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PLEASE CITE THE PUBLISHED VERSION

<https://doi.org/10.1111/1365-2664.13795>

PUBLISHER

Wiley

VERSION

AM (Accepted Manuscript)

PUBLISHER STATEMENT

This is the peer reviewed version of the following article: GUARESCHI, S. ... et al, 2021. Invasive species influence macroinvertebrate biomonitoring tools and functional diversity in British rivers. *Journal of Applied Ecology*, 58 (1), pp.135-147, which has been published in final form at <https://doi.org/10.1111/1365-2664.13795>. This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Use of Self-Archived Versions.

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REPOSITORY RECORD

Guareschi, Simone, Alex Laini, Judy England, Tim Johns, Martin Winter, and Paul Wood. 2021. "Invasive Species Influence Macroinvertebrate Biomonitoring Tools and Functional Diversity in British Rivers". Loughborough University. <https://hdl.handle.net/2134/13567499.v1>.

**Invasive species influence macroinvertebrate biomonitoring tools and functional diversity in
British rivers**

Simone Guareschi¹, Alex Laini², Judy England³, Tim Johns³, Martin Winter³, Paul J Wood¹

¹ Geography and Environment, Loughborough University, Loughborough, Leicestershire, LE11 3TU, England, UK

² Department of Chemistry, Life Sciences and Environmental Sustainability, University of Parma, Parco Area delle Scienze 11/A, 43124 Parma, Italy

³ Environment Agency, Horizon House, Deanery Rd, Bristol BS1 5AH, United Kingdom

Corresponding author: Simone Guareschi, email: S.Guareschi@lboro.ac.uk

SG ORCID: 0000-0003-2962-0863

Keywords: biological invasions, bioassessment, demon shrimp, fluvial systems, functional indices, waterbody assessment, freshwater, Dikerogammarus

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/1365-2664.13795

Abstract

1. Biological invasions could have major implications for the management and conservation of freshwater systems if they lead to a misclassification of waterbodies. However, there is limited understanding of the sensitivity of existing biomonitoring tools to invasive species in rivers; and even less known regarding how they influence community taxonomic and functional measures.

2. This research explores the response of freshwater macroinvertebrate communities to biological invasion using taxonomic and functional indices. Utilising a long-term dataset (spanning 2000-2019, 5,988 samples) from rivers in England, the performance of four biomonitoring tools (WHPT, WHPT-ASPT, LIFE, PSI) and two community functional indices (functional richness and redundancy) were examined before and after the colonisation of the invasive species, *Dikerogammarus haemobaphes* (Eichwald, 1841) (Crustacea: Gammaridae). This species represents a recent (first record 2012) and highly successful invader, allowing its range expansion within waterbodies to be examined in detail. Spatial (national and basin level) and seasonal (spring and autumn) effects were investigated using a Before–After Control–Impact (BACI) experimental framework and linear mixed effects models.

3. Results indicated that invasion by *D. haemobaphes* resulted in significant reductions to the WHPT index and functional diversity metrics (richness and redundancy) while more subtle patterns were observed for other metrics. Analysis of seasonal and individual river basins (River Trent and R. Thames) identified largely consistent responses. The establishment of *D. haemobaphes* also resulted in some modifications to the functional composition of aquatic communities primarily associated with voltinism and resistance form features.

4. *Synthesis and applications.* Our findings indicate that *D. haemobaphes* should be considered a significant pressure to riverine communities. These results have implications for biomonitoring which informs managerial actions as effects may not be detected using a single taxonomic index. Community functional measures are useful in characterising the effects of invasive species and may form a valuable part of the “toolbox” used for studying biological invasions in rivers. The research illustrates the need to consider the wider threats posed by invasive species on the long-term integrity of freshwaters and the efficacy of freshwater biomonitoring tools.

Abstract in Spanish: Las especies invasoras influyen en las herramientas de biomonitoreo de macroinvertebrados y en la diversidad funcional de los ríos británicos

Las invasiones biológicas pueden tener importantes repercusiones en la gestión y conservación de los ecosistemas fluviales si dan lugar a clasificaciones erróneas del estado de los cuerpos de agua. Sin embargo, el conocimiento de la sensibilidad de las herramientas de biomonitoreo frente a las especies invasoras en ríos, así como su influencia sobre las métricas taxonómicas y funcionales de la comunidad, es todavía limitado.

Esta investigación explora la respuesta de las comunidades de macroinvertebrados acuáticos a las invasiones biológicas utilizando índices taxonómicos y funcionales. Utilizando un conjunto de datos a largo plazo de ríos de Inglaterra (desde 2000 a 2019, con 5988 muestras), se examinó la respuesta de cuatro métricas de biomonitoreo (WHPT, WHPT-ASPT, LIFE, PSI) y dos índices funcionales (riqueza funcional y redundancia) antes y después del establecimiento de *Dikerogammarus haemobaphes* (Eichwald, 1841) (Crustacea: Gammaridae), un invasor reciente en los ríos de Inglaterra (primera cita en 2012). Se investigaron los efectos espaciales (a nivel nacional y de cuenca) y estacionales utilizando un diseño experimental “BACI” y modelos lineales mixtos.

Los resultados muestran que la invasión de *D. haemobaphes* provoca descensos significativos del índice WHPT y de las métricas de diversidad funcional, mientras que se observan patrones más sutiles en otras métricas. El análisis de los datos por cuencas (Trent y Thames) y por estación (primavera y otoño) identificó respuestas consistentes en gran medida. El establecimiento de *D. haemobaphes* dio lugar también a algunas modificaciones en la composición funcional de las comunidades, asociadas principalmente a los rasgos biológicos de voltinismo y formas de resistencia.

Estos resultados muestran que el establecimiento de poblaciones de *D. haemobaphes* puede considerarse como una presión significativa para las comunidades fluviales. Estos resultados tendrían implicaciones para el biomonitoreo y la gestión, ya que es posible que los efectos de la invasión no se detecten utilizando únicamente índices taxonómicos. Así, las métricas funcionales de la comunidad resultan de utilidad en la caracterización de los efectos de las especies invasoras, debiendo formar parte de la "caja de herramientas" utilizada para estudiar las invasiones biológicas en ríos. Esta investigación ilustra la necesidad de considerar, en sentido amplio, las amenazas que representan las especies invasoras sobre la integridad de los ecosistemas fluviales a largo plazo, así como la eficacia de las diferentes herramientas de biomonitoreo.

1. Introduction

The biological invasion of freshwater bodies by non-native invasive species is recognised as a growing pressure (Strayer, 2010; Gallardo, Clavero, Sánchez & Vilà, 2016) that may lead to biotic homogenization (Toussaint, Beauchard, Oberdorff, Brosse & Villéger, 2014), affecting species interactions and food web architecture (Twardochleb, Olden & Larson, 2013; Carbonell et al., 2017). Given the growing number of new invasive species globally (Seebens et al., 2017), the evaluation of their potential effects represents a major challenge for bioassessment science and aquatic ecology, with potential cascading effects for the management and conservation of freshwater systems if their presence leads to misclassification (e.g. Orendt, Schmitt, van Liefferinge, Wolfram & Deckere, 2010). Invasive species may constitute significant threats to the integrity of aquatic ecosystems that existing biomonitoring tools were not designed to address and may fail to detect their effects or presence within a waterbody (MacNeil, Briffa, Leuven, Gell & Selman, 2010; Vandekerkhove, Cardoso & Boon, 2013; Guareschi & Wood, 2019). Moreover, the integration of their presence and associated information into official biomonitoring and management frameworks has been applied heterogeneously internationally (Boon, Clarke & Copp, 2020). Aquatic macroinvertebrates are essential components of freshwater ecosystems (Suter & Cormier, 2015) and are currently among the most widely utilised indicators in freshwater biomonitoring by environmental agencies and legislators worldwide (Buss et al., 2015). Macroinvertebrate-based indices are usually based on the sensitivity of individual taxa to a specific pressure, such as organic pollution or flow stress, with taxa assigned sensitivity based on known / documented preferences (e.g. Hawkes, 1998; Extence, Balbi, Chadd, 1999). These scoring systems rarely recognize or integrate invasive invertebrate taxa, largely because their effect may be context-specific and the dynamics of their spread difficult to assess and integrate within legislation (Guareschi & Wood, 2019). For example, Mathers et al. (2016) warned that care should be taken when interpreting routine taxonomic macroinvertebrate biomonitoring data where non-native crayfish were known to be present; and Vandekerkhove, Cardoso & Boon (2013) advocated the need for optimization of methods for addressing the pressures exerted by invasive species.

The use of taxonomic-based datasets and metrics is crucial for biological conservation and long-term studies in ecology, however on their own they limit the ability to transfer or compare methodologies and results between regions (Van den Brink et al., 2011). To address this issue, community functional measures based on codified organism traits (e.g. body morphology, trophic habits and life-history strategies) have been developed to provide functional metrics that are geographically generalisable. These community functional metrics are unaffected by regional species lists and advance understanding beyond traditional taxonomic approaches (e.g. Villéger, Mason & Mouillot, 2008; Mouillot, Graham, Villéger, Mason & Bellwood, 2013; Soria et al., 2020). Functional responses have been increasingly tested in bioassessment and primary ecological research (e.g. Mondy, Villeneuve, Archambault & Usseglio-Polatera, 2012; Belmar et al., 2019), being recently identified as a promising approach for the assessment of impacts associated with biological invasion (Colin, Villéger, Wilkes, de Sostoa & Maceda-Veiga, 2018; Mathers et al., 2020a), but to date rarely used (Flood, Duran, Barton, Mercado-Molina & Trexler, 2020). To integrate environmental management and invasive species research better, wide-ranging studies considering multiple components of communities have been proposed (e.g. Matzek, Pujalet & Cresci, 2015). Moreover, the implications of aquatic invasive species on both taxonomically derived biomonitoring tools and community-based functional metrics have not been widely explored using long-term datasets comprising both invaded and control sites (see Mathers et al. 2020a for a recent trait-based example). To address these research gaps, this study aimed to examine the consequences of a successful invasive species, *Dikerogammarus haemobaphes* (Eichwald, 1841) (Crustacea: Gammaridae) on river biomonitoring tool behaviour and macroinvertebrate community functional characteristics using a long-term dataset (20-years) from British rivers. Being a relatively new invader to the UK (2012), but rapidly expanding its range, the study of this species over the period 2000-2019 represents a timely opportunity to examine the process of biological invasion in rivers. Specifically, this study sought to determine if the performance of four taxonomic-based biomonitoring tools used by regulatory agencies for freshwater system assessments and two functional indices (functional richness and redundancy) were affected following the establishment of the invasive amphipod (pre- and post-invasion assessments). In addition, this provided an opportunity to explore changes in the

macroinvertebrate functional composition following invasion by *D. haemobaphes*. Examination of this dataset at different spatial and seasonal scales would allow the identification of any shifts or changes to the results of biological indices and biomonitoring tools after the colonization of *D. haemobaphes* and facilitate an evaluation of the indirect ability of the tool(s) to detect any effects of the invasion on the wider freshwater macroinvertebrate community.

2. Materials and Methods

2.1 Biological data

Dikerogammarus haemobaphes, commonly known as “demon shrimp”, is an example of successful invasive species originating from the Ponto Caspian region and now recorded in many European areas (Bacela-Spychalska & van der Velde, 2013). The first British record of *D. haemobaphes* was in May 2012 from the River Severn (Aldridge, 2013) and it has subsequently spread rapidly, invading waterways throughout central England (Johns, Smith, Homann & England, 2018).

Data were obtained from the Environment Agency, the statutory regulator within England, which is responsible for monitoring and reporting the ecological quality and status of waterbodies. The dataset, considered in the analysis, comprised 5,988 samples from 259 lotic sampling sites (82 invaded sites and 177 control, details in Appendices S1 and S2 in Supporting information) across England, collected over a 20-year period (2000-2019 inclusive). For consistency, all 259 sites comprised at least 4 samples and were not subject to any other disturbance events (e.g. samples from pollution incidents) or contained other *Dikerogammarus* species. All benthic invertebrate samples were collected using a standard sampling protocol for river biomonitoring, comprising a 3-minute ‘kick-sample’ using a standard pond-net (ISO 7828-1985) covering all available habitats in proportion to their occurrence, followed by a 1-minute hand search (RIVPACS Macro-invertebrate Sampling Protocol, available at <http://www.eu-star.at/frameset.htm>).

Given that *D. haemobaphes* was first recorded in 2012, data from control sites for that year were not included in the current analysis. Samples collected prior to 1st January 2012 were considered “before” and data after 31st December 2012 as “after” invasion (n. total= 177). Invaded sample sites were

classified into before and after invasion based on the date of the first record of *D. haemobaphes* in the dataset at the site. Given that the majority of the data could be ascribed to two river basins (River Thames and R. Trent), the analysis was undertaken at two spatial scales: national level (all data) and for individual basins (2,651 samples from the R. Thames basin and 2,633 samples from the R. Trent basin). Assuming the potential effect of seasonality on macroinvertebrate occurrence (e.g. Mathers et al., 2016) analysis at the national level was also undertaken using spring (2,365 samples) and autumn datasets (2,291 samples) separately. These seasons are used routinely for biomonitoring purpose in UK rivers (UKTAG, 2014). Macroinvertebrate data were largely recorded at species level (60% of the entire taxa, n=962) and genus, while some Diptera larvae were resolved to family level and some taxa such as Hydracarina and Oligochaeta were recorded as such.

2.2 Biomonitoring tools: taxonomic and functional indices

Four standard taxonomic indices were calculated for each sample to obtain a comprehensive coverage of available biomonitoring tools: i) the Whalley, Hawkes, Paisley & Trigg index (WHPT Total: abundance-based adaptation of the globally utilised BMWP index, UKTAG, 2014) which is derived from the sensitivity of invertebrate families to organic pollution but can be affected by other factors; ii) the WHPT Average Score Per-Taxon (total WHPT value divided by the number of scoring taxa, coded WHPT-ASPT; Paisley, Trigg & Walley, 2014) which principally reflects organic pollution; iii) the Lotic Invertebrate index for Flow Evaluation (LIFE; Extence et al., 1999) which considers river flow and effects on instream communities; and iv) the Proportion of Sediment-sensitive Invertebrates (PSI; Extence et al., 2013), which provides a measure of community sensitivity to fine sediments deposition. All the above indices can be calculated using taxonomic data resolved to family level and are used by the Environment Agency to provide relative measures of lotic ecosystem conditions. The WHPT-ASPT is used, in conjunction with the number of WHPT scoring taxa, in the UK to characterise the ecological status of rivers in accordance with the requirements of the EU Water Framework Directive. The WHPT scoring system and other very similar approaches have been widely applied globally to characterise the ecological quality of flowing waterbodies (Buss et al., 2015; Guareschi, Laini & Sánchez-Montoya, 2017). The PSI and LIFE indices work with a specific

sensitivity lists and associated scoring systems and were specifically investigated because flow velocity, substrate composition and suspended sediment concentrations have been identified as potential abiotic parameters that may affect the distribution of *Dikerogammarus* species (Borza, Huber, Leitner, Remund & Graf, 2017). Functional diversity responses were investigated through calculating two metrics: i) functional richness (FRich) and ii) functional redundancy (FRed) for each sample. Both indices have been specifically identified as being sensitive to anthropogenic pressures in riparian and fluvial habitats (Bruno, Gutiérrez-Cánovas, Sánchez-Fernández, Velasco & Nilsson, 2016; Belmar et al., 2019). FRich is defined as the volume of functional multidimensional space occupied by all species in a community (following Villéger et al., 2008), while FRed reflects the relative resistance and resilience of an ecosystem to pressure (Hooper et al., 2005; McLean et al., 2019) and quantifies the degree to which species in a community share similar ecological features. FRed is calculated as the difference between taxonomic diversity (using the Gini–Simpson index) and functional diversity (using Rao’s quadratic entropy) following Pillar et al. (2013).

Both functional measures were characterized using the macroinvertebrate biological traits proposed by Tachet, Richoux, Bournaud & Usseglio-Polatera (2010) and Schmidt-Kloiber and Hering (2015) at the genus level. Taxa resolved to species level were aggregated to genus to be consistent with current trait classifications while data resolved to family were assigned values based on the average for the genus. Genus-level information is widely considered suitable to study the functional diversity and response of riverine communities (e.g., Gutiérrez-Cánovas, Sánchez-Fernández, Velasco, Millán & Bonada, 2015). This approach allowed the entire dataset covering 20 years to be utilized. Gower dissimilarity matrices (adapted for the fuzzy-coded traits, Pavoine, Vallet, Dufour, Gachet & Daniel, 2009) using all the 11 biological traits (63 modalities, Appendix S3) were used to calculate FRich. To calculate FRed five “effect traits” (size, dispersal, mode of locomotion, food consumed and feeding habits) were selected based on the review of Schmera, Heino, Podani, Erós & Dolédec (2017) and Hevia et al. (2017). An identical approach to measure both functional metrics was adopted by Bruno et al. (2019) and Laini et al. (2019) for alpine and temperate riverine community. However, to provide comprehensive coverage, functional redundancy considering all the biological traits was also calculated and inter-correlations among all measures examined.

2.3 Data analysis

To estimate potential changes in the biomonitoring tools examined (taxonomic and functional), data were analysed in a Before–After Control–Impact (BACI) design framework. To achieve this, each sample was placed into one of four groups: (i) control-before invasion, (ii) control-after invasion, (iii) invaded-before invasion date and (iv) invaded-after invasion date. The use of control sites (in the same climatic and geographic areas and for the same time-period) provided a complete BACI study design that is considered optimal when attempting to isolate the effect of an impact from natural variability (Christie et al., 2019). In this instance the potential impact considered was before and after the establishment of a new invasive species, *D. haemobaphes*. BACI analysis with a significant interaction (Before–After) * (Control–Impact) would indicate that following the establishment of *D. haemobaphes* population there had been an effect on the community measures at invaded sites. To determine the potential effects of the arrival of *Dikerogamamrus haemobaphes* on biomonitoring tools and functional metrics, they were analysed within a linear mixed models' framework to avoid pseudoreplication (LMM; package “lmerTest” Kuznetsova, Brockhoff & Christensen, 2017). LMMs are powerful tools for analysing complex datasets with multiple or clustered observations (Schielzeth et al., 2020) and were repeated at different spatial scales and seasons considering the interaction (BA*CI) as a fixed factor while sampling sites and year as random factors. All indices were standardised (subtracting the mean and dividing by the standard deviation) to facilitate comparison of the potential changes after the arrival of the invasive species. All models were validated by visually checking the graphical distribution of residuals for normality and homoscedasticity (Zuur et al., 2009). Correlations (Spearman rank correlations) among all indices were also investigated to assess their associations and inter-relatedness. To explore for potential differences in the functional structure of invertebrate communities, a fuzzy principal component analysis (FPCA, Chevenet, Dolédec & Chessel, 1994; Bruno et al., 2019) was performed on the Community-level Weighted Means matrix (CWM). CWM matrix with the proportion of each trait character from each sampling site was obtained by crossing the “taxon x traits” and “taxon x site” matrices. Squared-root transformation was applied on abundance data prior

to the calculation of the CWM matrix. Ordinations were performed separately for each dataset (national, basin, season scale) to explore differences in functional trait compositions between BACI levels using the first two axes. This enabled the potential shift in community functional composition to be identified (via the movements in centroid location before and after invasion for both the control and impact groups) in addition to changes in the size or shape of the functional space (via the convex hull – the minimum area enclosing all functional community values). Given the unbalanced number of samples between BACI levels, randomization samples were selected for each group using the least abundant sample group as the threshold (> 500 samples at national level and >100 at basin and seasonal level). The consistency of the outputs was assessed by testing changes in the slope of the connecting line between pairs of centroids and their distance over 1,000 simulations. All statistical analyses were performed using R statistical software v. 3.6.2 (R Core Team, 2019) and scripts used to calculate functional indices are available at <https://github.com/alexology/biomonitoR>.

3. Results

Taxonomic and functional indices were significantly but weakly correlated (maximum Spearman correlation coefficient was 0.37 between WHPT and FRich), indicating that the different indices represented distinct macroinvertebrate community characteristics. In contrast, the two measures of Functional redundancy (using all traits and the effects traits alone) were highly correlated ($r=0.91$, p -value < 0.001) and as a result only the one using effects traits were considered in subsequent analyses. A full list of the cross-correlation results among taxonomic and functional indices are presented in Appendix S4.

3.1 Implications at national level

A total of 5,988 samples comprising more than 8 million individual macroinvertebrate specimens were included in the analysis. The outputs from linear mixed models indicated significant changes in 4 of the 6 metrics considered (2 taxonomic and 2 functional indices) following the invasion of *D.haemobaphes* for the entire dataset - ‘all samples’ (Table 1). WHPT, FRich and FRed displayed

contrasting patterns between control and invaded sites with a significant reduction observed at invaded sites compared to stable or marginal increases at control sites (Figure 1). PSI displayed significant changes with limited or no increase after the arrival of the species compared to control samples (Figure 1). LIFE and WHPT-ASPT did not display significant changes.

When the two main sampling seasons were considered separately similar patterns and trends were recorded especially for autumn samples (Table 1, Appendix S5) when compared to ‘all samples’ at the national level. Significant differences (with reductions in index values at invaded sites) were observed between the control and invaded sites for WHPT, FRich and Fred, while the output for WHPT-ASPT, PSI and LIFE displayed similar trends and no significant changes.

The pattern for the spring sampling season was also comparable to the autumn, although the statistical significance was slightly reduced. Significantly different trends were recorded for WHPT and FRich (lower values at invaded sites), while the response for Functional redundancy was marginally significant ($p=0.05$, Table 1, Appendix S6).

Even though the general shape and size of the four functional composition plots were similar for each level at national scale, the FPCA ordination plots revealed some shifts when comparing the centroids of invaded and control sites on the first two axis; explaining 58.7% of the total variability (Figure 4). For the post invasion time period, samples from the control period migrated along the first axis (horizontally), primarily associated with “reproduction” (e.g. ovoviviparity) and “life cycle duration” characteristics. The centroids for the invaded samples shifted vertically on the second axis following the arrival of *D. haemobaphes*. The second axis was primarily associated with “resistance form” traits (e.g. lack of resistant life stage) and “number of life-cycles per year” (e.g. multi-voltinism). Trends and the associated traits were broadly comparable for the spring dataset (Figure 5, autumn dataset plots available in Appendix S7). The consistency of change in distance and slope between centroids (before and after of invaded and control samples) are presented in Appendix S8; and differences in slope results were confirmed via the randomization analysis at the national and seasonal levels.

3.2 Implications at regional level

The general patterns recorded for the River Thames and R. Trent basins were comparable and consistent with the results recorded at the national scale, with significant changes of WHPT, FRich and FRed in both river basins after the arrival of *D. haemobaphes*. Within the R. Trent basin (Figure 2, Appendix S9), the most marked reductions (at the invaded sites) were observed for WHPT, functional richness and functional redundancy, while LIFE, WHPT-ASPT and PSI displayed more subtle patterns. Similar patterns were recorded for the R. Thames basin (Figure 3, Appendix S9), where the most marked changes were observed for WHPT, PSI and both functional measures. Focussing on functional composition, in both river basins the invaded site centroids migrated on the second axis (associated with multi-voltinism and resistance life form / stages) when compared to control sites that migrated along the first axis (Appendices S10 and S11, first two axes explained >50% of the total variance in all instances). However, the centroid shifts were not always significant after randomization as there was some overlap in slope confidence intervals for both datasets. Interestingly, in the R. Trent basin 100% of the randomizations (1000 out of 1000) displayed greater distance between the centroids for the before and after periods for the invaded site samples (S8).

4. Discussion

The use of long-term datasets to enable the detection of temporal change has been increasingly advocated (e.g. Dafforn et al., 2016), and data from routine biomonitoring activities may provide appropriate long-term, high quality, standardized datasets for analysis of changes in the aquatic environment. For example, Vaughan and Ormerod (2012) identified an improving trend of water and biological quality over the period 1991–2008 using biomonitoring data for British rivers. The results of our long-term analysis (2000-2019) identify a similar pattern, with an increase or constant trend for most biological metrics when only control samples were considered. However, when the overall BACI dataset is considered, our results provide insights into how river communities are affected by a new invader as changes to both taxonomic metrics and functioning of instream macroinvertebrate communities were identified. Although we cannot exclude the potential interactions and cumulative effects of other historically recorded non-native species already present in British rivers (e.g. *Dreissena polymorpha*), our approach to the analysis of the effects of *D. haemobaphes* (covering 20

years around the date of the first record) may limit this influence. The results of this research support the fact that *D. haemobaphes* should be considered a significant threat to rivers it invades given the changes and reductions we observed for multiple types of indices (based on taxonomic and functional information, and on both presence and abundance data) and across spatial scales and seasons. This reinforces the need for specific research (e.g. periodic monitoring of population dynamic) and management activities (e.g. biosecurity protocols) focussed on invasive species. The effects recorded are probably related to the functional characteristics of successful invaders like *D. haemobaphes* including its flexible feeding habits, successful predatory behaviour (including intraguild predation), high fecundity and competition for resources with other species; or a combination of all these factors (Kinzler, Kley, Mayer, Waloszek & Maier, 2009; Bacela-Spychalska & van der Velde, 2013; Gallardo & Aldridge, 2013a; Constable & Birkby, 2016). Moreover, recent research has also indicated how *D. haemobaphes* may carry pathogens and parasites into its invasive range (e.g. *Cucumispora ornate*) that potentially threaten native species populations (Bojko et al., 2019). Great Britain has become increasingly affected by aquatic invertebrate invaders originating from the Ponto Caspian region (Gallardo & Aldridge, 2013a; Gallardo & Aldridge, 2013b; Gallardo & Aldridge, 2015). The extensive network of river channels and canals in the UK, coupled with inter-basin water transfers, have been recognized as migratory pathways that may have directly facilitated the internal dispersal of *D. haemobaphes* (Johns et al., 2018) and other invasive taxa (Gallardo & Aldridge, 2018). Our research facilitates a preliminary evaluation of the ability of current indices and tools to detect the effect of the invasion on the wider freshwater community. The performance of the Whalley, Hawkes, Paisley & Trigg index (WHPT - Paisley et al., 2014) in relation to invasive species has been presented for the first time in this research and it was shown to be particularly sensitive at all scales (national level, basin level and season) to the invasion of *D. haemobaphes*. A similar level of sensitivity was displayed by both functional metrics (with highly significant differences being recorded in 4 of the 5 sets of analysis). These results support those of Ćuk et al. (2019) who reported a negative effect of multiple invasive species on riverine biological monitoring tools, including the BMWP score, in Croatian rivers. Similarly, MacNeil et al. (2013) demonstrated how invasion by the co-generic *Dikerogammarus villosus* affected two macroinvertebrate-based indices, the MMIF (Multimetric

Macroinvertebrate Index Flanders, Gabriels, Lock, De Pauw & Goethals, 2010) and BMWP score (Hawkes, 1998) in Belgium.

It is particularly interesting to note that the response of the WHPT score and WHPT-ASPT, the latter being used directly for WFD ecological assessment in British rivers, displayed different trends despite being correlated with each other. The WHPT-ASPT displayed marginally significant changes at basin level (but not a reduction) at sites invaded by *D. haemobaphes* compared to control sites, although the trends were comparable. These results display a similar pattern to those of Mathers et al. (2016), who demonstrated that ASPT scores (derived from BMWP, Armitage, Moss, Wright & Furse, 1983) did not significantly change following invasion of signal crayfish (*Pacifastacus leniusculus*). Similarly, the Spanish adaptation of the index, IASPT (Iberian ASPT - Alba-Tercedor et al., 2002) has also been shown to be relatively insensitive to the presence of rare taxa (Guareschi et al., 2017) that may occur during the early stages of colonisation by non-native taxa.

These different patterns may primarily reflect the ASPT calculation method (average value of all scoring taxa), but also to the fact that it was originally designed to reflect taxa sensitivity to organic pollution (Sandin & Hering, 2004). The decline recorded in total WHPT score, but not WHPT-ASPT, may indicate that a pressure other than organic pollution was present. The BACI analysis indicates that the decline was most likely due to the invasion of *D. haemobaphes*. These results also highlight the need to recognise the purpose for which the individual tools were originally designed. Their appropriate use, ideally in combination (taxonomically based and community functional measures) may provide a greater understanding of the multiple pressures present in a waterbody.

The observed trends for the Proportion of Sediment-sensitive Invertebrates (PSI) and the Lotic-invertebrate Index for Flow Evaluation (LIFE) suggest that these indices are less sensitive to the establishment of *D. haemobaphes* populations than the other indices examined. In contrast, Mathers et al. (2016) demonstrated that abundance weighted biomonitoring tools such as LIFE and PSI were subject to significant inflation following *Pacifastacus leniusculus* invasion and its selective predation on low scoring taxa. When considered together, these results demonstrate the need for care when choosing and interpreting biomonitoring tools as the effects may also vary reflecting the individual characteristics of the invasive species. When compared to *P. leniusculus*, *D. haemobaphes* did not

show a marked seasonal variability in the effects on the wider community (except for functional redundancy measures), suggesting a constant pressure on riverine systems throughout the year. The response of functional indices following the invasion of *D. haemobaphes* indicated a consistent significant decreasing trend of both measures (at both the seasonal and spatial scales) at invaded sites, compared to relatively stable or slightly increasing values at control sites. Similarly, Michelan, Thomaz, Mormul & Carvalho (2010) demonstrated how plant community functional richness was significantly reduced as the biomass of *Urochloa subquadrifera* (an invasive macrophyte) increased in Brazilian waterbodies. In addition, Milardi, Gavioli, Soininen & Castaldelli (2019) demonstrated how alien species invasions may undermine regional functional diversity in Mediterranean stream fish communities. Focussing on British rivers, Mathers et al. (2020b) recently reported that the functional properties (e.g. richness) of macroinvertebrate communities can be negatively affected by signal crayfish invasion (but with differences at the regional level). This highlights the importance of considering community functional measures alongside other indices to facilitate understanding and interpretation of biological communities' results (e.g. Perez Rocha et al., 2019) also in aquatic biological invasions.

When the effect of community functional characteristics (at invaded sites) are considered alongside the reduction in WHPT scores temporally, it suggests that the arrival and establishment of *D. haemobaphes* in British rivers should be considered a long-term sustained pressure affecting riverine macroinvertebrate community structure and functioning. A reduction of functional richness may affect river ecosystem functioning via a reduction in productivity, nutrient cycling and litter decomposition. Moreover, the reduction in community functional redundancy may also reflect impairment of ecosystems resilience and sensitivity to disturbances (Angeler et al., 2016; McLean et al., 2019) and may indicate ecosystem and community vulnerability to future disturbances.

Instream functional measures (richness and redundancy) were particularly sensitive to biological invasion in our study and may be valuable tools in facilitating the detection of the establishment of an invasive species population, which could be used as part of a "toolbox" for the assessment of biological invasion of aquatic ecosystems. However, further research is recommended to examine the effects of multiple invasive species occurring at the same site, and in particular their effects on

community functioning and biological metrics so that specific indices that focus on biological invasions can be developed (see a an attempt in Arbaciauskas et al., 2008; Gallardo & Aldridge, 2013b) and better integrated into national and international legislation.

The analysis of functional trait composition appeared to identify more subtle trends than those obtained from specific functional metrics. Although the shifts following the establishment of the invading species appeared quantitatively limited, it is important to note that the changes recorded at invaded sites typically contrasted (i.e. different direction of change) or were characterised by different magnitudes of change (i.e. different distance between centroids) when compared to control sites.

These significant shifts were generally associated with multi-voltinism (number of cycles per year) at the national and seasonal scale, while more subtle patterns were observed at the individual basin level. Changes to voltinism patterns have been identified as a response to different stressors in both aquatic (Belmar et al., 2019) and terrestrial invertebrates (Altermatt, 2009). Similarly, when considering signal crayfish in UK, Mathers et al. (2020b) reported that taxa with one brood per year and/or that take over a year to complete a life cycle were negatively affected following invasions in some regions.

Considering the successful invasive characteristics displayed by *D. haemobaphes*, its expanding geographic distribution (numerous European countries) and the biomonitoring metrics applied, the results have widescale relevance for other invasive species and environmental contexts (e.g. freshwater ecologists, conservationists, environmental agencies, private consultancies). Our results provide an important starting point for the development of biological monitoring measures designed to detect the presence and impacts of invasive species. The development of such tools would advance global scientific research in the field and benefit numerous stakeholders providing a more complete understanding of the process and effects of biological invasion within river ecosystems.

Acknowledgment

S. Guareschi was supported by a Royal Society-Newton International Fellowship at Loughborough University (NIF\R1\180346). The authors would like to express their gratitude towards the various Environment Agency teams for collecting, preparing and extracting the data from the BIOSYS dataset, especially Carole Fitzpatrick. The views expressed in this paper are those of the authors and not

necessarily those of the Environment Agency of England. Mark Szegner is gratefully acknowledged for the preparation of Figure S2. The authors thank and acknowledge the insightful and constructive comments of two anonymous reviewers, the associate editor and editor which have improved the manuscript.

Authors' Contributions

SG and PJW conceived the idea and designed methodology; JE, TJ, MW provided the raw data; SG and AL analysed the data; JE, TJ, MW, PJW provided interpretations of results from a management perspective; SG led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Data availability statement

All site details and biological data can be obtained freely from the Environment Agency “Ecology and Fish Data Explorer” at <https://environment.data.gov.uk/ecology-fish/> selecting “invertebrates”. To obtain the data - specify the period required and select the geographic area on the map and input site codes (SITE_ID) found in first column of Appendix S1.

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Tables

Table 1. Linear Mixed Model (LMMs) results for all biological indices considered in the BACI analysis. (a) National level n.=5,988 samples; (b) Autumn dataset; (c) Spring dataset. Basin level results available in S9. ***p < 0.001; **p < 0.01; *p < 0.05

a) National level (all samples)					
WHPT Total					
	Sum Sq	DenDF	F value	<i>p-value</i>	
BA	0.55	51.0	1.60	0.2114	
CI	2.11	262.2	6.12	0.0140	*
BA:CI	35.08	4809.6	101.71	< 2e-16	***
WHPT-ASPT					
	Sum Sq	DenDF	F value	<i>p-value</i>	
BA	0.95	218.4	6.03	0.0148	*
CI	2.38	259.5	15.17	0.0001	***
BA:CI	0.02	5391.1	0.14	0.7057	
LIFE Index					
	Sum Sq	DenDF	F value	<i>p-value</i>	
BA	0.62	211.0	2.66	0.1045	
CI	9.69	261.0	41.45	5.77e-10	***
BA:CI	0.00	5388.1	0.00	0.9631	
PSI Index					
	Sum Sq	DenDF	F value	<i>p-value</i>	
BA	0.84	116.3	4.12	0.0447	*
CI	14.78	260.0	72.66	1.27e-15	***
BA:CI	1.77	5170.3	8.68	0.0032	**
Func. Richness					
	Sum Sq	DenDF	F value	<i>p-value</i>	
BA	8.82	57.5	13.12	0.0006	***
CI	0.04	267.1	0.07	0.7984	
BA:CI	44.90	4912.0	66.80	3.79e-16	***
Func. Redundancy					
	Sum Sq	DenDF	F value	<i>p-value</i>	
BA	0.98	37.6	1.40	0.2442	
CI	6.36	285.9	9.06	0.0028	**
BA:CI	26.26	4593.9	37.41	1.04e-09	***
b) Autumn dataset					
WHPT Total					
	Sum Sq	DenDF	F value	<i>p-value</i>	
BA	1.21	33.3	3.71	0.0626	
CI	2.32	271.3	7.12	0.0081	**
BA:CI	13.88	2012.3	42.57	8.61e-11	***
WHPT-ASPT					
	Sum Sq	DenDF	F value	<i>p-value</i>	

BA	2.86	40.8	19.83	6.43e-05	***
CI	1.69	263.4	11.67	0.0007	***
BA:CI	0.01	2013.9	0.06	0.8089	
LIFE Index					
	Sum Sq	DenDF	F value	<i>p-value</i>	
BA	1.93	56.1	8.56	0.0050	**
CI	7.90	267.5	35.00	1.01e-08	***
BA:CI	0.00	2036.5	0.00	0.9774	
PSI Index					
	Sum Sq	DenDF	F value	<i>p-value</i>	
BA	1.53	41.7	7.97	0.0073	**
CI	12.64	265.0	66.02	1.71e-14	***
BA:CI	0.46	2021.0	2.41	0.1206	
Func. Richness					
	Sum Sq	DenDF	F value	<i>p-value</i>	
BA	7.25	31.9	11.53	0.0019	**
CI	1.69	280.4	2.69	0.1021	
BA:CI	19.90	2062.5	31.65	2.09e-08	***
Func. Redundancy					
	Sum Sq	DenDF	F value	<i>p-value</i>	
BA	5.95	36.6	8.56	0.0059	**
CI	3.39	309.1	4.88	0.0278	*
BA:CI	11.31	2101.9	16.28	5.67e-05	***
c) Spring dataset					
WHPT Total					
	Sum Sq	DenDF	F value	<i>p-value</i>	
BA	0.01	55.0	0.02	0.8821	
CI	2.28	269.2	7.09	0.0082	**
BA:CI	8.14	2017.7	25.36	5.18e-07	***
WHPT-ASPT					
	Sum Sq	DenDF	F value	<i>p-value</i>	
BA	0.45	112.3	3.39	0.0684	
CI	2.32	263.4	17.40	4.12e-05	***
BA:CI	0.01	2078.4	0.05	0.8166	
LIFE Index					
	Sum Sq	DenDF	F value	<i>p-value</i>	
BA	0.40	103.0	1.99	0.1611	
CI	8.27	265.9	40.96	7.00e-10	***
BA:CI	0.12	2081.0	0.58	0.4447	
PSI Index					
	Sum Sq	DenDF	F value	<i>p-value</i>	
BA	0.21	89.6	1.16	0.2851	
CI	12.88	263.5	72.20	1.45e-15	***
BA:CI	0.19	2067.0	1.05	0.3065	
Func. Richness					
	Sum Sq	DenDF	F value	<i>p-value</i>	
BA	3.63	47.4	5.46	0.0237	*
CI	0.09	276.3	0.14	0.7082	
BA:CI	23.04	2041.4	34.70	4.49e-09	***

Func. Redundancy

	Sum Sq	DenDF	F value	<i>p-value</i>	
BA	2.19	37.1	3.31	0.0771	
CI	6.16	295.8	9.31	0.0025	**
BA:CI	2.54	1797.8	3.84	0.0502	.

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Figures

Figure 1. Responses of the 6 biological indices considered at the national scale (England). Control (red) and invaded samples (blue) are displayed before and after invasion of *D. haemobaphes*. Mean and 95% coefficient intervals are shown. Metrics standardised to z-scores on the y-axis. ***p < 0.001; **p < 0.01; *p < 0.05

Figure 2. Responses of the 6 biological indexes considered at the basin level (Trent river basin). Control (red) and invaded (blue) samples are displayed before and after invasion of *D. haemobaphes*. Mean and 95% coefficient intervals are shown. Metrics standardised to z-scores on the y-axis. *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$

Figure 3. Responses of the 6 biological indexes considered at the basin level (Thames river basin). Control (red) and invaded (blue) samples are displayed before and after invasion of *D. haemobaphes*. Mean and 95% coefficient intervals are shown. Metrics standardised to z-scores on the y-axis. ***p < 0.001; **p < 0.01; *p < 0.05

Figure 4. Fuzzy Principle Components Analysis (FPCA) ordination showing a) the centroids for macroinvertebrate community functional trait composition at the national level (England); b) the main biological traits driving community functional composition. First 2 axes account for 58.7% of the statistical variation. Each BACI level is enclosed in a convex hull corresponding to all samples in the group. (%) = percentage contribution of each modality to the trait-based ordination. After Control = red circle; After Invaded = light blue triangle; Before Control = orange square; Before Invaded = dark blue cross. For FPCA plots at basin level please see S10- S11.

Figure 5. Fuzzy Principle Components Analysis (FPCA) ordination showing a) the centroids of functional community composition for the spring dataset (England); b) the main biological traits in driving the functional composition ordination. Each BACI level is enclosed in a convex hull corresponding to all replicate samples. (%) = percentage contribution of each modality to the trait-based ordination. After Control = red circle; After Invaded = light blue triangle; Before Control = orange square; Before Invaded = dark blue cross.

Supplementary Information (Appendix code S)

S1. Environmental Agency of England macroinvertebrate sampling site dataset used in this study (n=259). Information for water bodies, river basin and treatment (control or invaded) is also provided.

S2. Map of study sites employed with main catchment boundaries (Trent and Thames) delineated. Solid triangle=Control site; Open triangle= Invaded sites

S3. Macroinvertebrate biological traits examined within this study (from Tachet et al., 2010 and Schmidt-Kloiber and Hering, 2015).

S4. Spearman rank correlations among all the biological indices analysed.
***p < 0.001; **p < 0.01; *p < 0.05

S5. Responses of the 6 biological indexes analysed for the Autumn dataset (England). Control (red) and invaded (blue) samples are displayed before and after the invasion of *D. haemobaphes*. Mean and 95% coefficient intervals are shown. Metrics standardised to z-scores on the y-axis. ***p < 0.001; **p < 0.01; *p < 0.05

S6. Responses of the 6 biological indexes analysed for the Spring dataset (England). Control (red) and invaded (blue) samples are displayed before and after the invasion of *D. haemobaphes*. Mean and 95% coefficient intervals are shown. Metrics standardised to z-scores on the y-axis. ***p < 0.001; **p < 0.01; *p < 0.05

S7. Fuzzy Principle Components Analysis (FPCA) ordination showing a) the centroids of functional community composition for the autumn dataset (England); b) the main biological traits in driving the functional composition ordination. Each BACI level is enclosed in a convex hull corresponding to all replicate samples. (%) = percentage contribution of each modality to the trait-based ordination. After Control = red circle; After Invaded = light blue triangle; Before Control = orange square; Before Invaded = dark blue cross.

S8. Results of the 1,000 simulations to assess the consistency of the outputs for function composition analysis at all levels (national, basin, season). Slope and distance between the before and after of the invaded and control samples have been assessed. Mean and 95% coefficient intervals are shown. Distance= n. of time the distance between centroids (before vs after) is greater for invaded samples (1=100%).

S9. Linear Mixed Model (LMMs) results for all biological indices considered in the BACI analysis at the basin (Trent and Thames) level. *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$

S10. Fuzzy Principle Components Analysis (FPCA) ordination showing a) the centroids for macroinvertebrate community functional trait composition for the River Trent basin; b) the main biological traits in driving the functional composition ordination. Each BACI level is enclosed in a convex hull corresponding to all replicate samples. (%) = percentage contribution of each modality to the trait-based ordination. After Control = red circle; After Invaded = light blue triangle; Before Control = orange square; Before Invaded = dark blue cross.

S11. Fuzzy Principle Components Analysis (FPCA) ordination showing a) the centroids of functional macroinvertebrate community composition for the River Thames basin dataset; b) the main biological traits driving community functional composition ordination. Each BACI level is enclosed in a convex hull corresponding to all replicate samples in the group. (%) = percentage contribution of each modality to the trait-based ordination. After Control = red circle; After Invaded = light blue triangle; Before Control = orange square; Before Invaded = dark blue cross.

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Supplementary Information (Appendix code S)

S1. Environmental Agency of England macroinvertebrate sampling site dataset used in this study (n=259). Information for water bodies, river basin and treatment (control or invaded) is also provided.

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S4. Spearman rank correlations among all the biological indices analysed.
***p < 0.001; **p < 0.01; *p < 0.05

S5. Responses of the 6 biological indexes analysed for the Autumn dataset (England). Control (red) and invaded (blue) samples are displayed before and after the invasion of *D. haemobaphes*. Mean and 95% coefficient intervals are shown. Metrics standardised to z-scores on the y-axis. ***p < 0.001; **p < 0.01; *p < 0.05

S6. Responses of the 6 biological indexes analysed for the Spring dataset (England). Control (red) and invaded (blue) samples are displayed before and after the invasion of *D. haemobaphes*. Mean and 95% coefficient intervals are shown. Metrics standardised to z-scores on the y-axis. ***p < 0.001; **p < 0.01; *p < 0.05

S7. Fuzzy Principle Components Analysis (FPCA) ordination showing a) the centroids of functional community composition for the autumn dataset (England); b) the main biological traits in driving the functional composition ordination. Each BACI level is enclosed in a convex hull corresponding to all replicate samples. (%) = percentage contribution of each modality to the trait-based ordination. After Control = red circle; After Invaded = light blue triangle; Before Control = orange square; Before Invaded = dark blue cross.

S8. Results of the 1,000 simulations to assess the consistency of the outputs for function composition analysis at all levels (national, basin, season). Slope and distance between the before and after of the invaded and control samples have been assessed. Mean and 95% coefficient intervals are shown. Distance= n. of time the distance between centroids (before vs after) is greater for invaded samples (1=100%).

S9. Linear Mixed Model (LMMs) results for all biological indices considered in the BACI analysis at the basin (Trent and Thames) level. *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$

S10. Fuzzy Principle Components Analysis (FPCA) ordination showing a) the centroids for macroinvertebrate community functional trait composition for the River Trent basin; b) the main biological traits in driving the functional composition ordination. Each BACI level is enclosed in a convex hull corresponding to all replicate samples. (%) = percentage contribution of each modality to the trait-based ordination. After Control = red circle; After Invaded = light blue triangle; Before Control = orange square; Before Invaded = dark blue cross.

S11. Fuzzy Principle Components Analysis (FPCA) ordination showing a) the centroids of functional macroinvertebrate community composition for the River Thames basin dataset; b) the main biological traits driving community functional composition ordination. Each BACI level is enclosed in a convex hull corresponding to all replicate samples in the group. (%) = percentage contribution of each modality to the trait-based ordination. After Control = red circle; After Invaded = light blue triangle; Before Control = orange square; Before Invaded = dark blue cross.

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35402	WEY (NORTH)	Thames	control
82078	WEY (SOUTH)	Thames	control
35791	WHITEWATER (LODDON)	Thames	control
35792	WHITEWATER (LODDON)	Thames	control
96883	AMPNEY BROOK	Thames	control
36186	COLN	Thames	control
36204	COLN	Thames	control
36133	EVENLODE	Thames	control
35491	KENNET	Thames	control
36079	KENNET	Thames	control
36072	LAMBOURN	Thames	control
36073	LAMBOURN	Thames	control
35007	LEACH	Thames	control
35965	OG	Thames	control
35443	PANG	Thames	control
35939	SOR BROOK	Thames	control
35911	THAME	Thames	control
35799	WINDRUSH	Thames	control
35528	WYE (THAMES)	Thames	control
35782	WYE (THAMES)	Thames	control
49859	ALFRETON BROOK	Trent	control
48583	AMBER	Trent	control
52265	AMBER	Trent	control
53167	AMBER	Trent	control
53845	AMBER	Trent	control
53846	AMBER	Trent	control
97161	ASHOP	Trent	control
47616	ASHWELL BROOK	Trent	control
47156	BAGTHORPE BROOK	Trent	control
50336	BAILEY BROOK	Trent	control
50817	BAKER LANE BROOK	Trent	control
52380	BECK DYKE	Trent	control
47672	BENTLEY BROOK	Trent	control
50623	BEVERCOTES BECK	Trent	control
51073	BLACK BROOK	Trent	control
52189	BLACK BROOK	Trent	control
47661	BOTTLE BROOK	Trent	control
50583	BOTTLE BROOK	Trent	control
52534	BOTTLE BROOK	Trent	control
53857	BRADFORD BROOK	Trent	control
50020	BRADGATE BROOK	Trent	control
47612	BROADBRIDGE DYKE	Trent	control
52704	BROADBRIDGE DYKE	Trent	control
48599	BROUGHTON ASTLEY BROOK	Trent	control
51466	COCKER BECK	Trent	control
46924	DERWENT (LT)	Trent	control
47057	DERWENT (LT)	Trent	control
47138	DERWENT (LT)	Trent	control
50454	DERWENT (LT)	Trent	control
51342	DERWENT (LT)	Trent	control
51495	DERWENT (LT)	Trent	control
53543	DERWENT (LT)	Trent	control

53618	DERWENT (LT)	Trent	control
53873	DERWENT (LT)	Trent	control
47206	DEVON	Trent	control
51330	DEVON	Trent	control
51838	DOVER BECK	Trent	control
52988	DOVER BECK	Trent	control
153886	DOVER BECK	Trent	control
49026	EAU	Trent	control
50016	EAU	Trent	control
49700	ECCLESBOURNE (River ECCLESBOURNE)	Trent	control
52718	ECCLESBOURNE (River ECCLESBOURNE)	Trent	control
48442	EYE	Trent	control
49040	EYE	Trent	control
52587	EYE	Trent	control
52768	FLEET	Trent	control
154963	GILT BROOK	Trent	control
154964	GILT BROOK	Trent	control
154965	GILT BROOK	Trent	control
47538	GRACE DIEU BROOK	Trent	control
52301	GRACE DIEU BROOK	Trent	control
48568	GREET	Trent	control
50964	GREET	Trent	control
52726	HARBY BROOK	Trent	control
51253	HATFIELD WASTE DRAIN	Trent	control
51064	HEATH END BROOK	Trent	control
53594	IDLE	Trent	control
78923	JAGGERS CLOUGH	Trent	control
47617	LANGHAM BROOK	Trent	control
53858	LATHKILL	Trent	control
50048	LEEN	Trent	control
51280	LEEN	Trent	control
53426	LEEN	Trent	control
52417	LUBBESTHORPE BK (EX BIAM)	Trent	control
50963	MACKWORTH BROOK	Trent	control
51044	MARKEATON BROOK	Trent	control
47100	MAUN	Trent	control
51324	MAUN	Trent	control
50765	MEDEN	Trent	control
51331	MIDDLE BECK	Trent	control
47592	NEW BROOK	Trent	control
51204	NOE	Trent	control
47526	NORMANTON / THURLASTON BK	Trent	control
52332	NORMANTON / THURLASTON BK	Trent	control
53424	OLDCOTES DYKE	Trent	control
53214	OUSE DYKE	Trent	control
52322	PICKWELL BROOK EAST	Trent	control
48605	PICKWELL BROOK WEST	Trent	control
50094	POULTER	Trent	control
51896	POULTER	Trent	control
52480	POULTER	Trent	control
52504	POULTER	Trent	control
153578	RADBOURNE BK	Trent	control

49170	RAINWORTH WATER	Trent	control
49363	RAINWORTH WATER	Trent	control
153552	RAMSLEY BROOK	Trent	control
53705	RANSKILL BROOK	Trent	control
53835	ROTHLEY BROOK	Trent	control
48913	RYTON	Trent	control
49366	RYTON	Trent	control
51015	SENCE (SOAR)	Trent	control
52426	SENCE (SOAR)	Trent	control
52727	SHILTON BROOK	Trent	control
52772	SMITE	Trent	control
52495	SOAR	Trent	control
47122	SOMERBY BROOK	Trent	control
51065	STAUNTON HAROLD BROOK	Trent	control
48272	SYSTON BROOK	Trent	control
52501	THE BECK	Trent	control
51118	THURLASTON BROOK	Trent	control
53757	TORNE	Trent	control
53150	WALTON BROOK	Trent	control
47147	WHETSTONE BROOK	Trent	control
47615	WHISSENDINE BROOK	Trent	control
50146	WOOD BROOK	Trent	control
50349	WYE (LT)	Trent	control
53811	WYE (LT)	Trent	control
53819	WYE (LT)	Trent	control
56208	KINGS DYKE	Ouse	impact
56235	OUSE (KYM)	Ouse	impact
56310	OUSE (OUZEL)	Ouse	impact
56407	OUSE (OUZEL)	Ouse	impact
56143	OUSE (ST IVES)	Ouse	impact
56164	OUSE (ST IVES)	Ouse	impact
56439	OUZEL	Ouse	impact
56453	TOVE	Ouse	impact
55583	GWASH	Welland	impact
55669	NENE	Nene	impact
55673	NENE	Nene	impact
55676	NENE	Nene	impact
55678	NENE	Nene	impact
55694	NENE	Nene	impact
55697	NENE	Nene	impact
55698	NENE	Nene	impact
55722	PITSFORD ARM/FAXTON BROOK	Nene	impact
75913	AVON (LS - SOWE TO BIDFORD)	Severn	impact
48445	AVON (LS - ABOVE SOWE)	Severn	impact
53305	AVON (LS - ABOVE SOWE)	Severn	impact
154944	AVON (LS - ABOVE SOWE)	Severn	impact
158736	AVON (LS - ABOVE SOWE)	Severn	impact
53026	CARLTON BROOK	Trent	impact
47062	LEAM	Severn	impact
49704	LEAM	Severn	impact
51122	LEAM	Severn	impact
46926	River Trent	Trent	impact

47273	River Trent	Trent	impact
51394	River Trent	Trent	impact
51508	River Trent	Trent	impact
48476	SOW	Trent	impact
50369	SOW	Trent	impact
52917	TAME (UT)	Trent	impact
48459	EREWASH	Trent	impact
50294	IDLE	Trent	impact
47275	SOAR	Trent	impact
48468	SOAR	Trent	impact
48888	SOAR	Trent	impact
49307	SOAR	Trent	impact
53754	SOAR	Trent	impact
46894	Trent	Trent	impact
47046	Trent	Trent	impact
47063	Trent	Trent	impact
48580	Trent	Trent	impact
49039	Trent	Trent	impact
50916	Trent	Trent	impact
51467	Trent	Trent	impact
47608	WOOD BROOK	Trent	impact
52341	WOOD BROOK	Trent	impact
48328	AVON (LS - BELOW BIDFORD)	Severn	impact
51827	AVON (LS - BELOW BIDFORD)	Severn	impact
53747	AVON (LS - BELOW BIDFORD)	Severn	impact
51524	MEESE	Severn	impact
51368	SALWARPE	Severn	impact
52933	SALWARPE	Severn	impact
51208	SEVERN	Severn	impact
48200	SEVERN (LS)	Severn	impact
51037	SEVERN (LS)	Severn	impact
51327	SEVERN (LS)	Severn	impact
172484	SEVERN (LS)	Severn	impact
51807	STOUR (US)	Severn	impact
153543	STOUR (US)	Severn	impact
67296	DOUGLAS	Ribble	impact
69695	DOUGLAS	Ribble	impact
66940	GOWY	Mersy	impact
68460	GOWY	Mersy	impact
66935	WEAVER	Mersy	impact
34013	COLNE BROOK	Thames	impact
33600	LEE (NAV.SUB A)	Thames	impact
34158	LEE (THAMES)	Thames	impact
82437	ODNEY WEIR STREAM	Thames	impact
35886	THAMES (SE AREA)	Thames	impact
35889	THAMES (SE AREA)	Thames	impact
81960	THAMES (SE AREA)	Thames	impact
98142	THAMES TIDEWAY (THAMES SOUTH-EAST AREA)	Thames	impact
36232	CHERWELL	Thames	impact
36242	CHERWELL	Thames	impact
36248	CHERWELL	Thames	impact
36261	CHERWELL	Thames	impact

36262
150066
34459

CHERWELL
CHERWELL
SEACOURT STREAM

Thames
Thames
Thames

impact
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Table S . Macroinvertebrate traits examined within this study (from Tachet et al., 2010).

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

S8. Results of the 1,000 simulations to assess the consistency of the outputs for function composition analysis at all levels (national, basin, season).

Slope and distance between the before and after of the invaded and control samples have been assessed.

Mean and 95% coefficient intervals are shown.

Distance= n. of time the distance between centroids (before vs after) is greater for invaded samples (1=100%).

Variable	National level	Trent Basin	Thames Basin	Spring	Autumn
distance	0.278	1	0.339	0.438	0.362
control slope	0.0001312770	0.0004761103	0.0002167011	0.0001821799	0.0002038475
ctrl_2.5	0.0000061544	0.0000210572	0.0000109909	0.0000079018	0.0000077152
ctrl_97.5	0.0006084933	0.0068468330	0.0019105820	0.0010641470	0.0008432789
invaded slope	0.0050451060	0.0138276369	0.0052501630	0.0037383440	0.0081910243
inv_2.5	0.0016019010	0.0041364090	0.0013485020	0.0014455900	0.0025293910
inv_97.5	0.1377811340	0.3073714660	0.1210929770	0.0858765200	0.1846265000

S9. Linear Mixed Model (LMMs) results for all biological indices considered in the BACI analysis at the basin (Trent and Thames) level.

BA : CI= interaction (Before–After) : (Control–Impact). *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$

TRENT					
WHPT					
Variable	Sum Sq	DenDF	F value	<i>p-value</i>	
BA	0.0785	40.76	0.2585	0.6139	
CI	0.4292	133.77	1.4139	0.2365	
BA:CI	5.5323	2491.64	18.2243	2.04E-05	***
WHPT- ASPT					
Variable	Sum Sq	DenDF	F value	<i>p-value</i>	
BA	3.2109	40.02	26.4891	7.40E-06	***
CI	0.617	132.23	5.0898	0.025709	*
BA:CI	0.8702	2484.66	7.1785	0.007427	**
LIFE					
Variable	Sum Sq	DenDF	F value	<i>p-value</i>	
BA	1.62697	48.04	8.9184	0.004433	**
CI	2.53097	133.28	13.8739	0.000288	***
BA:CI	0.98441	2490.78	5.3962	0.020261	*
PSI					
Variable	Sum Sq	DenDF	F value	<i>p-value</i>	
BA	1.8233	42.25	10.7194	0.00212	**
CI	3.5142	133.03	20.6607	1.22E-05	***
BA:CI	0.0873	2487.47	0.5132	0.47383	
Func. Richness					
Variable	Sum Sq	DenDF	F value	<i>p-value</i>	
BA	22.424	48	33.8088	4.81E-07	***
CI	0.07	135.06	0.1055	0.7458	
BA:CI	53.262	2496.5	80.3034	< 2.2E-16	***
Func. Redundancy					
Variable	Sum Sq	DenDF	F value	<i>p-value</i>	
BA	0.5501	39.46	0.7849	0.381	
CI	0.0001	138.05	0.0001	0.9916	
BA:CI	12.2417	2514.07	17.4653	3.03E-05	***
THAMES					
WHPT					
Variable	Sum Sq	DenDF	F value	<i>p-value</i>	
BA	0.2952	42.2	0.8284	0.3679	
CI	0.6282	83.01	1.7629	0.1879	
BA:CI	14.9286	2500.7	41.8925	1.16E-10	***
WHPT - ASPT					
Variable	Sum Sq	DenDF	F value	<i>p-value</i>	
BA	2.06459	59.46	9.8703	0.002618	**
CI	0.32542	81.83	1.5557	0.215849	
BA:CI	1.01764	2510.58	4.8651	0.027496	*
LIFE					
Variable	Sum Sq	DenDF	F value	<i>p-value</i>	
BA	0.6664	56.54	2.0796	0.154799	
CI	3.7544	82.27	11.7165	0.000968	***
BA:CI	0.7303	2507.8	2.279	0.131266	

PSI

Variable	Sum Sq	DenDF	F value	<i>p-value</i>	
BA	0.8672	54.46	3.0463	0.086564	.
CI	8.4074	82.41	29.5334	5.47E-07	***
BA:CI	3.6285	2509.44	12.7463	0.000363	***

Func. Richness

Variable	Sum Sq	DenDF	F value	<i>p-value</i>	
BA	4.3097	43.92	6.3064	0.015784	*
CI	3.5397	91.3	5.1797	0.025191	*
BA:CI	7.1517	2514.7	10.4651	0.001232	**

Func. Redundancy

Variable	Sum Sq	DenDF	F value	<i>p-value</i>	
BA	1.3785	40.33	1.8505	0.181278	
CI	6.5824	93.65	8.8361	0.003754	**
BA:CI	14.8446	2516.47	19.927	8.40E-06	***

A)

B)

- A - C
- ▲ A - I
- B - C
- + B - I