20	220	30 – 100	
5	Lowland, slow flow	5	25	20	50	230	< 30	High

2.2. Model development and comparison to TRP

The research of Paisley et al. (2003; 2011) was used to construct a single score – the Total Reactive Phosphorus Index (TRPI). This score indicates the TRP impact on the macroinvertebrate community. The strength of the statistical association of macroinvertebrate families with TRP, based on modelling for different seasons and river types (Paisley et al. 2003; 2011), was used to assign macroinvertebrate families into sensitivity groups (Supplementary A), adopting the principle of the Lotic-invertebrate index for Flow Evaluation (LIFE), Community Conservation Index (CCI) and PSI scores used in the UK for assessment of flow stress, conservation value, and fine sediment pressures, respectively (Extence et al. 1999; Chadd and Extence, 2004; Extence et al. 2013). Sensitivity groups A and B indicate high and moderate sensitivity to TRP, respectively, whereas categories C and D indicate tolerance and high tolerance to TRP, respectively (Table 2). The purpose of the sensitivity categories is to weight the abundance of a macroinvertebrate family according to its sensitivity or tolerance to TRP.

The classification was then used to develop a TRPI score, using the same computational structure as the PSI (Extence et al. 2013). The resultant score describes the percentage of TRP sensitive taxa present in a sample, and is calculated as:

$$TRPI = \frac{\sum Nutrient\ scores\ for\ Groups\ A\ \&\ B}{\sum Nutrient\ scores\ for\ Groups\ A, B, C, D} \times 100$$

To calculate the TRPI, the taxa comprising the sample must be partitioned into their respective sensitivity group using Supplementary Material A. The grouping of invertebrates depends on the river type, which can be determined by examination of Table 1. When selecting from the table, weighting should be given to the closest substrate composition at the sample site, followed by alkalinity and altitude. In addition, look-up tables are dependent on the season the sample was collected (spring or autumn). Once river type and season have been identified, the correct look-up table can be selected from Supplementary Material A. The nutrient score for each group is then calculated using Table 2, which is abundance weighted, following the principle of other UK biomonitoring tools (e.g., PSI and LIFE score). The TRPI score ranges from 0, indicating that TRP-sensitive taxa are absent from the sample and, therefore, the site is likely to be heavily TRP impacted, to 100, which indicates 100% of the community is TRP-sensitive and, therefore, the site is likely to have limited TRP concentrations (Table 3).

Table 2: *TRP tolerance bandings and the nutrient score associated with each, which is dependent on the abundance of that family.*

Group	TRP Tolerance Definition	Log Abundance			
		1 - 9	10 – 99	100 - 999	1000+
A	Taxa highly sensitive to TRP	2	3	4	5
B	Taxa moderately sensitive to TRP	1	2	3	4
C	Taxa tolerant to TRP	1	2	3	4
D	Taxa very tolerant to TRP	2	3	4	5
E	Taxa indifferent to TRP or excluded from methods for other reasons	-	-	-	-

Table 3: *Interpretative bandings of the TRPI, ranging from 0 to 100.*

TRPI	Nutrient Condition
81 - 100	Very low TRP
61 – 80	Low TRP
41 – 60	Moderate TRP
21 – 40	High TRP
0 - 20	Very High TRP

2.3. Model testing and utility in comparison to other metrics

The ability of the TRPI to characterise TRP effects at a site was tested by correlating TRPI with measured chemical concentration of TRP at the same site. Correlations of TRPI to TRP were performed using two separate data-sets, both comprising data collected from across England. The first was collected by the authors at 88 sites across England between 2013 and 2015, providing 156 data points as most sites were sampled in spring and autumn (Figure 1; Supplementary Material B). These represented a range of TRP concentrations and geographical locations. TRPI was calculated using macroinvertebrate data collected using EA standard protocol 3-minute kick samples followed by 1-minute hand searching (Environment Agency, 2009). The TRP was calculated as a seasonal average concentration derived from EA monthly spot measurements at the same location. The second data set constituted 76 sites from across England, monitored by the EA in 2015 for chemical TRP concentrations, TDI, MTR and family-level macroinvertebrate community data, which were used to calculate TRPI and other commonly used macroinvertebrate indices (Figure 1; Supplementary Material C). These sites did not have the same range of TRP concentration as the author-collected database but had the advantage of concurrent measurements of TDI, MTR, chemical TRP and invertebrate community in the same season by the EA following standard protocols (Holmes et al. 1999; UKTAG 2013). Therefore, both data sets were examined to provide multiple opportunities to validate TRPI index. For both data-sets, scores from spring and autumn were included within the same correlation because TRPI accounts for seasonality in the metric calculation and, therefore, the scores are comparable.

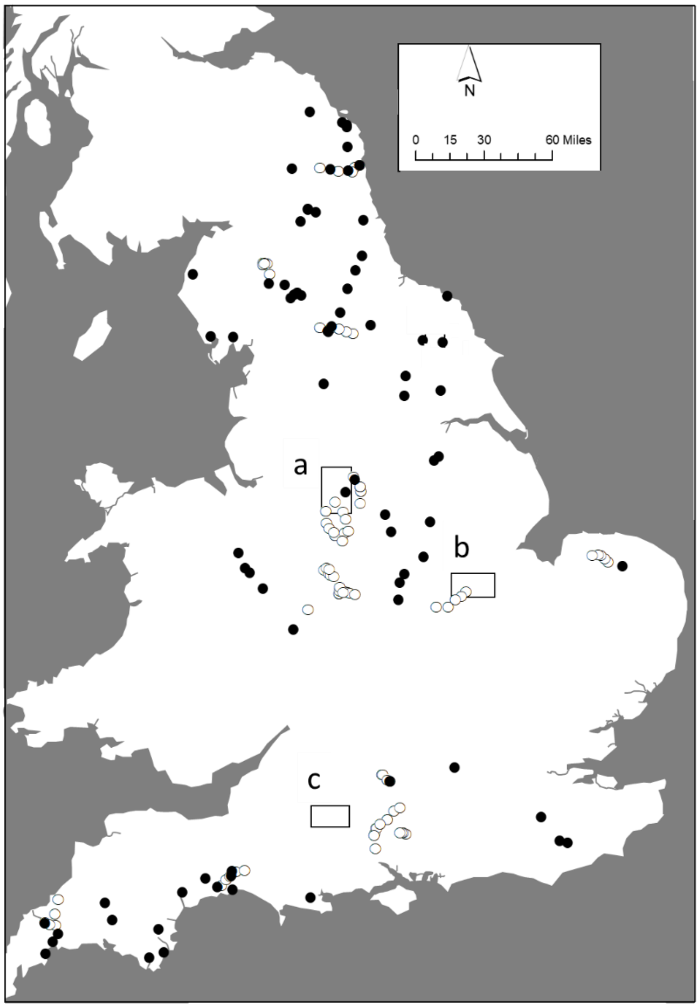

Figure 1: Map of sites included in the analysis. Open circles are author sampled sites and filled circles are EA sites. Rectangles indicate case study rivers: River Dove (a), River Welland (b) and River Wylye (c).

An increasing strength of correlation between biological metrics of TRP (e.g. TDI, MTR and TRPI) and measured chemical TRP was not necessarily deemed to indicate a greater utility because each score potentially characterises a different aspect of instream TRP effects, i.e. TRPI specifically aims to indicate the effect of TRP on the invertebrate community whereas TDI indicates the effect on algal communities.

Therefore, significant positive correlation between variables with TRP was considered a success, with an expectation for closer associations at higher TRP concentrations, where P is more likely to be the dominant control on biological communities.

TRPI was also examined directly in association with 9 other benthic macroinvertebrate biomonitoring scores, detailed below. Here, close similarity between metrics with TRPI would indicate redundancy in the utility of one of the biological metrics as they are supposed to be identifying different pressures. The proportion of Ephemeroptera, Plecoptera, Trichoptera (EPT) in a sample has been used internationally as an ecological indicator of water quality (Stanford and Spacie, 1994). The Biological Monitoring Working Park (BMWP) score (Armitage et al. 1983) scores 76 macroinvertebrate families based on their sensitivity to organic pollution and until recently formed the basis of WFD classification in the UK along with the Average Score Per Taxon (ASPT), derived from the BMWP score divided by the total number of scoring families (Armitage et al. 1983). In 2013, the BMWP and ASPT were updated by integrating abundance weighting into its derivation into the Whalley, Hawkes, Paisley and Trigg (WHPT) score, which takes the BWMP family sensitivity score and weights it by the abundance of that family found in the sample (Whalley and Hawkes, 1997; Paisley et al. 2013; 2014). When the WHPT is divided by the total number of scoring taxa, this gives the WHPT ASPT. Given the established nature of this progression of metrics in the UK, all are still derived and therefore all are tested here. In addition, more stressor-specific metrics were tested, including the LIFE score (flow pressure; Extence et al. 1999), PSI score (fine sediment pressure; Extence et al. 2013) and the

Saprobic Index, which is used in continental Europe to assess organic pollution stresses (Roulaffs et al. 2004).

2.4. Case study test sites

Given the limitations of correlative comparisons in understanding metric performance, a series of case studies were developed using historic macroinvertebrate and TRP data. These case studies were used to identify whether TRPI was related to TRP at a site scale, and whether other biological metrics provide a better characterisation of, or are correlated to, TRPI.

The case studies presented here are for the: River Wylye, Wiltshire; River Welland, Northamptonshire and; the River Dove, Staffordshire (Figure 1). An overview of the case study site geography and background information is provided in supplementary material D. The case studies were selected to represent a range of TRP loadings and trajectories and to represent different regional, geological, hydrological and land use scenarios.

3. Results

3.1. Statistical relationship between family-level macroinvertebrate community data and TRP (Objective 1)

There was a statistically significant relationship between TRPI and measured TRP concentrations across the 76 EA monitoring sites (r = 0.72) and the 156 additional samples in England (r = 0.86) (Table 4). The smaller sample of EA sites showed a linear decrease in TRPI with increasing TRP concentration, whereas the 156 sampled sites showed an exponential decline in TRPI with increasing TRP, most likely because the latter covered a greater range of TRP values. In both cases, there was a clustering of points at low TRP values.

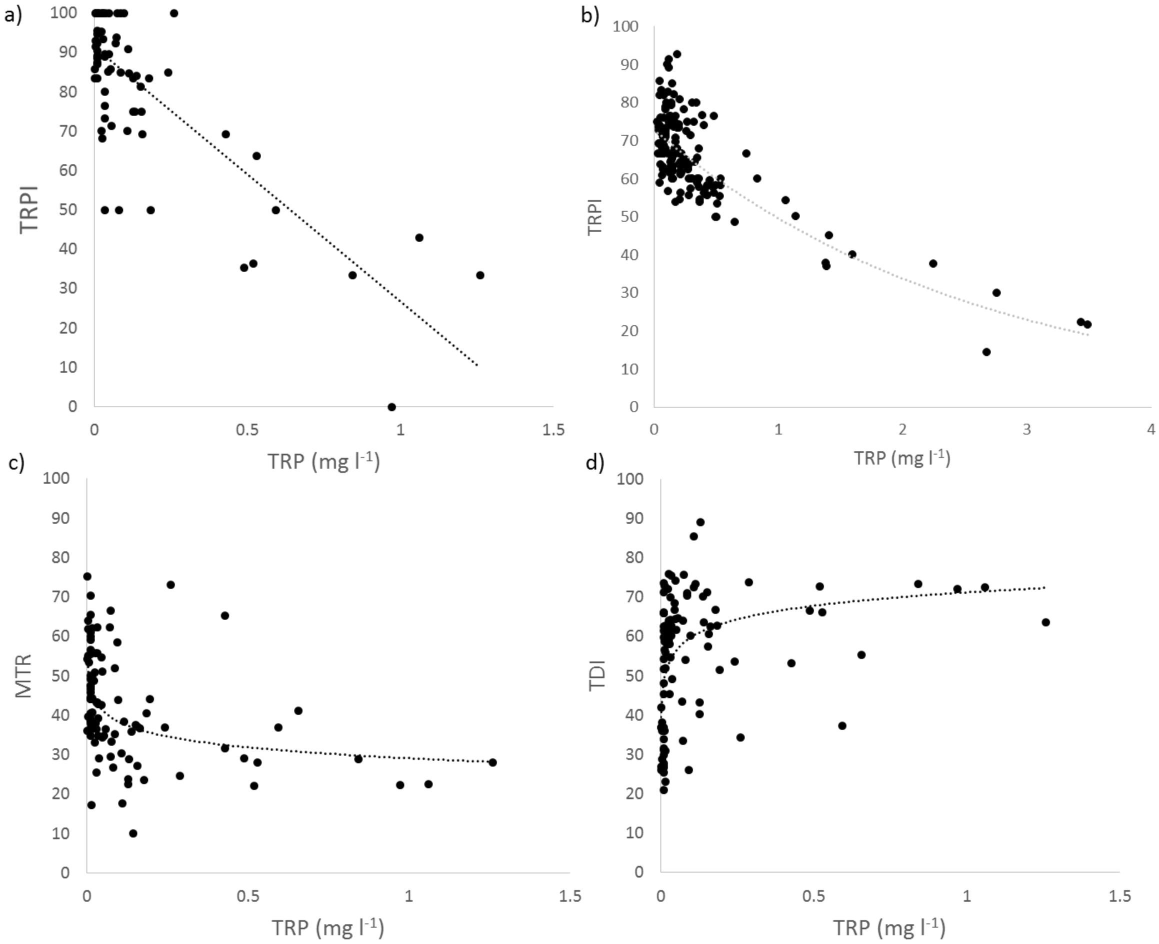

Figure 2: Scatter plots with linear and exponential lines of best showing a) TRPI against TRP measured at 76 sites by the EA in 2015, b) TRPI against TRP at 88 sites measured by the authors in spring and autumn, c) MTR against TRP and, d) TDI against TRP derived from the same 76 sites as TRPI in panel a.

Table 4: Correlation coefficients (*r*) and equations between TRP (mg l^{-1}) and TRPI; between TRPI and the MTR and TDI; and between TRPI and 8 commonly used biomonitoring indices in the UK. TRPI was correlated to TRP at 156 sites sampled by the authors and separately on 76 sites sampled by the EA where diatoms (TDI) and macrophytes (MTR) were also recorded. Number of data points is shown by *n*. All correlations were statistically significant.

x	**Y**	***n***	***r***	**Equation**
TRP (mg l^{-1})	TRPI	76	-0.72	Linear
TRP (mg l^{-1})	TRPI	156	-0.86	Exponential
TRP (mg l^{-1})	MTR	76	-0.47	Log
TRP (mg l^{-1})	TDI	76	0.47	Log
TDI	MTR	76	-0.27	Linear
TDI	TRPI	76	-0.52	Linear
MTR	TRPI	76	0.40	Linear
BMWP	TRPI	76	0.46	Linear
ASPT	TRPI	76	0.63	Linear
WHPT	TRPI	76	0.51	Linear
WHPT ASPT	TRPI	76	0.67	Linear
EPT	TRPI	76	0.44	Linear
PSI	TRPI	76	0.64	Linear
LIFE	TRPI	76	0.63	Linear
Saprobic	TRPI	76	-0.55	Linear

3.2. Comparison between TRPI to other biological measures of eutrophication, including diatom and macrophyte community composition (Objective 2)

The TDI and MTR were both correlated to TRP with significant, exponential relationships (Table 4). Ultimately, the relationships were relatively weak (*r* = 0.47 and *r* = 0.47, respectively) with biomonitoring values spread widely at low TRP values, especially for the TDI. The correlation between MTR and TDI was linear, significant and negative, and was anticipated given that they are both indicators of the same stressor with inverse scales (e.g. 100% indicates high impact for TDI and low impact for MTR). However, the relationship was associated with substantial scatter ($r = 0.58$). Similarly, TRPI was significantly correlated to both TDI ($p < 0.01$) and MTR ($p < 0.01$) but with weak associations in both instances ($r = 0.35$ and $r = 0.39$, respectively).

3.3. Comparison between TRPI and other, existing metrics

To determine the degree of collinearity and potential redundancy among indices, the TRPI was correlated with other commonly used macroinvertebrate community indices measured at 76 sites in England (Table 4). Significant correlations exist for TRPI with all metrics ($p < 0.01$), with r ranging from 0.44 (EPT) to 0.67 (WHPT ASPT); however, all relationships were weaker than that between TRPI and the target stressor TRP ($r = -0.72$). The highest other relationships are with WHPT ASPT ($r = 0.67$) and PSI ($r = 0.64$). The latter is indicative of elevated fine sediment, which can be related to elevated P which can be attached to sediment particles, particularly from agricultural fields (Owens and Walling, 2002).

3.4. Case studies

3.4.1. The River Wylye, Wiltshire (River Type 3)

The River Wylye is failing its WFD phosphate criteria, with a Moderate rating in 2016. It also has a Moderate rating for macrophytes and phytobenthos, but a High rating for macroinvertebrates and other water quality indicators, including ammonia and dissolved oxygen (DO). Chemical TRP measurements by Wessex Water indicated that TRP concentrations in the River Wylye have been reduced since the 1990's due to phosphate stripping from upstream sewage works discharges. TRPI calculated using both spring and autumn macroinvertebrate communities has consistently increased between 1991 and 2011, from low to very low TRPI values (Figure 2). This indicates that the macroinvertebrate community composition has shifted towards greater proportion of TRP sensitive families in association with declining concentrations of TRP over the same period.

Despite following the same broad trend over the 20-year monitoring period, the correlation between TRP and TRPI was relatively weak for both spring and autumn datasets ($r = 0.32$ and 0.45, respectively; Figure 2b). This is because whilst TRPI mirrors the declining trend and shorter-term fluctuations in TRP, the magnitude of fluctuations between years was not predicted well. Correlations for MTR ($n = 11$) and TDI ($n = 7$) against measured TRP indicated no significant correlation in either case and they incorrectly indicate increasing TRP pressure as TRP declines.

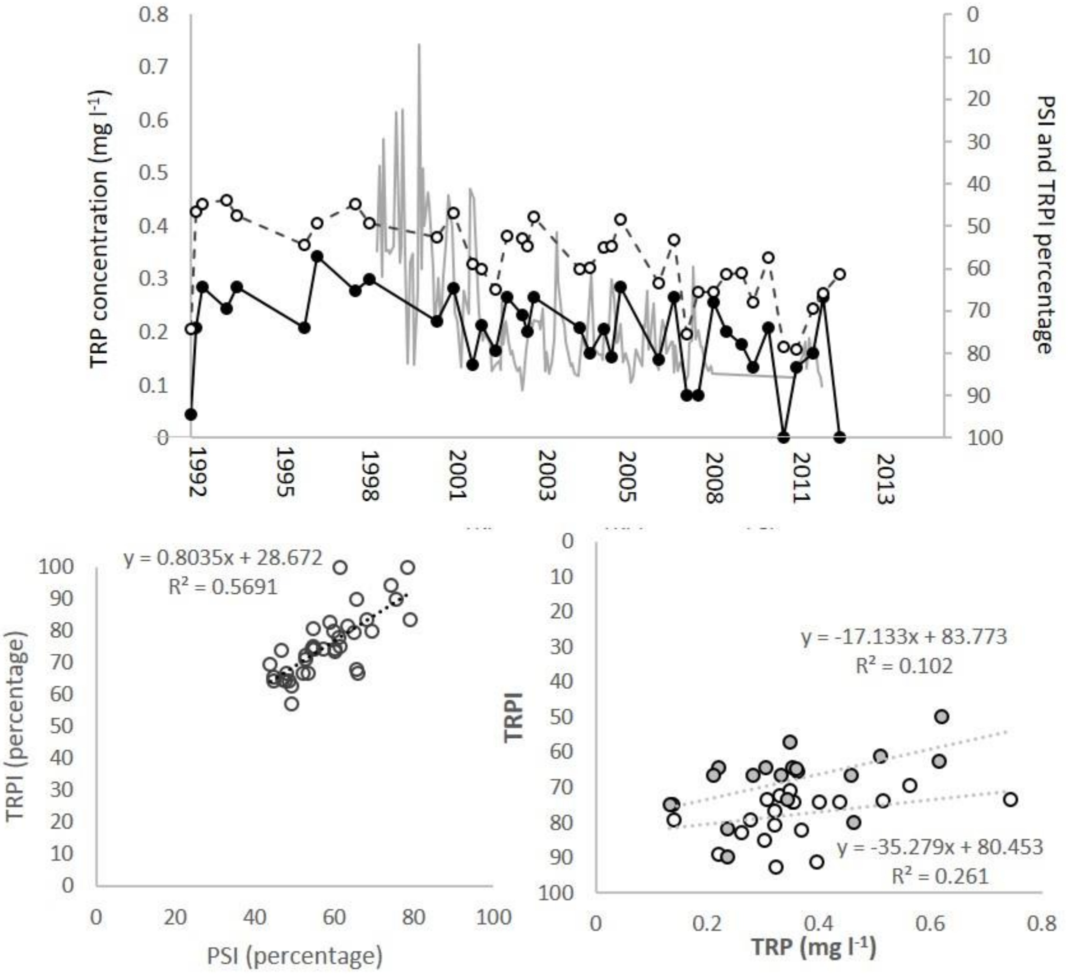

Figure 2: TRP conditions on the River Wylye at Norton Bavant. a) TRPI values (full circles) and PSI (open circles) from 1991 to 2011 with TRP concentration overlaid (grey line) over the same period. Note the y-axis is inverted so TRPI and PSI gradients follow TRP, with unimpacted conditions occurring at low TRP concentrations and impacted conditions are high values. b) Correlation between PSI and TRPI. c) Annual average TRP (mg l^{-1}) over the 12 months preceding the biotic score correlated against TRPI from spring (open) and autumn (closed) samples.

The PSI follows a similar increasing gradient to the TRPI, improving from moderately sedimented to slightly sedimented invertebrate community. There is a significant and relatively strong correlation between PSI and TRPI ($r = 0.75$, $p < 0.01$), although the correlation between PSI and TRP is weaker ($r = 0.31$) than that of TRPI. The saprobic index and WHPT are also significantly correlated to TRPI but with weaker relationships ($r = -0.38$ and $r = -0.65$, respectively). Other metrics are not correlated to TRPI (Supplementary E).

3.4.3. River Welland, Northamptonshire (River Type 4).

The River Welland at Collyweston, Rockingham and Harringworth all indicated a broad decline in TRP from 2001 to 2015 (Figure 4). Measured TRP levels ranged from 0.1 to 5.5 mg l^{-1} across the three sites, resulting in a Poor WFD classification. At each site, the TRPI displayed a gradual shift in macroinvertebrate community composition from highly impacted to low impacted communities sensitive to TRP. This was broadly consistent with TRP measurements, where winter peaks occurred before 2003 but declined thereafter due to nutrient management interventions (Rockingham $r = 0.49$; Harringworth $r = 0.41$; Collyweston $r = 0.68$). There was evidence of a lag in response at Harringworth, which had the highest TRP concentrations, because TRPI values drop 2 years after a substantial drop in TRP (Figure 4b). At Rockingham, the community composition indicated a change to increasing sensitivity to TRP, although a peak in TRP concentrations in 2015 (to 1.4 mg l^{-1}) was associated with a sudden rise in TRPI in spring 2015 from a low (68%) to moderately impacted community (48%) (Figure 4a). Despite differences in absolute TRP concentrations (e.g. peaks of 1 mg l^{-1} at Collyweston and peaks of 6 mg l^{-1} at Harringworth) the TRPI values were broadly comparable between sites. For all three sites, autumn TRPI was higher than spring TRPI.

Across the three sites there was no correlation between TRP or other biological metrics, including PSI (Supplementary E). However, PSI did follow a similar trajectory to TRPI and TRP and was significantly correlated to TRPI ($p < 0.01$, $r = 0.48$). Similarly, the WHPT shows an improving trend over the same period and across the same sites but was not significantly correlated to either TRP or TPRI.

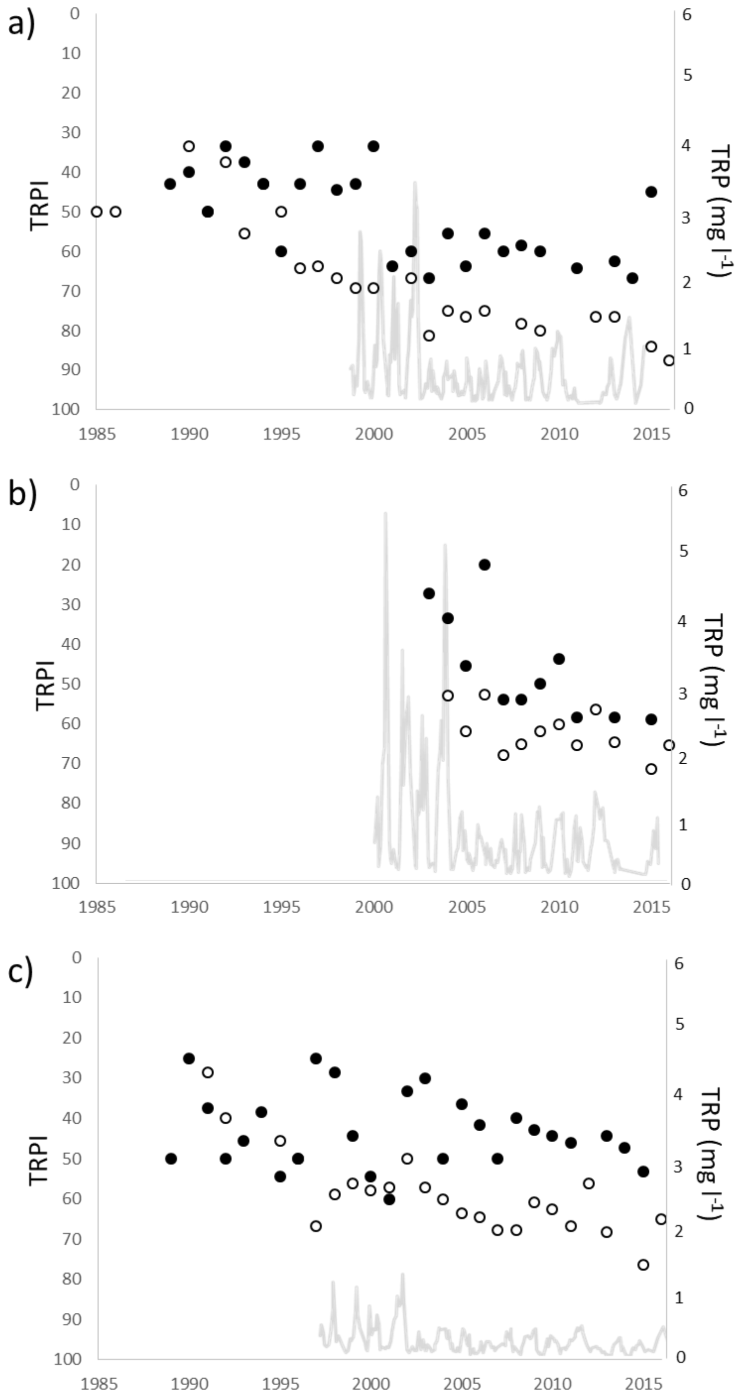

Figure 4: Spring (open) and Autumn (filled) TRPI values at Rockingham (a), Harringworth (b) and Collyweston (c) on the River Welland. TRP measures (grey line) are also indicated. Note the inverted y-axis for TRPI so improvements follow the same direction as improvements in TRP.

3.3.4. River Dove (River Type 2)

TRPI on the River Dove indicated heavily impacted conditions, with an increase in impact with distance from the source resulting in a gradient across the 35 sites (Figure 5). This was supported by TDI measurements which indicated a similar downstream pattern. However, at a subset of 3 sites, monthly spot measures made by the EA for the past 15 years indicate TRP levels were low relative to the other case studies (max = 0.102 mg l^{-1}) (Figure 6). TRPI does not correlate with other macroinvertebrate biological metrics (Supplementary E), including the PSI. Other metrics indicate good macroinvertebrate conditions, for example, the PSI indicates slightly sedimented or unimpacted conditions (Figure 6).

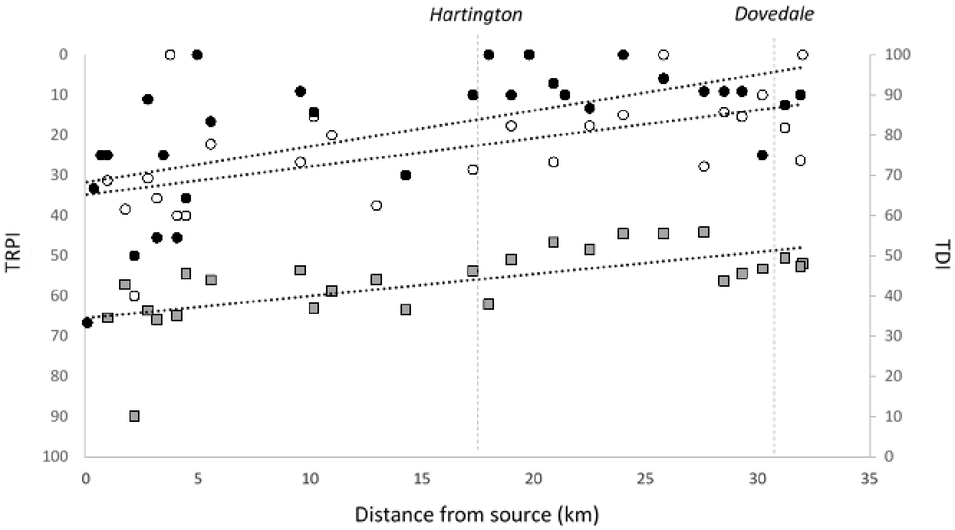

Figure 5. The TRPI on spring (open) and autumn (closed) circles at sites on the River Dove with increasing distance downstream Squares indicate the TDI, calculated on diatom community at the same sites, at the same time. The graph shows both metrics increasing with downstream distance, indicating increased TRP stress. Note the

inverted y-axis for TRPI so improvements follow the same direction as improvements in TDI.

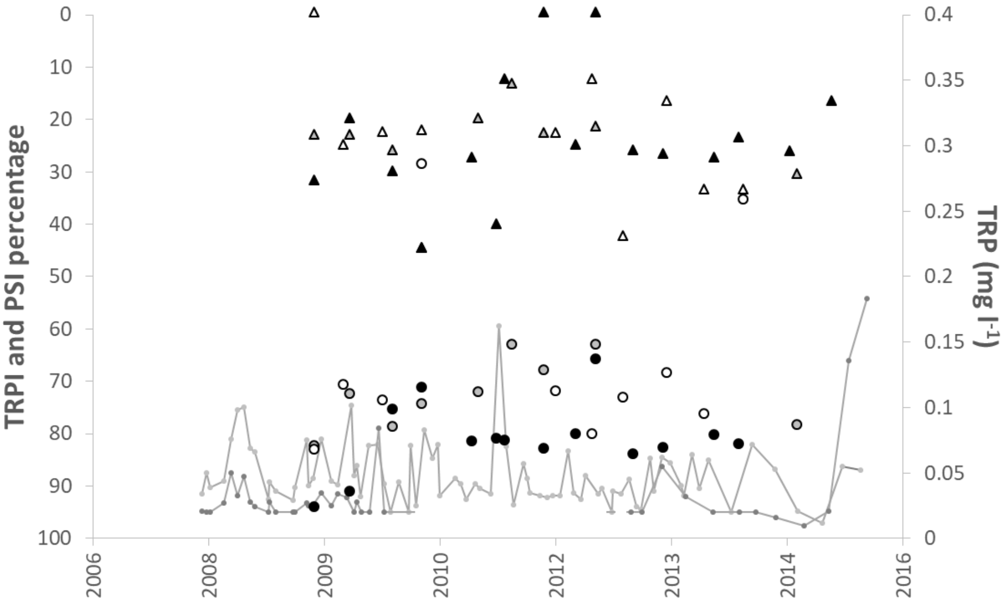

Figure 6. TRP measured at Hartington (light grey line) and Mayfield (dark grey line) with the PSI (circles) and TRPI (triangles) measured through time at three sites on the River Dove: Hartington (19 km from source – grey symbols); Dovedale (31.2 km from source – black symbols), and Mayfield (40 km from source – open symbols). Note the inverted y-axis for TRPI so improvements follow the same direction as improvements in TRP.

4. Discussion

4.1. Metric construction and consistency

We demonstrated the feasibility of using family-level macroinvertebrate community data to assess the effects of TRP on macroinvertebrate communities. The results derived using the TRPI methodology indicate comparable patterns to those obtained using other measures of TRP stress in the UK based on macrophytes and diatoms but with a stronger association to TRP. In addition, TRPI has the benefit of being calculated using routinely collected data and the ability to be retrospectively applied to historic data. Differences between the metrics may reflect that macrophytes, diatoms and invertebrates possibly integrate the effect of TRP over different timescales.

The TRPI threshold values indicated that site condition was dependent on substrate, alkalinity and altitude. This reflects the influence of geology and weathering rates on background P levels and is consistent with legislative thresholds for chemical TRP levels in the UK (UKTAG 2013). The UK legal thresholds were determined using diatom, macrophyte and chemical nutrient concentration data collected across the UK (UKTAG 2013). Legal thresholds are more stringent for upland sites and, in their development, the only environmental factors found to be good predictors of TRP concentrations, based on reference sites, were alkalinity and altitude (UKTAG 2013).

The interacting effects of substrate, altitude and alkalinity probably explain much of the scatter in the relationships between TRP and other indices in Table 4 given that TRP may exert different pressures on the community, depending on river type. The relatively strong correlations between TRPI and TRP across 76 and 156 samples (r = -0.71 and -0.86, respectively) was encouraging given that TRP effect may be evident on invertebrate communities at different concentrations dependent on river type, although the strong correlation may reflect the limited data available for small, upland, fast-flowing streams (Type 1 and 2 rivers).

The response by the macroinvertebrate community to TRP concentration is more clearly demonstrated in the case studies. TRPI values recorded indicate that the macroinvertebrate community in the River Dove appears to be heavily impacted by TRP levels less than 0.1 mg l^{-1}, whereas in the R. Welland the community indicate only low levels effects despite being an order-of-magnitude higher. This reflects the upland limestone characteristic (Type 2 in the TRPI river typology) of the R. Dove and as such would be predicted to have naturally lower TRP levels and a more TRP-sensitive invertebrate community than lowland streams. This is consistent with UK legal thresholds which state that in a river such as the Dove, TRP values above 0.03 mg l^{-1} would be considered moderately impacted under WFD rather than high or good condition (UKTAG 2013). The relative lack of monitoring on Type 1 and Type 2 streams in the UK (small, upland streams) may mask considerable issues because the results here suggest relatively low concentrations of P could be having substantial effects on ecological communities in some areas. This finding also supports the conclusions of UKTAG (2013) that indicate that previous standards for High and Good Ecological Status under WFD resulted in a large number of mismatches between classifications, with biological indicators failing more frequently than chemically measured P.

The wider implications of the differential sensitives of macroinvertebrates within different river-types are that the typology must be carefully implemented by users (environmental regulators and end-users) to avoid inaccurate classification. Incorrect classification of a river type could dramatically influence the TRPI score. For example, if the regression between TRPI and TRP from 88 sites (Table 4) is re-calculated but with data points attributed to one river type higher than their current designation, there is no significant relationship between variables ($p > 0.656$) and sites can change category from “very low” to “high” impact.

4.2. Metric performance

Given that the effect of TRP on macroinvertebrate communities is frequently indirect, the relationships observed are relatively strong. The datasets presented displayed similar relationships between TRPI and TRP. The exponential relationship in the 88 sites spanning three-years (2013-2015) indicated a clustering of points at low TRPI values. This was expected given that at low TRP values other pressures are probably more important in controlling macroinvertebrate community composition.

TRPI displayed broad consistency with TDI and MTR scores. It has been suggested that diatom communities in streams are more responsive than macroinvertebrates to nutrient enrichment (eutrophication), because of the direct effect of nutrients on growth and abundance of plants (Soininen and Kononen, 2004). In the current study, TRPI displayed a stronger association with TRP than TDI or MTR and provides evidence that macroinvertebrate communities are more responsive to changing TRP that previously thought. The associations for TDI obtained in this study were consistent with the literature. For example, Bae et al. (2011) reported a Spearman Rank correlation of TDI with Total P of 0.49 and with phosphate 0.42. This finding is supported by case study results where, for example, TRPI characterised changing TRP concentrations on the River Wylye more effectively than either TDI or MTR, although this may also reflect the relatively low number of data points influencing the correlation (Figure 2c). The results derived using TRPI have the potential benefit over other existing metrics given that the recognition of different river types (specified in the methodology) allows the differentiation of pressures among rivers.

4.3. Metric utility and comparison to other metrics

TRPI offers additional information to other water quality biomonitoring indices used in the UK. Moderately strong correlations were observed between TRPI and other water quality indices, but stronger correlations existed between other, already well established, metrics used in the UK, such as LIFE and PSI scores (r = 0.97). This result was anticipated given that some water quality indices (e.g. BMWP, WHPT) are designed to quantify faunal responses to organic pollution and are likely to pick up P pressures and, where P pressure is low, other stressors are also likely to be low (e.g. fine sediment, other organic pollutants – Piggott et al. 2012). The strongest associations recorded were with WHPT ASPT, with strong correlations also observed for other metrics with a weighted average score – e.g., PSI. Case studies also indicated a similarity between TRPI and PSI but this was relative weak (with the notable exception of the R. Wylye). This association is likely because of the close relationship between fine sediment and phosphorous pollutants (Owens & Walling, 2002), with P often bound to fine sediment particles. However, the River Dove case study indicates the possibility of differential P and fine sediment pressures, with PSI indicating slight sedimentation or unimpacted conditions whereas TRPI indicates the invertebrate community is suffering from elevated TRP pressure. This interpretation is supported by the TDI score which also indicates elevated P and the chemical measurements of

TRP, which despite being lower than other case studies, represent impacted conditions within the alkalinity and altitude categories of the River Dove (UKTAG 2013). Therefore, a multi-metric approach, utilising multiple indices simultaneously would be appropriate, with TRPI used as part the suite of indices derived using the same invertebrate dataset, to screen for multiple pressures (Clews and Ormerod, 2009).

4.4. P impacts on invertebrates and biomonitoring potential

The case studies presented in this study indicate that macroinvertebrate community response followed the average decline in TRP rather than any short-term fluctuations. This probably reflects the invertebrate community responding to conditions integrated over their life history up to the point of sampling. Some differences may be associated with acclimation of individuals to TRP concentrations, indirect feedbacks (Maidstone and Parr, 2002), as well as magnitude of TRP concentrations. As a result, associations between TRPI and TRP in individual case studies were typically statistically significant, but weak.

TRPI appears to respond to relatively subtle changes in TRP, such as on Costa Beck (Figure 3), despite relatively small absolute changes in TRP concentrations compared to background levels. This is surprising given TRP is unlikely to be the dominant stressor at low to moderate concentrations and when the community is relatively un-impacted. The reasons for this close association in some instances are currently unclear, but could relate to the interaction of multiple stressors, and suggest further research is required to understand the direct, causal implications of P on macroinvertebrate communities.

Paisley et al (2003; 2011) considered all 76 scoring BMWP macroinvertebrate families of which 46 had significant associations with TRP (i.e. $p < 0.1$) for at least one river type and season. As River Type increases from 1 to 5, the number of taxa with a strong association with TRP (significant to 5%) was reduced, as was the strength of relationships. This is partially related to the changing macroinvertebrate fauna associated with different river types and particularly the effect of substrate composition.

TRPI was designed based on the assumption that TRP would have largely indirect effects on the macroinvertebrate community; however, the strength of association between TRPI and TRP implies that TRP may have a more direct impact on the invertebrate community than previously thought. Some recent research has demonstrated that the survival of *Serratella ignita* eggs to hatching is directly impacted by moderate TRP levels (0.1 mg l^{-1}) (Everall *et al*., 2018). This implies that a more causal, trait-based approach could be developed if the direct mechanisms by which TRP impacts invertebrate communities can be established.

The statistically-derived sensitivity of taxa to TRP is complex, with some families being sensitive at some times of year or in some river types, when compared to others. For example, Gammaridae are very tolerant of TRP for River Type 2 but very appear sensitive within River Type 5. This may be because of other co-occurring difference between these river types. For example, Type 5 rivers are likely to be macrophyte and fine sediment dominated and Type 2 rivers relatively macrophyte poor, as well as differences in substrate particle size due to downstream fining of sediment and thermal regime variability due to downstream warming. Research has demonstrated that multiple stressors can have unexpected results, for example, insect larvae were less affected by fine sediment when organic matter was prevalent in the study of Doretto et al. (2017) and other stressors, such as fine sediment or warm water can alter the response of organisms subject to nutrient stress (Piggott et al., 2012). To unravel these complex interactions, future work would usefully focus on the direct, causal interactions between elevated nutrient concentrations and invertebrate persistence, on larval, adult and egg stages. Increasing the resolution to species level or focusing on particular taxonomic traits which are lost in the presence of elevated P may enable a better understanding of P impacts on macroinvertebrates, and improvement of the biomonitoring potential of TRPI (e.g. see Monk et al. 2012).

5. Conclusions

The TRPI showed a strong association with TRP concentrations which, for national and local datasets, was stronger than the association with the diatom community (TDI) or macrophyte composition (MTR). Therefore, TRPI provides an effective method for identifying areas of potential TRP stress upon benthic communities in the UK. The ability of macroinvertebrate communities to integrate impacts over time provides an advantage over direct monitoring of P levels, which are temporally and spatially variable and, therefore, relatively expensive and logistically intensive to monitor. TRPI also has the advantage that it can be calculated retrospectively using existing national biological databases, allowing P enrichment trends to be tracked over periods of time.

The results suggest that in some instances macroinvertebrate community structure has a stronger than expected response to organic loading in rivers, responding even where TRP levels are only moderately elevated. However, aspects of the statistical relationship between TRP and the macroinvertebrate community are not fully understood, such as the seasonal differences in sensitivity of some taxa. More information is required to establish the direct effects of P on benthic macroinvertebrates. Additionally, TRPI interpretation is highly sensitive to alkalinity, substrate size and altitude and would be improved with additional information from small, upland streams (type 1 and 2) where TRP is likely to have an ecological effect even at very low concentrations.

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Supplementary A: TRP tolerance groupings for invertebrate family groupings for each river type and season. The MI indicates the “mutual information” in explaining TRP from the analysis of Paisley et al. (2003). The % indicates the significance of relationship between the taxa and TRP (i.e. 1 = significant to 1%, 5 = significant to 5%). ± indicates whether the taxa is a positive or negative indicator of TRP, also indicated by the description.

River Type 1

River type	Season	Taxon	MI	%	±	Description	TRP group
1	Spring	Chloroperlidae	0.0453	1	−	v.sensitive	A
1	Spring	Nemouridae	0.0579	1	−	v.sensitive	A
1	Spring	Perlidae	0.0271	5	−	sensitive	B
1	Spring	Asellidae	0.0292	5	+	tolerant	C
1	Spring	Caenidae	0.027	5	+	tolerant	C
1	Spring	Chironomidae	0.0272	5	+	tolerant	C
1	Spring	Erpobdellidae	0.0299	5	+	tolerant	C
1	Spring	Leptoceridae	0.027	5	+	tolerant	C
1	Spring	Sphaeriidae	0.027	5	+	tolerant	C
1	Spring	Baetidae	0.0391	1	+	v. tolerant	D
1	Spring	Elmidae	0.0386	1	+	v. tolerant	D
1	Spring	Ephemerellidae	0.0517	1	+	v. tolerant	D
1	Spring	Gammaridae	0.0514	1	+	v. tolerant	D
1	Spring	Hydrobiidae	0.0743	1	+	v. tolerant	D
1	Spring	Leptophlebiidae	0.0385	1	+	v. tolerant	D
1	Autumn	Nemouridae	0.0357	1	−	v.sensitive	A
1	Autumn	Heptageniidae	0.0296	5	−	sensitive	B
1	Autumn	Perlidae	0.0293	5	−	sensitive	B
1	Autumn	Perlodidae	0.0258	5	−	sensitive	B
1	Autumn	Rhyacophilidae	0.0279	5	−	sensitive	B
1	Autumn	Gyrinidae	0.0284	5	+	tolerant	C
1	Autumn	Lymnaeidae	0.0249	5	+	tolerant	C
1	Autumn	Simuliidae	0.0287	5	+	tolerant	C
1	Autumn	Ancylidae	0.0552	1	+	v. tolerant	D
1	Autumn	Asellidae	0.0317	1	+	v. tolerant	D
1	Autumn	Elmidae	0.0667	1	+	v. tolerant	D
1	Autumn	Erpobdellidae	0.0483	1	+	v. tolerant	D
1	Autumn	Gammaridae	0.0408	1	+	v. tolerant	D
1	Autumn	Hydrobiidae	0.076	1	+	v. tolerant	D
1	Autumn	Hydropsychidae	0.0465	1	+	v. tolerant	D

River Type 2

River type	Season	Taxon	MI	%	±	Description	TRP group
2	Spring	Leptoceridae	0.0836	1	+	v. tolerant	D
2	Spring	Rhyacophilidae	0.0792	1	-	v.sensitive	A
2	Spring	Gammaridae	0.0700	1	+	v. tolerant	D
2	Spring	Hydropsychidae	0.0646	1	+	v. tolerant	D
2	Spring	Glossiphoniidae	0.0575	1	+	v. tolerant	D
2	Spring	Hydroptilidae	0.0535	1	+	v. tolerant	D
2	Spring	Baetidae	0.0529	1	+	v. tolerant	D
2	Spring	Erpobdellidae	0.0489	1	+	v. tolerant	D
2	Spring	Heptageniidae	0.0484	1	-	v.sensitive	A
2	Spring	Taeniopterygidae	0.0467	5	-	sensitive	B
2	Spring	Elmidae	0.0456	5	+	tolerant	C
2	Spring	Sphaeriidae	0.0438	5	+	tolerant	C
2	Spring	Hydrobiidae	0.0399	5	+	tolerant	C
2	Spring	Leptophlebiidae	0.0366	10	+	insig tol.	E
2	Spring	Ephemerellidae	0.0362	10	+	insig tol.	E
2	Autumn	Planorbidae	0.0789	1	+	v. tolerant	D
2	Autumn	Leuctridae	0.0579	1	-	v.sensitive	A
2	Autumn	Simuliidae	0.0550	1	+	v. tolerant	D
2	Autumn	Hydropsychidae	0.0541	1	+	v. tolerant	D
2	Autumn	Leptophlebiidae	0.0512	1	+	v. tolerant	D
2	Autumn	Sphaeriidae	0.0500	1	+	v. tolerant	D
2	Autumn	Tipulidae	0.0476	5	-	sensitive	B
2	Autumn	Erpobdellidae	0.0473	5	+	tolerant	C
2	Autumn	Ephemeridae	0.0466	5	+	tolerant	C
2	Autumn	Elmidae	0.0464	5	+	tolerant	C
2	Autumn	Lepidostomatidae	0.0435	5	+	tolerant	C
2	Autumn	Lymnaeidae	0.0431	5	-	sensitive	B
2	Autumn	Calopterygidae	0.0408	5	+	tolerant	C
2	Autumn	Sericostomatidae	0.0370	10	-	insig sens.	E
2	Autumn	Ephemerellidae	0.0363	10	-	insig sens.	E

River Type 3

River type	Season	Taxon	MI	%	±	Description	TRP group
3	Spring	Chironomidae	0.0632	1	−	v.sensitive	A
3	Spring	Ephemerellidae	0.113	1	−	v.sensitive	A
3	Spring	Rhyacophilidae	0.0825	1	−	v.sensitive	A
3	Spring	Sericostomatidae	0.0727	1	−	v.sensitive	A
3	Spring	Simuliidae	0.0726	1	−	v.sensitive	A
3	Spring	Baetidae	0.0524	5	−	sensitive	B
3	Spring	Chloroperlidae	0.0533	5	−	sensitive	B
3	Spring	Gammaridae	0.0562	5	−	sensitive	B
3	Spring	Heptageniidae	0.0471	5	−	sensitive	B
3	Spring	Lepidostomatidae	0.05	5	−	sensitive	B
3	Spring	Nemouridae	0.0504	5	−	sensitive	B
3	Spring	Leptophlebiidae	0.0512	5	+	tolerant	C
3	Spring	Sphaeriidae	0.0529	5	+	tolerant	C
3	Spring	Caenidae	0.0629	1	+	v. tolerant	D
3	Spring	Neritidae	0.0774	1	+	v. tolerant	D
3	Autumn	Sericostomatidae	0.0672	1	−	v.sensitive	A
3	Autumn	Chironomidae	0.059	5	−	sensitive	B
3	Autumn	Elmidae	0.0509	5	−	sensitive	B
3	Autumn	Heptageniidae	0.0485	5	−	sensitive	B
3	Autumn	Rhyacophilidae	0.059	5	−	sensitive	B
3	Autumn	Asellidae	0.0501	5	+	tolerant	C
3	Autumn	Neritidae	0.0512	5	+	tolerant	C
3	Autumn	Brachycentridae	0.0807	1	+	v. tolerant	D
3	Autumn	Planorbidae	0.06	1	+	v. tolerant	D
3	Autumn	Baetidae	0.0445	10	−	insig sens.	E
3	Autumn	Caenidae	0.045	10	+	insig tol.	E
3	Autumn	Gammaridae	0.0479	10	−	insig sens.	E
3	Autumn	Goeridae	0.044	10	−	insig sens.	E
3	Autumn	Leuctridae	0.0473	10	−	insig sens.	E
3	Autumn	Tipulidae	0.0455	10	−	insig sens.	E

River type 4

River type	Season	Taxon	MI	%	±	Description	TRP group
4	Spring	Rhyacophilidae	0.1356	1	-	v.sensitive	A
4	Spring	Gammaridae	0.0844	1	-	v.sensitive	A
4	Spring	Ephemerellidae	0.0688	1	-	v.sensitive	A
4	Spring	Perlodidae	0.0615	1	-	v.sensitive	A
4	Spring	Dendrocoelidae	0.0613	1	-	v.sensitive	A
4	Spring	Calopterygidae	0.0582	1	+	v. tolerant	D
4	Spring	Asellidae	0.0569	5	+	tolerant	C
4	Spring	Caenidae	0.0547	5	+	tolerant	C
4	Spring	Leptoceridae	0.0541	5	+	tolerant	C
4	Spring	Heptageniidae	0.0528	5	-	sensitive	B
4	Spring	Unionidae	0.0525	5	+	tolerant	C
4	Spring	Leuctridae	0.0523	5	-	sensitive	B
4	Spring	Lepidostomatidae	0.0485	5	-	sensitive	B
4	Spring	Sphaeriidae	0.0485	5	+	tolerant	C
4	Spring	Baetidae	0.0484	5	-	sensitive	B
4	Autumn	Caenidae	0.0837	1	+	v. tolerant	D
4	Autumn	Calopterygidae	0.0731	1	+	v. tolerant	D
4	Autumn	Coenagriidae	0.0638	1	+	v. tolerant	D
4	Autumn	Rhyacophilidae	0.0571	5	-	sensitive	B
4	Autumn	Elmidae	0.0546	5	-	sensitive	B
4	Autumn	Sericostomatidae	0.0539	5	-	sensitive	B
4	Autumn	Ephemeridae	0.0527	5	-	sensitive	B
4	Autumn	Chironomidae	0.0477	5	-	sensitive	B
4	Autumn	Psychomyiidae	0.0477	5	-	sensitive	B
4	Autumn	Asellidae	0.0460	5	+	tolerant	C
4	Autumn	Ancylidae	0.0456	10	+	insig tol.	E
4	Autumn	Sialidae	0.0436	10	+	insig tol.	E
4	Autumn	Limnephilidae	0.0418	10	-	insig sens.	E
4	Autumn	Planariidae	0.0416	10	-	insig sens.	E
4	Autumn	Neritidae	0.0374	10	+	insig tol.	E

River type 5

River type	Seasonal	Taxon	MI	%	±	Description	TRP group
5	Spring	Gammaridae	0.061	1	−	v.sensitive	A
5	Spring	Tipulidae	0.0731	1	−	v.sensitive	A
5	Spring	Ephemerellidae	0.0504	5	−	sensitive	B
5	Spring	Heptageniidae	0.0568	5	−	sensitive	B
5	Spring	Valvatidae	0.0461	5	+	tolerant	C
5	Spring	Calopterygidae	0.0673	1	+	v. tolerant	D
5	Spring	Ancylidae	0.0376	10	+	insig tol.	E
5	Spring	Baetidae	0.0423	10	−	insig sens.	E
5	Spring	Caenidae	0.0454	10	+	insig tol.	E
5	Spring	Goeridae	0.0443	10	−	insig sens.	E
5	Spring	Hydroptilidae	0.0438	10	+	insig tol.	E
5	Spring	Limnephilidae	0.0427	10	−	insig sens.	E
5	Spring	Notonectidae	0.0429	10	+	insig tol.	E
5	Spring	Simuliidae	0.039	10	−	insig sens.	E
5	Spring	Sphaeriidae	0.039	10	−	insig sens.	E
5	Autumn	Gammaridae	0.0798	1	−	v.sensitive	A
5	Autumn	Hydrobiidae	0.0583	1	−	v.sensitive	A
5	Autumn	Rhyacophilidae	0.0583	1	−	v.sensitive	A
5	Autumn	Sphaeriidae	0.0776	1	−	v.sensitive	A
5	Autumn	Glossiphoniidae	0.0528	5	−	sensitive	B
5	Autumn	Calopterygidae	0.0484	5	+	tolerant	C
5	Autumn	Valvatidae	0.0667	1	+	v. tolerant	D
5	Autumn	Brachycentridae	0.0438	10	+	insig tol.	E
5	Autumn	Elmidae	0.0409	10	−	insig sens.	E
5	Autumn	Heptageniidae	0.0447	10	−	insig sens.	E
5	Autumn	Limnephilidae	0.0413	10	−	insig sens.	E
5	Autumn	Nemouridae	0.0405	10	−	insig sens.	E
5	Autumn	Oligochaeta	0.0439	10	−	insig sens.	E
5	Autumn	Physidae	0.0399	10	−	insig sens.	E
5	Autumn	Planariidae	0.0394	10	-	Insig. Sens.	E

Supplementary Material B

Table of sites sampled used in correlative analysis. 88 sites were sampled seasonally. The site location, river and geographical region are included with a latitude and longitude. The date is the date of the invertebrate sample in all cases.

Site	River	Region	Latitude	Longitude	Date
Forge Farm	Black Bourne Footherley Brook	Worcestershire	52.613001	-1.8670388	29/11/2012
Footherley STW	Black Bourne Footherley Brook	Worcestershire	52.616593	-1.864074	29/11/2012
Footherley Hall	Black Bourne Footherley Brook	Worcestershire	52.631865	-1.8551617	29/11/2012
Thickbroom Farm	Black Bourne Footherley Brook	Worcestershire	52.629094	-1.8034615	29/11/2012
Hints	Black Bourne Footherley Brook	Worcestershire	52.622743	-1.7709915	29/11/2012
Lower Bangley	Black Bourne Footherley Brook	Worcestershire	52.618206	-1.7503365	29/11/2012
Fazeley	Black Bourne Footherley Brook	Worcestershire	52.609996	-1.6986962	29/11/2012
Chesterfield	Black Bourne Footherley Brook	Worcestershire	52.645353	-1.8580731	29/11/2012
Wall	Black Bourne Footherley Brook	Worcestershire	52.65704	-1.8580354	29/11/2012
Little Hay	Black Bourne Footherley Brook	Worcestershire	52.621928	-1.8197424	29/11/2012
Forge Farm	Black Bourne Footherley Brook	Worcestershire	52.613001	-1.8670388	17/06/2013
Footherley STW	Black Bourne Footherley Brook	Worcestershire	52.616593	-1.864074	17/06/2013
Footherley Hall	Black Bourne Footherley Brook	Worcestershire	52.631865	-1.8551617	17/06/2013
Thickbroom Farm	Black Bourne Footherley Brook	Worcestershire	52.629094	-1.8034615	17/06/2013
Hints	Black Bourne Footherley Brook	Worcestershire	52.622743	-1.7709915	17/06/2013
Lower Bangley	Black Bourne Footherley Brook	Worcestershire	52.618206	-1.7503365	17/06/2013
Fazeley	Black Bourne Footherley Brook	Worcestershire	52.609996	-1.6986962	17/06/2013
Wall	Black Bourne Footherley Brook	Worcestershire	52.65704	-1.8580354	17/06/2013
Little Hay	Black Bourne Footherley Brook	Worcestershire	52.621928	-1.8197424	17/06/2013
Cherry Slade	Cannock Chase Forest streams	Staffordshire	52.763272	-2.0206604	16/03/2015

Birches Valley	Cannock Chase Forest streams	Staffordshire	52.746631	-1.9710262	17/03/2015
Seven Springs	Cannock Chase Forest streams	Staffordshire	52.730292	-1.9562583	17/03/2015
Hare’s Hill	Cannock Chase Forest streams	Staffordshire	52.723748	-1.921204	17/03/2015
Abrahams Valley	Cannock Chase Forest streams	Staffordshire	52.777064	-1.993272	18/03/2015
Stafford Brook SSSI	Cannock Chase Forest streams	Staffordshire	52.768978	-1.9684474	18/03/2015
Whitford Bridge	River Axe	Devon	50.753527	-3.0472249	28/05/2015
Cloakham Bridge	River Axe	Devon	50.789305	-2.9995472	28/05/2015
Wadbrook Bridge	River Axe	Devon	50.81062	-2.9629713	28/05/2015
Forde Abbey	River Axe	Devon	50.844123	-2.9073863	28/05/2015
Seaborough	River Axe	Devon	50.848952	-2.8160991	28/05/2015
Whitford Bridge	River Axe	Devon	50.753527	-3.0472249	16/09/2015
Cloakham Bridge	River Axe	Devon	50.789305	-2.9995472	16/09/2015
Wadbrook Bridge	River Axe	Devon	50.81062	-2.9629713	16/09/2015
Forde Abbey	River Axe	Devon	50.844123	-2.9073863	16/09/2015
Seaborough	River Axe	Devon	50.848952	-2.8160991	16/09/2015
Polbrook Bridge	River Camel	Cornwall	50.490783	-4.7999834	27/05/2015
Nanstallon	River Camel	Cornwall	50.474762	-4.6926022	27/05/2015
Dunmere Bridge	River Camel	Cornwall	50.477479	-4.7525382	27/05/2015
Wenford Bridge	River Camel	Cornwall	50.544486	-4.7040413	27/05/2015
Slaughter Bridge	River Camel	Cornwall	50.638772	-4.6751381	27/05/2015
Polbrook Bridge	River Camel	Cornwall	50.490783	-4.7999834	15/09/2015
Nanstallon	River Camel	Cornwall	50.474762	-4.6926022	15/09/2015
Dunmere Bridge	River Camel	Cornwall	50.477479	-4.7525382	15/09/2015
Wenford Bridge	River Camel	Cornwall	50.544486	-4.7040413	15/09/2015
Slaughter Bridge	River Camel	Cornwall	50.638772	-4.6751381	15/09/2015
Tittesworth	River Churnet	Staffordshire	53.139851	-2.0029493	15/01/2014
Dimmings Dale	River Churnet	Staffordshire	52.985202	-1.9061184	15/01/2014
Coombes Valley	River Churnet	Staffordshire	53.065242	-1.9969746	16/01/2014

Blackbank	River Churnet	Staffordshire	53.028382	-1.9641746	16/01/2014
Cotton Dell	River Churnet	Staffordshire	53.005886	-1.9179956	16/01/2014
Dydon Wood	River Churnet	Staffordshire	53.000363	-1.8062521	16/01/2014
Tittesworth	River Churnet	Staffordshire	53.139851	-2.0029493	07/05/2014
Dimmings Dale	River Churnet	Staffordshire	52.985202	-1.9061184	07/05/2014
Coombes Valley	River Churnet	Staffordshire	53.065242	-1.9969746	07/05/2014
Blackbank	River Churnet	Staffordshire	53.028382	-1.9641746	07/05/2014
Cotton Dell	River Churnet	Staffordshire	53.005886	-1.9179956	07/05/2014
Dydon Wood	River Churnet	Staffordshire	53.000363	-1.8062521	07/05/2014
Warkworth Ford	River Coquet	Northumberland	55.338175	-1.6291127	11/06/2015
Guyzance Mill	River Coquet	Northumberland	55.32517	-1.675805	11/06/2015
Felton	River Coquet	Northumberland	55.296653	-1.7092986	11/06/2015
Cragend Farm	River Coquet	Northumberland	55.301848	-1.8653661	11/06/2015
Holystone	River Coquet	Northumberland	55.321033	-2.0683941	11/06/2015
Warkworth Ford	River Coquet	Northumberland	55.338175	-1.6291127	08/09/2015
Guyzance Mill	River Coquet	Northumberland	55.32517	-1.675805	08/09/2015
Felton	River Coquet	Northumberland	55.296653	-1.7092986	08/09/2015
Cragend Farm	River Coquet	Northumberland	55.301848	-1.8653661	08/09/2015
Holystone	River Coquet	Northumberland	55.321033	-2.0683941	08/09/2015
Calver	River Derwent	Derbyshire	53.266493	-1.6315698	01/05/2015
Grindleford	River Derwent	Derbyshire	53.294971	-1.6348657	01/05/2015
Lydgate Farm	River Derwent	Derbyshire	53.358576	-1.7034838	01/05/2015
Calver	River Derwent	Derbyshire	53.266493	-1.6315698	14/10/2015
Grindleford	River Derwent	Derbyshire	53.294971	-1.6348657	14/10/2015
Lydgate Farm	River Derwent	Derbyshire	53.294971	-1.6348657	14/10/2015
Manor House Farm	River Dever	Hampshire	51.174677	-1.3799597	24/04/2015
Bransbury	River Dever	Hampshire	51.174404	-1.3811077	24/04/2015
Bransbury	River Dever	Hampshire	51.174404	-1.3811077	29/09/2015
Manor House Farm	River Dever	Hampshire	51.174677	-1.3799597	29/09/2015
Hartington RB	River Dove	Derbyshire	53.135528	-1.8209962	15/04/2014
Rochester	River Dove	Derbyshire	52.950123	-1.8300068	08/05/2015
Mayfield	River Dove	Derbyshire	53.012754	-1.7632112	08/05/2015
Milldale	River Dove	Derbyshire	53.088364	-1.7938532	08/05/2015
Rochester	River Dove	Derbyshire	52.950123	-1.8300068	11/09/2015
Mayfield	River Dove	Derbyshire	53.012754	-1.7632112	11/09/2015
Milldale	River Dove	Derbyshire	53.088364	-1.7938532	11/09/2015

Hollinsclough	River Dove	Derbyshire	53.198774	-1.9075548	11/09/2015
Great Salkeld	River Eden	Lancashire	54.717501	-2.6871766	24/04/2015
Great Salkeld	River Eden	Lancashire	54.70444	-2.6908354	24/04/2015
Hunsonby	River Eden	Lancashire	54.712939	-2.6490707	24/04/2015
Little Salkeld	River Eden	Lancashire	54.716654	-2.6747902	24/04/2015
Eden Mount	River Eden	Lancashire	54.709474	-2.676285	24/04/2015
Temple Sowerby	River Eden	Lancashire	54.646326	-2.6150784	24/04/2015
Great Salkeld	River Eden	Lancashire	54.717501	-2.6871766	09/09/2015
Great Salkeld	River Eden	Lancashire	54.70444	-2.6908354	09/09/2015
Hunsonby	River Eden	Lancashire	54.712939	-2.6490707	09/09/2015
Little Salkeld	River Eden	Lancashire	54.716654	-2.6747902	09/09/2015
Eden Mount	River Eden	Lancashire	54.709474	-2.676285	09/09/2015
Temple Sowerby	River Eden	Lancashire	54.646326	-2.6150784	09/09/2015
Ovington Mill	River Itchen	Hampshire	51.082979	-1.1946779	21/04/2015
Yavington	River Itchen	Hampshire	51.090178	-1.2220538	21/04/2015
Chilland	River Itchen	Hampshire	51.091119	-1.2366169	21/04/2015
Chilland Mill	River Itchen	Hampshire	51.08968	-1.2546458	21/04/2015
Ovington Mill	River Itchen	Hampshire	51.082979	-1.1946779	30/09/2015
Yavington	River Itchen	Hampshire	51.090178	-1.2220538	30/09/2015
Chilland	River Itchen	Hampshire	51.091119	-1.2366169	30/09/2015
Chilland Mill	River Itchen	Hampshire	51.08968	-1.2546458	30/09/2015
Great Shefford	River Lambourn	Berkshire	51.463356	-1.430599	14/04/2015
Weston	River Lambourn	Berkshire	51.461275	-1.4241907	14/04/2015
Hunts Green	River Lambourn	Berkshire	51.429224	-1.3775433	14/04/2015
Woodspeen	River Lambourn	Berkshire	51.419197	-1.3476232	14/04/2015
Great Shefford	River Lambourn	Berkshire	51.463356	-1.430599	01/10/2015
Weston	River Lambourn	Berkshire	51.461275	-1.4241907	01/10/2015
Hunts Green	River Lambourn	Berkshire	51.429224	-1.3775433	01/10/2015
Woodspeen	River Lambourn	Berkshire	51.419197	-1.3476232	01/10/2015
Houghton	River Test	Hampshire	51.087541	-1.5083687	24/09/2013
Longstock West	River Test	Hampshire	51.123263	-1.4927008	24/09/2013
Longstock East	River Test	Hampshire	51.122614	-1.4881356	24/09/2013
Abbey Mill	River Test	Hampshire	50.990546	-1.5050052	03/06/2015
Bossington	River Test	Hampshire	51.074457	-1.5146878	03/06/2015
Fullerton	River Test	Hampshire	51.149551	-1.4555411	03/06/2015
Whitchurch	River Test	Hampshire	51.230406	-1.3164236	03/06/2015
Polhampton	River Test	Hampshire	51.251144	-1.250721	03/06/2015
Abbey Mill	River Test	Hampshire	50.990546	-1.5050052	30/09/2015
Bossington	River Test	Hampshire	51.074457	-1.5146878	30/09/2015
Fullerton	River Test	Hampshire	51.149551	-1.4555411	30/09/2015

Whitchurch	River Test	Hampshire	51.230406	-1.3164236	30/09/2015
Polhampton	River Test	Hampshire	51.251144	-1.250721	30/09/2015
Kilgram Bridge	River Ure	Yorkshire	54.269129	-1.7069727	21/05/2015
Ulshaw Bridge	River Ure	Yorkshire	54.280262	-1.7776354	21/05/2015
Wensley Bridge	River Ure	Yorkshire	54.300168	-1.9568797	21/05/2015
Bishopdale Brook	River Ure	Yorkshire	54.300122	-1.8600744	21/05/2015
Worton Bridge	River Ure	Yorkshire	54.307893	-2.0695712	21/05/2015
Hawes	River Ure	Yorkshire	52.512558	-2.1912459	21/05/2015
Kilgram Bridge	River Ure	Yorkshire	54.269129	-1.7069727	03/09/2015
Ulshaw Bridge	River Ure	Yorkshire	54.280262	-1.7776354	03/09/2015
Wensley Bridge	River Ure	Yorkshire	54.300168	-1.9568797	03/09/2015
Bishopdale Brook	River Ure	Yorkshire	54.300122	-1.8600744	03/09/2015
Worton Bridge	River Ure	Yorkshire	54.307893	-2.0695712	03/09/2015
Hawes	River Ure	Yorkshire	52.512558	-2.1912459	03/09/2015
Collyweston Bridge	River Welland	Leicestershire	52.620252	-0.5390524	14/05/2015
Wakerley	River Welland	Leicestershire	52.587183	-0.59088653	14/05/2015
Harringworth	River Welland	Leicestershire	52.568557	-0.65290802	14/05/2015
Rockingham	River Welland	Leicestershire	52.521723	-0.72656231	14/05/2015
Weston-by-Welland	River Welland	Leicestershire	52.523033	-0.85475663	14/05/2015
Collyweston Bridge	River Welland	Leicestershire	52.620252	-0.5390524	23/09/2015
Wakerley	River Welland	Leicestershire	52.587183	-0.59088653	23/09/2015
Harringworth	River Welland	Leicestershire	52.568557	-0.65290802	23/09/2015
Rockingham	River Welland	Leicestershire	52.521723	-0.72656231	23/09/2015
Weston-by-Welland	River Welland	Leicestershire	52.523033	-0.85475663	23/09/2015
Bintree Mill	River Wensum	Norfolk	52.776686	0.95553038	28/05/2015
Senmore Bridge	River Wensum	Norfolk	52.798846	0.92534217	28/05/2015
Pensthorpe Natural Park	River Wensum	Norfolk	52.821647	0.8858887	28/05/2015
Fakenham Common	River Wensum	Norfolk	52.825923	0.85262074	28/05/2015
Bintree Mill	River Wensum	Norfolk	52.776686	0.95553038	25/09/2015
Senmore Bridge	River Wensum	Norfolk	52.798846	0.92534217	25/09/2015

Pensthorpe Natural Park	River Wensum	Norfolk	52.821647	0.8858887	25/09/2015
Fakenham Common	River Wensum	Norfolk	52.825923	0.85262074	25/09/2015
Doughton Bridge	River Wensum	Norfolk	52.826138	0.79255433	25/09/2015
Dove House Farm	River Wye	Staffordshire	53.18862	-1.6367868	22/05/2013

Supplementary Material C

Table of sites sampled by the Environment Agency for invertebrates, macrophytes, diatoms and TRP concentration in 2015. The site location, river and geographical region are included with a latitude and longitude. The date reported if for the macroinvertebrate collection sample in all cases.

Site	River	Region	Latitude	Longitude	Date
Eades Mill	Blackwater	Eastern	52.74869	1.102605	10/07/2015
Westhouses	Westwood Brook	East	53.11289	-1.37419	30/06/2015
Shipley Gate	Erewash	East	53.0042	-1.31143	26/06/2015
Shatton	Noe	East	53.33986	-1.69649	17/08/2015
Rolleston	Halloughton Dumble	East	53.064	-0.90342	28/08/2015
Owston Ferry	Ferry Drain	East	53.48158	-0.79715	01/09/2015
Newton Ferry	Bradgate Brook	East	52.68348	-1.22861	22/06/2015
Nether Broughton	Dalby Brook	East	52.84267	-0.97373	10/07/2015
Misterton	Idle	East	53.45729	-0.84987	20/08/2015
Huncote	Thurlaston Brook	East	52.57133	-1.24234	20/07/2015
Mill Farm Quorn	Quorn Brook	East	52.73721	-1.17735	17/09/2015
Millers Dale	Monks Dale Stream	East	53.25731	-1.78937	15/07/2015
Yeaton RB	War Brook	West	52.77262	-2.84449	30/07/2015
Hordley	Tetchill Brook	West	52.86928	-2.91846	30/07/2015
Cound Bridge	Cound Brook	West	52.64646	-2.65492	10/08/2015
Oak Cottage	Dowles Brook	West	52.38483	-2.33887	06/08/2015
Lower Isle of Bicton	Severn	West	52.74392	-2.79841	03/07/2015
Shipley Wood	Shipley Burn	North East	55.45338	-1.76145	11/08/2015
Warkworth Ford	Coquet	North East	55.33818	-1.62944	06/08/2015
Swarland Fence	Swarland Burn	North East	55.30567	-1.75432	25/06/2015
Thropton	Wreigh Burn	North East	55.31389	-1.95362	16/07/2015
Jesmond Dene	Ouseburn	North East	54.98718	-1.58811	17/06/2015
Chollerton	Erring Burn	North East	55.03752	-2.10826	12/08/2015
Simonburn	Simon Burn	North East	55.05591	-2.20202	12/08/2015
Byreness	Rede	North East	55.31651	-2.37726	25/08/2015
U/S Gaunless	Wear	North East	54.66834	-1.67518	17/07/2015
Langley Moor	Deerness	North East	54.76276	-1.60536	17/07/2015
A67 Bridge	Langley Beck	North East	54.55317	-1.75966	23/07/2015
Spindlestone	Waren Burn	North East	55.59498	-1.76538	30/06/2015
Crag Mill	Belford Burn	North East	55.61105	-1.81423	29/06/2015
Twizel Mill	Till	North East	55.67861	-2.18224	06/08/2015
Proctor's Bridge / Swamill	Proctors Burn	North East	55.06031	-2.19817	12/08/2015

Standalone Cottage	Honeycrook Burn	North East	54.97922	-2.27811	13/08/2015
U/S Warren Burn Confluence	Newlands Burn	North East	55.57996	-1.76522	30/06/2015
Redmire	Apedale Beck	Yorkshire	54.31381	-1.93676	21/07/2015
Sandsend	East Row Beck	Yorkshire	54.50016	-0.6718	30/06/2015
Marske	Marske Beck	Yorkshire	54.39946	-1.84216	12/08/2015
Morton on Swale	Swale	Yorkshire	54.32054	-1.51152	11/09/2015
Muscoates	Ellerker Beck	Yorkshire	54.22194	-0.95109	16/06/2015
Skelton	Hurns Gutter	Yorkshire	53.99695	-1.14043	04/09/2015
Near Moor Close Farm	The Syme	Yorkshire	54.20482	-0.73339	06/07/2015
Holme Green	Fleet	Yorkshire	53.8682	-1.15763	11/06/2015
U/S River Aire	Eller Beck Skipton	Yorkshire	53.94863	-2.02483	18/08/2015
Hayton Grange	The Beck/Bielby Beck	Yorkshire	53.89827	-0.76418	02/09/2015
D/S Eshington Bridge	Bishopdale Beck	Yorkshire	54.28613	-1.97766	14/07/2015
D/S Burton Bridge	Walden Beck	Yorkshire	54.27966	-1.97269	14/07/2015
Langton	Hilton Beck	North	54.57512	-2.4485	12/08/2015
NY825129	Argill Beck	North	54.51107	-2.27124	19/06/2015
Soulby 25m D/S Ford	Scandal Beck	North	54.49405	-2.38172	09/07/2015
U/S B6276	Swindale Beck (Eden)	North	54.52736	-2.31409	30/06/2015
Near Hall Garth	Swindale Beck (Eden)	North	54.51571	-2.35155	09/07/2015
Black Beck	Black Beck (Duddon)	North	54.24573	-3.25006	19/08/2015
Leven U/S Low Wood Bridge	Leven	North	54.24451	-3.00595	18/08/2015
Marron - Bridge U/S STW	Marron	North	54.63546	-3.45896	19/08/2015
Morland Beck at Newby	Morland Beck	North	54.58484	-2.6211	09/09/2015
Stonegate	Tidebrook	Kent & E.Sussex	51.01777	0.354759	17/08/2015
Etchingham	Dudwell	Kent & E.Sussex	51.00654	0.435717	17/08/2015
Penshurst Clappers Sluice	Eden (Kent)	Kent & E.Sussex	51.17293	0.173855	06/08/2015
Pentewan Bridge	St Austell River	Cornwall	50.29225	-4.78534	18/08/2015
St Blazey Bridge	Par River	Cornwall	50.3676	-4.71163	18/08/2015
Greenlanes Bridge	Lyd	Cornwall	50.62869	-4.20519	08/07/2015
Grogley	Camel	Cornwall	50.48348	-4.80081	22/07/2015
Restormel	Fowey	Cornwall	50.42118	-4.66534	30/07/2015
Grenofen Bridge	Walkham	Cornwall	50.51828	-4.13049	15/07/2015
100m U/S Road Bridge Bowcombe	Small Brook	Devon	50.28703	-3.75445	08/07/2015
Cottarson 50m D/S weir	Otter	Devon	50.79823	-3.21031	17/06/2015
150m U/S Ford Heathhayne	Coly	Devon	50.74387	-3.08674	22/06/2015

25m U/S Bridge Mill Green	Lim	Devon	50.72779	-2.93561	22/06/2015
50m U/S Bridge Buddlewall	Blackwater River	Devon	50.81519	-2.95226	24/06/2015
60m U/S Fishacre Bridge	Am Brook	Devon	50.46786	-3.66503	09/09/2015
Near Old Mill House	Grindle Brook	Devon	50.70482	-3.43693	30/06/2015
20m U/S Blackpool Bridge	Blackpool Stream	Devon	50.31998	-3.613	16/07/2015
20m D/S Bridge Perry Street	Forton Brook	Devon	50.84554	-2.94419	08/07/2015
Holme Bridge	Dorset Frome	South Wessex	50.67959	-2.15586	13/07/2015
Above Thames, Bray	The Cut	South East	51.49993	-0.68707	18/08/2015
Bagnor	Lambourn	West	51.42077	-1.35146	12/08/2015

Supplementary Material D

River Wylye

The River Wylye is chalk stream in the south of England in Wiltshire (51.183645; -2.1310766; Figure 1). The river flows through two SSSIs and a National Nature Reserve, although the reach used at Norton Bavant is outside the boundaries of these designations. The Wylye has a catchment of 470 km^2, with a 112 km^2 catchment upstream of the Norton Bavant study reach. The upstream catchment receives approximately 900 mm of rainfall annually and land use is predominately arable and grassland, with around 13% woodland cover.

The channel is approximately 10 m wide, with adjacent agriculture and grassland, with isolated trees. The study reach has altitude of 97 masl, with the maximum elevation in the catchment being 284 m. The reach is gravel-bedded with alkalinity averaging 203 mg l^{-1} (range 140 to 249) over the past 16 years, based on EA monthly spot measurements since 2000. Therefore, the river has the closest fit to a River Type 3. Gauged discharge is recorded on the river less than 50 m upstream from the study reach by the EA, with data made available through the National River Flow Archive (NRFA). This data indicates the river has mean flow of 1.11 m^3 s^{-1} with a Q95 of 0.46 m^3 s^{-1} and Q10 of 2.11 m^3 s^{-1}

At the study reach, the EA collect monthly spot samples of chemical water quality and spring and autumn macroinvertebrate samples. The river has been classified as Moderate under WFD targets for chemical and ecological quality, in particular because of high phosphate concentrations. EA monthly spot samples indicate that the stream pH averages 8.1 and the suspended solid concentration averages 9.4 mg l^{-1} (max. 110 mg l^{-1}). Dissolved oxygen averages 105%, dropping to 90 – 95% through winter. Nitrate averages 6.46 mg l^{-1} (max. 8.92 mg l^{-1}) with nitrite averaging 0.05 mg l^{-1} (max 0.153 mg l^{-1}) and ammonia 0.05 mg l^{-1} (max 0.27 mg l^{-1}).

Diatom and macrophyte samples have also been collected by the EA and used to calculate TDI and MTR infrequently at sites within 3 km of the study reach. In addition, Wessex Water recorded TRP levels in a 20 year assessment from 1991 to 2011, monitoring the response of targeted phosphate concentration improvements from the year 2000 in the study reach.

River Welland

The River Welland is a lowland stream in eastern England (Northamptonshire). The case study includes three sites separated by 7 km at Rockingham, Harringworth and Collyweston. The catchment upstream of these sites is 400 km^2 with land-use predominately arable agriculture. The catchment receives 644 mm rainfall a year.

The channel at the sampling sites is approximately 5 m wide and the substrate is predominately gravel at all sites. The site altitude ranges from 50 masl at Rockingham to 25 masl at Collyweston. The average alkalinity is 201 mg l^{-1} at Rockingham, 186 mg l^{-1} at Harringworth and 205 mg l^{-1} at Collywesten, indicating a River Type 3 at all sites. The adjacent land use to the sites is arable agriculture with 10% woodland cover and all are on the edge of villages with populations between 200 and 500 people. The flow is gauged at Barrowden, 3 km downstream from Harringworth, with an average flow recorded of 1.96 $m^3 s^{-1}$ between 1968 and 2016 (Q95 = 0.23 $m^3 s^{-1}$; Q10 = 4.29 $m^3 s^{-1}$).

At all three sites, routine EA macroinvertebrate and chemical data was used from between 2000 – 2016 and is currently ranked Moderate under WFD. It scores good or high for all physico-chemical variables with the exception of phosphate, which is currently ranked Poor.

River Dove

The River Dove is an upland stream flowing over limestone in central England, in the Peak District National Park. The Upper Dove Catchment is entirely within the National Park boundary and also contains a National Nature Reserve and SSSI. It is internationally recognised for fly fishing as it is where Izaak Newton wrote "The Compleat Angler" in 1653. Sampling sites at 35 locations from the source of the Dove to just downstream of the confluence with the River Manifold were sampled by the authors (Nick Everall). The upstream catchment area is 238 km^2. The land use is predominately cattle-grazed grassland with 4% woodland cover. The catchment receives 1098 mm rainfall a year.

The channel at the sampling sites range from 1 m to 18 m wide and the substrate is predominately gravel and cobbles. The sites range in altitude from 348 – 150 masl. The flow is gauged 1 km upstream from the confluence with the Manifold (Izaak Walton Gauging Station), with an average flow of 1.92 $m^3 s^{-1}$ (Q95 = 0.54 $m^3 s^{-1}$; Q10 = 3.52 $m^3 s^{-1}$).

Sites were monitored by the authors (Nick Everall) for diatoms and invertebrates in winter 2009 and spring 2010. In addition, routine EA macroinvertebrate and chemical data were used from three sites between 2000 and 2016. These were Hartington (19 km from source) and Dovedale (31.2 km from source) and Mayfield (40 km from source). It is currently rated Moderate under WFD. All physico-chemical variables and macroinvertebrates are ranked High, but a Moderate overall classification is in place because of a moderate fish population.

Supplementary Material E

Linear correlation equations and *r*-values for TRPI vs 10 other macroinvertebrate biomonitoring metrics recorded in each case study river. Significant relationships are in bold.

	River Welland		***River Wylye***		***River Dove***	
	Equation	**r**	**Equation**	**r**	**Equation**	**r**
Saprobic	y = -27.37x + 93.83	-0.31	y = -15.06x + 102.48	-0.38	y = 4.01x + 16.51	0.05
PSI	**y = 0.60x + 17.57**	**0.43**	**y = 0.80x + 28.67**	**0.75**	y = -0.04x + 26.81	-0.05
LIFE	**y = 17.92x – 84.38**	**0.42**	**y = 17.96x – 57.72**	**0.67**	y = 3.10x – 0.96	0.12
BMWP	y = 0.11x + 20.96	0.23	**y = -0.24x + 116**	**-0.73**	y = -0.02x + 26.91	-0.06
ASPT	y = 8.59x – 8.24	0.30	y = 3.83x + 52.92	0.11	y = 4.74 – 6.15	0.18
NTAXA	y = 0.49x + 22.94	0.15	**y = -1.32x + 113.92**	**-0.73**	y = -0.22x + 28.95	-0.1
EPT	y = 0.95x + 26.23	0.26	y = -0.04x + 76.00	-0.02	y = 0.04x + 23.00	0.03
Abund	y = 0.006x + 31.79	0.20	y = -0.0003x + 75.80	-0.04	y = -0.0004x + 24.27	-0.03
WHPT	y = 0.14x + 17.55	0.30	**y = -0.21x + 114.04**	**-0.65**	Y = -0.01x + 26.22	-0.05
WHPT ASPT	y = 10.84x – 17.34	0.38	**y = 15.79x – 17.36**	**0.47**	Y = 2.80x + 4.87	0.13