Ecological Indicators 107 (2019) 105619

Contents lists available at ScienceDirect

Ecological Indicators

journal homepage: www.elsevier.com/locate/ecolind

Macroinvertebrate community structure as an indicator of phosphorus enrichment in rivers

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ARTICLE INFO

Keywords:
Orthophosphate
Macroinvertebrate
Biomonitoring
Eutrophication
Organic pollutant
Nutrient enrichment

ABSTRACT

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 communities. The objective of this paper was 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 invertebrate community and chemical data from two datasets, one consisting 88 sites across England and the other 76 sites, both sampled in spring and autumn using the same methodology between 2013 and 2015. There was a significant association between TRPI and TRP concentrations that was stronger than other biological indices of elevated phosphorus, including the TDI (diatoms) and MTR (macrophytes), currently available in the UK. 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.

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 discharges 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 pressures (Javie et al., 2013; Emelko et al., 2016). As a result, there is currently no standard macroinvertebrate methodology available to characterise or identify P impacts 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.

More commonly, freshwater algae and macrophytes are used to assess nutrient loadings because they require several macronutrients for growth, particularly nitrogen and P (Conley et al., 2009). Excessive nutrient loading can lead to prolific 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

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https://doi.org/10.1016/j.ecolind.2019.105619
Received 25 February 2019; Received in revised form 25 June 2019; Accepted 4 August 2019
1470-160X/

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 may be complex.

It is widely acknowledged that nutrient enrichment can reduce instream faunal biodiversity (Smith, 2003; Hilton et al., 2006; Bini et al., 2014) and, in particular, decrease 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. Piggott et al., 2012). There is evidence that invertebrate communities respond to strong nutrient gradients (Smith et al., 2007; Yuan, 2010; Heiskary and Bouchard, 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 across 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 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).

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.

A strong case can therefore be made for the development of a biomonitoring tool for quantifying the degree to which riverine TRP concentrations impact upon the macroinvertebrate community in the UK. Such a metric would complement existing eutrophication indicators for WFD classification (e.g. TDI, MTR) and align with other macroinvertebrate community based indices developed for other stressors (e.g. Proportion of Sediment-sensitive Invertebrates [PSI]; Extence et al., 2013; Turley et al., 2016). 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:

Explore whether there is a statistical relationship between family-level macroinvertebrate community data and TRP at the national scale;

Compare the strength of macroinvertebrate-TRP relationships with traditional biological measures of eutrophication, including diatom and macrophyte community composition;

Use case studies and national data, to assess whether a TRP macroinvertebrate biomonitoring index provides additional information to that available using existing metrics, such as evidence of ecological effects not detecting using traditional metrics;

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 prior, published analysis that identified macroinvertebrate taxa 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 spring and autumn 1995 across England, Wales and Northern Ireland, to determine which invertebrate families were potential indicators of P status. The dataset had 6695 records, including both spring (February–July) and autumn (28th August–November) samples, and covered a range of nutrient concentrations from $< 0.001\ \mathrm{mg\,l^{-1}}$ to over $0.5\ \mathrm{mg\,l^{-1}}$. 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 (Paisley et al., 2003). This was justified because their analysis accounted for spring and autumn separately so seasonally specific water quality measures were deemed most suitable. Biological data comprised the abundance of macroinvertebrates based on the 76 BMWP scoring families (Whalley and Hawkes, 1997), collected using nationally standard 3-minute kick samples and hand search (Environment Agency, 2009). Paisley et al. (2003) then 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. These river types were differentiated using neural network analysis, which identified altitude, alkalinity and substrate composition as the key controls on macroinvertebrate community response to TRP (Paisley et al., 2011). The five site typology represents

Table 1
Characteristics of the 5 river types that differentiate TRP indicator invertebrates after Paisley et al. (2011). Descriptions are only included as a qualitative indication of the broad type of river that is most likely associated with each river type. To determine river type, focus should be given first to the composition of the substrate, then the alkalinity and finally to the altitude.

River type	Description	Composition of substrate (% by area)				Alkalinity ($\mathrm{mg\,L^{-1}}$)	Altitude (m)
		Boulders	Pebbles	Sand	Silt		
1	Upland, fast-flow	50	40	5	5	30	> 100
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

Table 2
TRP tolerance bandings and the nutrient score associated with each, which is dependent on the abundance of that family. The group is determined using supplementary table A, which requires information on river type and season of sample collection.

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	–	–	–	–

a progression from fast-flowing upland streams to slow-flowing lowland streams, with generally increasingly alkalinity and fining of substrate particle size (Table 1).

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 effect on the macroinvertebrate community. The strength of the statistical association of macroinvertebrate families with TRP (from 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) and PSI scores for assessment of flow stress and fine sediment pressures, respectively (Extence et al., 1999; Extence et al., 2013). The sensitivity grouping of families depends on the river type (Table 1), which must be known to partition macroinvertebrate families into appropriate groups and allow comparison of TRPI values between different river types. 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 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 the total score made up by TRP sensitive taxa, 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).

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 information 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 (March–June) and autumn (September–November) (Supplementary Material B). Seasonal values were used as separate replicates because TRPI accounts for seasonality in the calculation of the score. These data represented a range of TRP concentrations (0–4.6 mg l^{-1}) and geographical locations (Fig. 1). TRPI was calculated using macroinvertebrate data collected using EA standard protocol 3-minute kick samples followed by 1-minute hand searching different habitats being sampled with effort proportional to extent (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

Table 3
Proposed 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

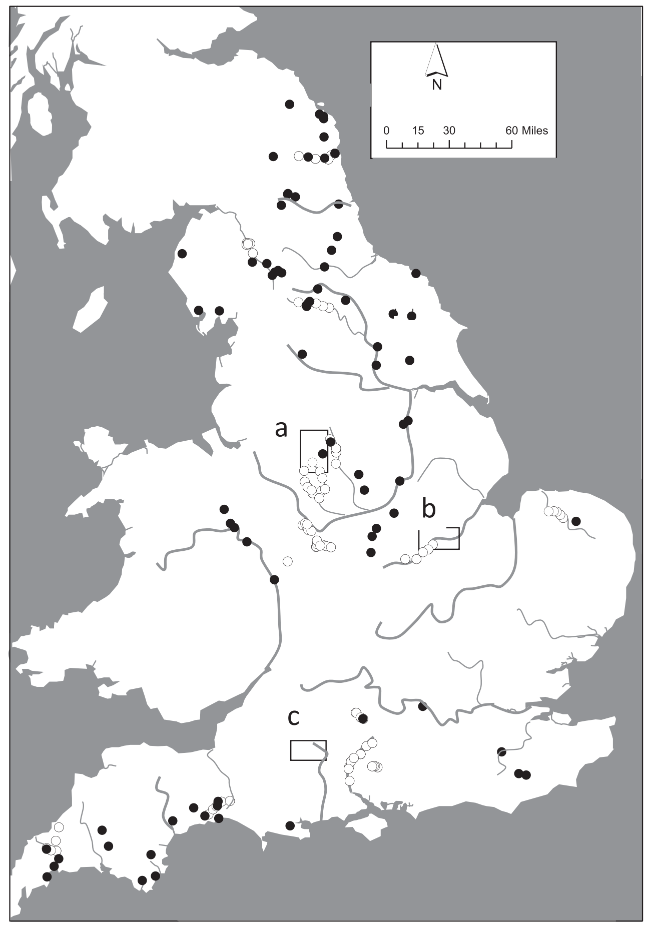

Fig. 1. Map of sites included in the analysis. Open circles are author sampled sites and filled circles are EA sites. Rectangles indicate case study rivers: River Dove (a), River Welland (b) and River Wylye (c).

community data, which were used to calculate TRPI and other commonly used macroinvertebrate indices (Fig. 1; Supplementary Material C). These sites did not have the same range of TRP concentration as the author-collected database (0–1.4 mg l^{-1}) 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 the 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.

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 diatom 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 designed to identify different pressures. The proportion of Ephemeroptera, Plecoptera, Trichoptera (EPT) in a sample has been used internationally as an ecological indicator of water quality and ecosystem health (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; Turley et al., 2016; Extence et al., 2017) and the Saprobic Index, which is used in Europe to assess organic pollution stresses (Rolauffs et al., 2004).

2.4. Case study test sites

Given the potential 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 (Fig. 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 (0.1–1 mg l^{-1}) and trajectories and to represent different regional, geological, hydrological and land use scenarios.

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 ($p < 0.01$).

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. Results

3.1. Statistical relationship between family-level macroinvertebrate community data and TRP

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. The results tentatively suggest that, nationally and across all sampled rivers, the proposed TRP bandings (Table 3) represent concentrations of 0–0.1 (very low); 0.1–0.4 (low); 0.4–0.6 (moderate); 0.6–1 (high) and > 1.5 (very high); however, there is scatter, particularly at low TRP values, and values are dependent on river type.

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

The TDI and MTR were both correlated with TRP significantly and displayed 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 both are indicators of the same stressor with inverse scales (e.g. 100% indicates high impact for TDI and low impact for MTR). However, the relationship included considerable 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 strongest relationships were with WHPT ASPT ($r = 0.67$) and PSI ($r = 0.64$). The latter is indicative of elevated fine sediment and this 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 the regional water supply company, Wessex Water, indicated that TRP concentrations in the River Wylye have been reduced since the 1990s due to phosphate stripping from upstream sewage works discharges and general investment. TRPI calculated using both spring and autumn macroinvertebrate communities has consistently increased between 1991 and 2011, from low to very low TRPI values (Fig. 3). 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; Fig. 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 misleadingly indicate increasing TRP pressure as TRP declines.

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 with TRPI but with weaker relationships ($r = -0.38$ and $r = -0.65$, respectively). Other metrics are not correlated with TRPI (Supplementary E).

3.4.2. 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 (Fig. 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 (Fig. 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%) (Fig. 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

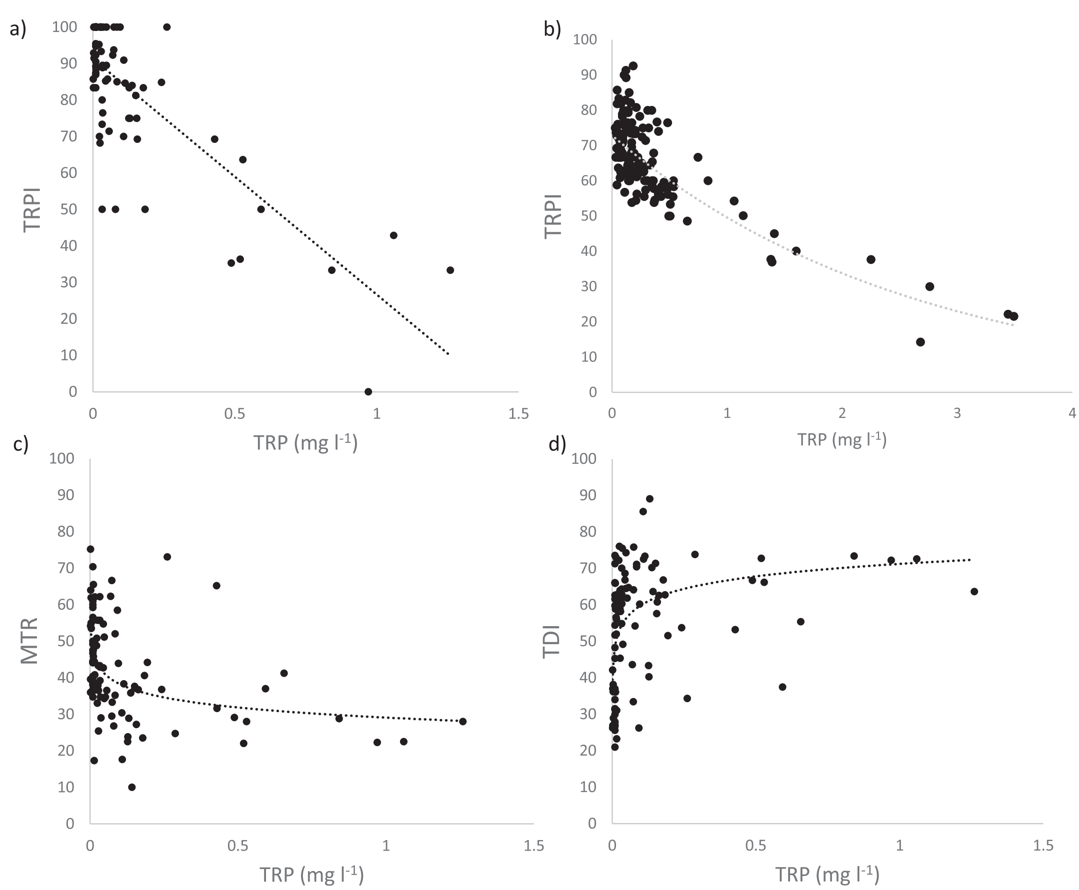

Fig. 2. Scatter plots with linear and exponential lines of best showing a) TRPI against TRP measured at 76 sites by the EA in 2015, b) TRPI against TRP at 88 sites measured by the authors in spring and autumn, c) MTR against TRP and, d) TDI against TRP derived from the same 76 sites as TRPI in panel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

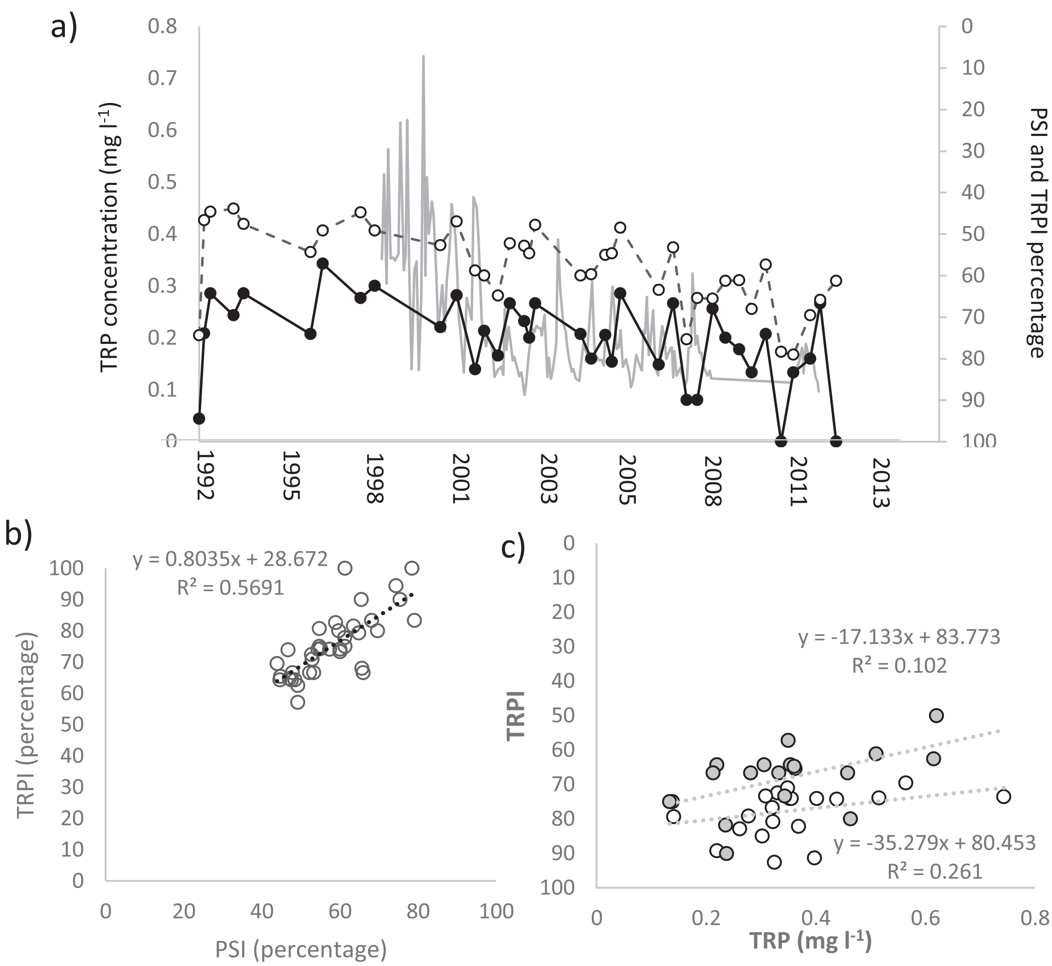

Fig. 3. TRP conditions on the River Wylye at Norton Bavant. a) TRPI values (full circles) and PSI (open circles) from 1991 to 2011 with TRP concentration overlaid (grey line) over the same period. Note the y-axis is inverted so TRPI and PSI gradients follow TRP, with unimpacted conditions occurring at 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.

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.

3.4.3. 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 (Fig. 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}) (Fig. 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 (Fig. 6).

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 the fact that macrophytes, diatoms and invertebrates possibly integrate the effect of TRP over varying timescales, due to their differing individual residence times in rivers, relative mobility levels and life cycles (Johnson & Hering 2010).

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

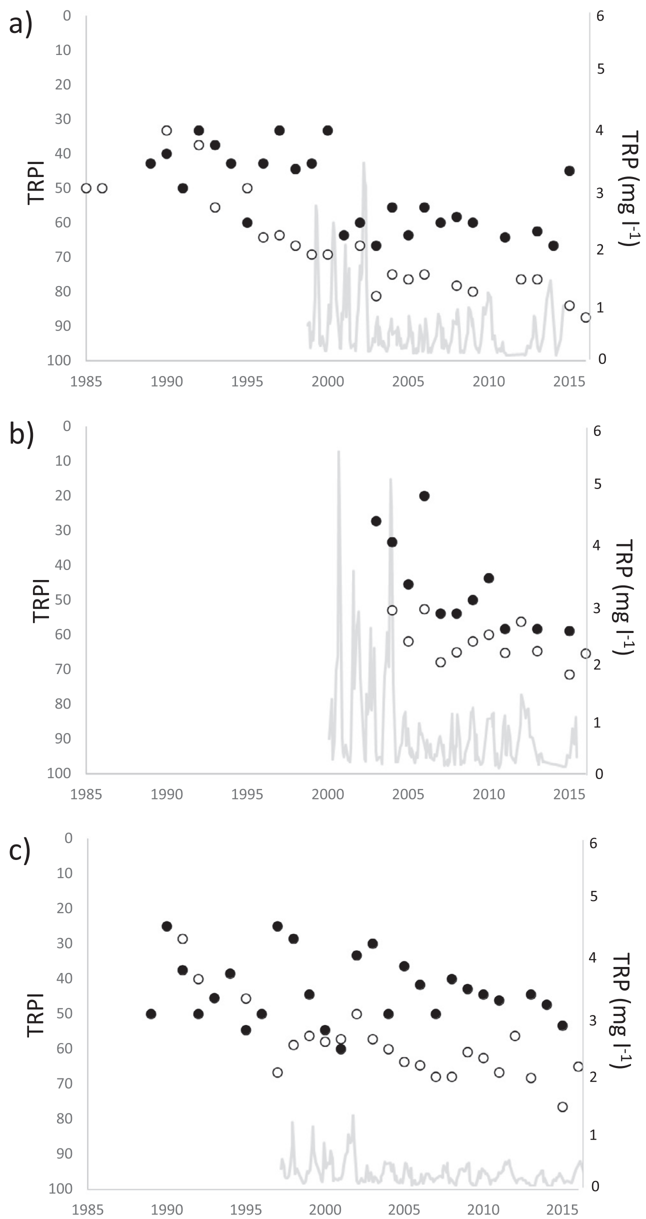

Fig. 4. Spring (open) and Autumn (filled) TRPI values at Rockingham (a), Harringworth (b) and Collyweston (c) on the River Welland. TRP measures (grey line) are also indicated. Note the inverted y-axis for TRPI so improvements follow the same direction as improvements in TRP.

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) and differences in flow history and habitat structure.

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 level

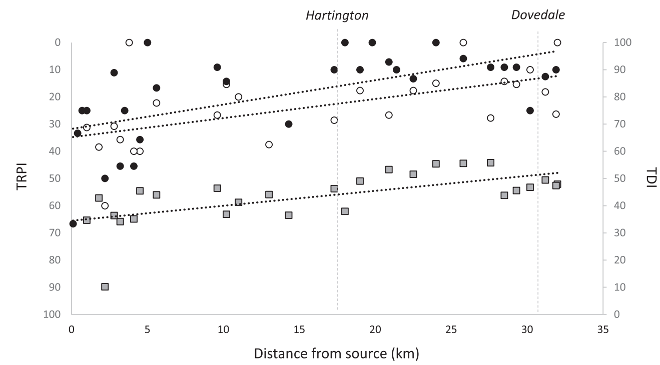

Fig. 5. The TRPI on spring (open) and autumn (closed) circles at sites on the River Dove with increasing distance downstream Squares indicate the TDI, calculated on diatom community at the same sites, at the same time. The graph shows both metrics increasing with downstream distance, indicating increased TRP stress. Note the inverted y-axis for TRPI so improvements follow the same direction as improvements in TDI.

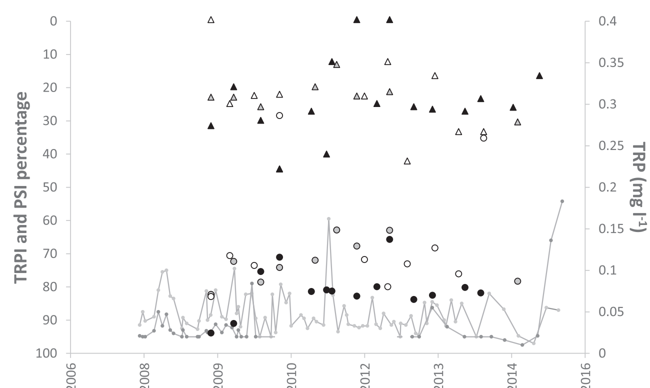

Fig. 6. TRP measured at Hartington (light grey line) and Mayfield (dark grey line) with the PSI (circles) and TRPI (triangles) measured through time at three sites on the River Dove: Hartington (19 km from source – grey symbols); Dovedale (31.2 km from source – black symbols), and Mayfield (40 km from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

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 based on the River Dove suggest relatively low concentrations of P could have 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 recalculated 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). However, there is evidence that MTR and TDI perform less well in river type 4 and 5 (i.e., lowland, slow flowing rivers), at least partially because of the difficulty in untangling the impacts of physical condition from changes in water chemistry (Szoszkiewicz et al. 2006; Steffen et al., 2014). 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 (Fig. 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 has the potential to provide 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, UK metrics, such as LIFE and PSI ($r = 0.97$). 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 key indices simultaneously would be appropriate, with TRPI used as a component of 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 pattern probably arises because the invertebrate community is 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 the magnitude of TRP concentrations. As a result, associations between TRPI and TRP in individual case studies were typically statistically significant, but weak. In some cases, there was also association between PSI and TRPI, which likely relates to the TRP commonly being bound onto fine sediment, with elevated fine sediment and elevated TRP often co-occurring (Owens & Walling, 2002). However, it should be noted this was not always the case, for example the River Dove case study, which showed evidence of TRP pressure but without concomitant fine sediment pressure.

TRPI appears to respond to relatively subtle changes in TRP, such as on Costa Beck (Fig. 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. This suggests further research is required to understand the direct, causal implications of P on macroinvertebrate communities, which could relate to the fact that elevated levels of normally limiting nutrients, including phosphorus, in food can decrease the growth rate of animals (Boersma and Elser, 2006). For example, Evans-White et al. (2009) found elevated P impacted macroinvertebrate communities, particularly shredders and collector-gatherers, potentially due to elevated P altering food quality. In support of this, Halvorsen et al. (2015) found elevated P in experimental mescosms reduced growth rates of the caddisfly *Pycnopsyche lepida* feeding on leaf litter.

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 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 appear very 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 with coarser sediments. 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 should ideally 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 both alongside other invertebrate metrics and 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 strongly influenced by 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.

Acknowledgements

We are grateful to Richard Chadd and Shirley Medgett at the Environment Agency for assistance in testing the TRPI methodology and providing helpful feedback. We thank The Wessex Chalk stream and Rivers Trust for permission to use their data and Natural England, Staffordshire Wildlife Trust and the Salmon and Trout Conservation UK for support and feedback during the development of this monitoring tool.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2019.105619.

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